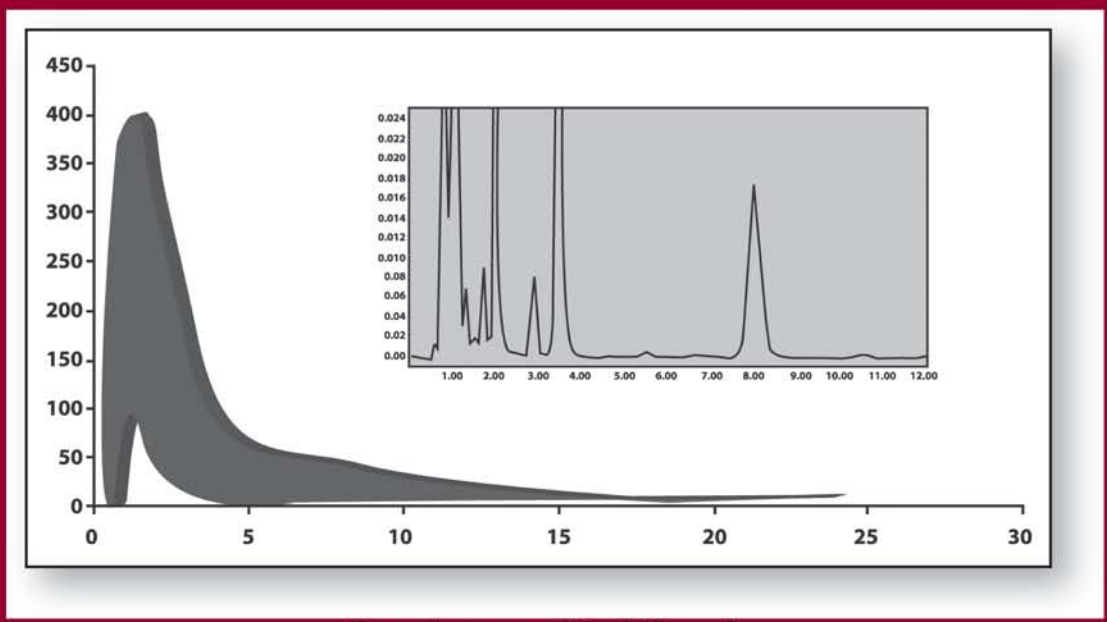
DRUGS AND THE PHARMACEUTICAL SCIENCES VOLUME 171

Handbook of Bioequivalence Testing



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Handbook of Bioequivalence Testing

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Handbook of Bioequivalence Testing

Sarfaraz K. Niazi
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I dedicate this book to a long-time friend and professional associate,
Abdul Razzaq Yousef, who heads Gulf Pharmaceutical Industries;
his vision about the health care business
as well as his dynamism and leadership in the industry
are refreshing and stimulating.
His encouragement in the writing of this book was invaluable.

Preface

The Drug Price Competition and Patent Term Restoration Act of 1984 (Pub. L. No. 98-417) (the Hatch-Waxman Amendments) created section 505(j) of the Act, which established the current Abbreviated New Drug Application approval process. The showing that must be made for an Abbreviated New Drug Application to be approved is quite different from what is required in a New Drug Application. A New Drug Application applicant must prove that the drug product is safe and effective. An Abbreviated New Drug Application applicant does not have to prove the safety and effectiveness of the drug product because an Abbreviated New Drug Application relies on the finding the Food and Drug Administration has made that the reference listed drug is safe and effective. Instead, an Abbreviated New Drug Application applicant must demonstrate, among other things, that its drug product is bioequivalent to the reference listed drug (2 1 U.S.C. 355(j)(2)(A)(iv)). The scientific premise underlying the Hatch-Waxman Amendments is that, in most circumstances, bioequivalent drug products may be substituted for each other. A generic drug is bioequivalent to the listed drug if "the rate and extent of the absorption of the drug do not show a significant difference from the rate and extent of absorption of the listed drug when administered at the same molar dose of the therapeutic ingredient under similar experimental conditions in either a single dose or multiple doses" (21 U.S.C. 355(j)(8)(B)(i)).

The 21st Century Initiative by the U.S. Food and Drug Administration has resulted in several major changes in how it would conduct its business in the future. This includes the Office of New Drug Chemistry's Risk-Based Quality Systems and the Risk-Based Good Manufacturing Practices Initiative. Bioequivalence testing of multisource drug products occupies a significant portion of Abbreviated New Drug Application filings and the U.S. Food and Drug Administration has recently initiated several actions to streamline the bioequivalence trials; these initiatives include "Waivers of *In Vivo* Demonstration of Bioequivalence" and the "Biopharmaceutics Classification System." However, there remains a need to open the entire issue of bioequivalence testing in light of formal risk-based testing requirements and through appreciation of the complexities involved in testing bioequivalence. For example, the Food and Drug Administration removed the bioavailability protocols that it used to list on its Web site starting in 1997 to take away the impression that only the listed protocols are valid or even the better choice.

With the advancement of science as envisioned in the Process and Analytical Technology initiative, new vistas are opening in creating rationale for better tools to emulate bioequivalence. Whereas a large number of modifications to dissolution methodologies, the dissolution media, and methods of testing have been reported in the literature, use of multiphasic dissolution systems that will characterize the thermodynamic activity of drug at the site of absorption are yet to be developed. These simple systems are likely to simulate the absorption surface better than the use of more elaborate models, ranging from parallel artificial membrane permeation assay to Caco-2 systems. Once the dissolution medium is capable of picking up the difference in the transport of free drug molecules across a lipophilic barrier, the thermodynamic activity is established, and when compared to a reference listed drug, the comparability of the two products is well established.

The U.S. Food and Drug Administration allows waiver of bioequivalence for several drugs; this should continue and the list expanded to include those where there is a sufficient merit in the actual use of the product over a period of time. For all other products where the U.S. Food and Drug Administration currently requires demonstration of bioequivalence, the

multisource product manufacturer will be allowed to present surrogate methods that take a fresh look at the emulation of absorption and may include the use of nanosensors embedded in the drug product, multiphasic dissolution systems, and a host of other possibilities that have just begun to open with new technologies arriving. Of great importance is that these new technologies will appear in conducting complex studies such as those involving food effects, topical drugs, inhalation devices, and even botanical drugs. The field of biological drugs is another area that is fast evolving. The U.S. Food and Drug Administration is developing guidelines for "biosimilar" or "follow-on" biological products and has not been able to conclude what tests would constitute demonstration of bioequivalence. Whereas these products are administered through routes that provide lesser barriers in the entry of drug to the body, the differences are related to antigenicity potential, which needs a clinical evaluation; however, studies have demonstrated that minute differences in the structure of protein drugs including dimerization, 3rd and 4th degree structural changes, and easily picked up in partitioning studies since these studies truly represent the thermodynamic potential which is quickly changed even where minor differences in the structures, often too small to be detected by even the most sophisticated instruments; in most instances, the use of instrumentation itself disturbs the structure enough to make the studies meaningless. This almost borders on the Heisenberg's principle of uncertainty.

The field of bioequivalence testing continues to offer many challenges to the generic drug industry. With fast escalating costs of these studies, particularly where repeated studies are required, the generic industry would do well by designing better studies. Successful bioequivalence reviews have their root in careful planning well before the study is conducted. Inevitably, this planning reduces the need for costly bioequivalence study repeats, reduces the number of review cycles, and compresses the Abbreviated New Drug Application approval process; incidentally, the regulatory agencies also like this because it reduces their burden of work. Experienced investigators and study monitors know well that whenever issues arise, it is important to resolve them complete and effectively as soon as they appear and follow this simple trail to make the project more efficient:

1. Evaluate the provisions of all regulatory guidance with respect to the drug being developed and design study protocols accordingly, and have these commented on by the regulatory agency through correspondence. The Food and Drug Administration used to have bioequivalence protocols listed on its Web site, but that was changed in 1996 when the Food and Drug Administation decided that the issues relating to bioavailability and bioequivalence are complex, the intent being to encourage sponsors to come up with more relevant studies. The protocols, however, can still be obtained through the Freedom of Information Web site (http://www.fda.gov/opacom/backgrounders/foiahand.html) or through commercial contractors. Complete details of all communications with the regulatory agencies should be summarized in the section, "Clinical Protocols," in the final bioequivalence report. It would be of great hindsight value if this report contains details of all correspondence, written or oral, as well.
2. Non-conventional dosage forms, when involved, create a substantial delay in review unless the sponsor follows any existing guidelines and additionally follows through with comments from the Food and Drug Administration; this applies particularly to such dosage forms as nasal sprays, metered dose inhalers, and topical drugs.
3. Ensure appropriate selection of the Reference Listed Drug, carefully considering and identifying the dosage strengths to be studied when pursuing approval of multiple strengths of a given product. Know that departure from maximum dosing level will require filing an Investigational New Drug application.
4. Auditing Contract Research Organization for Good Clinical Practice and Good Laboratory Practice compliance; preservation of raw data and application of good document practice and proper retention of study samples is assured. Review the guidelines of inspection of clinical facilities, particularly those that pertain to bioavailability studies; all audits must conform to the requirements of inspections of the Food and Drug Administration—these are available on the Food and Drug Administration Web site.

5. Beware of "outliers" as one can not simply remove them from a data set; you must have a solid justification and/or re-dosing of the outlier with a subset of the original patient population. The Contract Research Organization must additionally have Standard Operating Procedures governing sample plasma analysis, which must require supporting stability data.
6. Redesigning failed studies should be done to avoid giving the impression that the sponsor is "testing into compliance," by first fully understanding the causes of failure and addressing these through reformulation and adjustment of protocol with justification. Know that while the agencies do not currently require but they may soon require it and even now still demand to know about any failed studies prior to the study being submitted. Obviously, if a study is failing, this should not be part of any Abbreviated New Drug Application, even though a "close call" case can be made through statistical descriptions and other arguments.
7. When requesting a waiver, clearly define the dissolution test and the related quality control measures taken; to compare with the RLD request information through the Freedom of Information Web site (see above), submit written requests to OGD when necessary and now that the Food and Drug Administration has begun publishing dissolution tests on its Web site; see Appendix 3 of the book.
8. Preparing a readily readable, clear, and comprehensive bioequivalence report goes a long way in expediting the review; make sure that each section contains a detailed table of contents and link these entries to the text for easy navigation. A quality audit of application prior to submission is essential, as suggested in Chapter 13.

The *Handbook of Bioequivalence Testing* is a practical treatise for all who are involved in planning and conducting such studies. The roots to this book go back to the late 1970s when I wrote The *Textbook of Biopharmaceutics and Clinical Pharmacokinetics*, the first book on the subject. Several good books followed this teaching textbook, and I felt no compelling reason, despite the suggestions of my publishers, to write another book on the subject. Almost 30 years later, I find that there is a need to take a comprehensive approach to testing bioequivalence of drug products. The Food and Drug Administration has undergone major changes in its thinking on new suggestions to do these studies, and I find it exciting to design challenging studies. Generic companies generally do not want to go against established norms, and there are good reasons for that; however, the new Food and Drug Administration is ready to listen to alternate methods and even petition to waive these studies. I believe once a company fully understands the nature of the drug being developed, it will be possible to create tests that would qualify as alternates to any biological testing. It is for this reason that I have included several classical teaching chapters in this book.

Chapter 1, "Bioequivalence Testing Rationale," provides a historical perspective to the development of regulations that have led to today's regulatory requirements. The guidelines that govern submissions related to bioequivalence studies are discussed along with therapeutic code classification by the Food and Drug Administration. An overview of bioequivalence testing requirements and approaches establishes a scientific rationale for products that must demonstrate bioequivalence. This topic is discussed in much greater depth in later chapters. Regulatory expectations are described in terms of what the Food and Drug Administration considers to be the factors of variability contributing to bioequivalence variation, and various measurement indices and techniques are described. Included in this chapter is the rationale for bioequivalence estimation along with a listing of drugs with historic bioequivalence problems. Broad guidelines to single-dose, multiple-dose, and fed studies are described along with pharmacological and clinical end-point studies that can be substituted for the traditional blood level studies. The use of precise and accurate analytical methods along with guidelines of validation are described. Pharmacokinetic and statistical considerations in experiment design are reviewed in this chapter as well. Errors in bioequivalence measurement are highlighted. Studies related to animals, locally administered drugs, and drugs given topically are also described. This chapter serves as a primer to the *Handbook of Bioequivalence Testing*.

Chapter 2, "Regulatory Aspects of Bioequivalence Testing," deals with the regulations that govern the submission of studies to support marketing authorization applications worldwide, along with a detailed discussion on the most controversial and misunderstood aspects of regulatory submissions. A new concept of risk-based bioequivalence is introduced, health risk categories are defined, and examples of drugs presenting complex situations in bioequivalence measurement are presented. The concept of bioequivalence surrogates is detailed along with methods of absorption profiling.

Chapter 3, "Pharmacokinetic/Pharmacodynamic Modeling," fulfils the scientific inquiry needs addressed in the earlier chapter for surrogates of bioequivalence testing. A detailed mathematical treatment along with common assumptions are described. Bioequivalence and systemic exposure modeling equations are described to help create models. Deconvulation techniques, including computer software use, is described. Pharmacological evaluation of bioavailability is discussed.

Chapter 4, "Waiver of BA/BE Studies," is a pivotal chapter for both generic as well as innovator sponsors. Changing trends at the regulatory level allow sponsors to make a strong case for reducing the scope of bioavailability/bioequivalence studies; this is an extremely important factor for the industry. This chapter includes a detailed description of the biopharmaceutics classification system with several recent modifications to the concept. Pharmacokinetic studies include a discussion of absolute bioavailability, which, though of lesser importance to the generic sponsor, brings new possibilities where the drug delivery system is modified (improved). Details of all available surrogate methods to substitute bioavailability/bioequivalence data are described.

Chapter 5, "Regulatory Review Process," describes the events that take place during regulatory audits. This is crucial for contract research organizations to understand what the regulatory authorities want to see when they land at the facility conducting the work.

Chapter 6, "Statistical Evaluation of Bioequivalence Data," is a detailed description of the statistical models, in theory and in practice, and the software available, along with a full-length data analysis exercise. A list of available software and recommendations on choosing calculation support is provided.

Chapter 7, "Physicochemical Properties Affecting Bioequivalence," describes all those factors that are responsible for introducing bioequivalence variability from the Active Pharmaceutical Ingredients viewpoint. Although this chapter comes late in the order of presentation, it is placed here because the sponsor needs to understand why factors that may seem remote may have caused failure of bioequivalence. Details of dissolution models are also provided.

Chapter 8, "Drug Delivery Factors," takes the previous chapter on the properties of Active Pharmaceutical Ingredients to the properties of the drug delivery systems chosen. Factors affecting drug release and assessment of these characteristics are provided in detail. This will be of great value to the formulation scientists both at the generic as well as the innovator laboratories. Detailed discussions of mechanisms of absorption are also provided in light of release factors from dosage forms, but these principles also apply to several chapter discussions where the drugs with potential bioequivalence problems are identified.

Chapter 9, "Bioanalytical Method Validation," addresses a key requirement of regulatory filing—the use of fully validated analytical methods. Various guidelines from regulatory authorities along with real-time examples of validation are presented here.

Chapter 10, "Good Clinical Practice," describes how these rules apply to all bioavailability/bioequivalence studies since volunteers are humans, even though they may not be tested for therapeutic response. The Declaration of Helsinki begins this chapter and then provides the required compliance details and a long trail of documents needed to certify that the testing facility is Good Manufacturing Practice compliant.

Chapter 11, "Good Laboratory Practices for Nonclinical Laboratory Studies," applies to all those aspects that relate to the use of laboratory facilities and includes data handling requirements. These are often the main areas of noncompliance during the audit visits. Audit-related details are provided.

Chapter 12, "Computer and Software Validation," deals with a topic of great importance as electronic submissions and the use of automated systems to collect and analyze the data are

becoming norms. Although the Contract Research Organization is likely use to use off-the-shelf and commercial hardware, it is important to understand what questions to ask when seeking validation proof. All phases of data collection, storage, and analysis are subject to strict regulations.

Chapter 13, "Bioequivalence Reports," provides a practical guide to writing reports for regulatory submissions along with several examples of reports submitted to regulatory authorities.

Appendix 1, "Glossary of Terms," provides a ready reference to various definitions, particularly where these have specific meaning in the regulatory authority interpretation.

Appendix 2, "Bioequivalence Testing Literature," is a review of current trends in bioequivalence testing. Abstracts of the most relevant published papers are presented here.

Appendix 3, "Dissolution Testing Methods of Approved Drugs," is a current listing of methods reported to the Food and Drug Administration for the approval of New Drug Applications. This is current as of early 2007.

The theme of this book remains a handbook rather than an exhaustive treatise on the topics related to methods used to minimize bioequivalence variation, designing experiments to test bioequivalence, and securing biowaivers and preparing submission reports. The bibliographies quoted in the book should be of help to the reader, but material is enclosed here in sufficient depth to allow for a clear understanding of the difficult path that leads to bioequivalence demonstration.

One of the most useful parts of this handbook is the examples of bioequivalence reports, form templates, and presentation styles of reports. I am greatly indebted to my long-time friend and colleague, Dr. Ruwaydah Dham of Gulf Pharmaceutical Industries, for giving me permission to reproduce these reports and for her incessant encouragement; the analytical validation report has been redacted for confidentiality purposes. The work was conducted at the world-famous International Pharmaceutical Research Center in Jordan under the able guidance of Dr. Naji Najib; this laboratory is among the best fully accrediated laboratories in the world (http://www.iprc.com.jo/main.asp).

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Sarfaraz K. Niazi

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1 Bioequivalence Testing Rationale and Principles

BACKGROUND

Applicants submitting a new drug application (NDA) or new animal drug application (NADA) under the provisions of section 505(b) in the Federal Food, Drug, and Cosmetic Act (FDC Act) are required to document bioavailability (BA) [21 CFR 320.21(a)]. If approved, an NDA drug product may subsequently become a reference listed drug (RLD). Under section 505(j) of the Act, a sponsor of an abbreviated new drug application (ANDA) or abbreviated new animal drug application (ANADA) must document first pharmaceutical equivalence and then bioequivalence (BE) to be deemed therapeutically equivalent to an RLD. Defined as relative BA, BE is documented by comparing the performance of the generic (test) and listed (reference) products. (Pharmaceutical equivalents are drugs that have the same active ingredient in the same strength, dosage form, and route of administration, and have comparable labeling and meet compendia or other standards of identity, strength, quality, purity, and potency.)

In addition to the standard chemistry, manufacturing, and control (CMC) tests, the active bulk drug substance for an NDA should be studied and controlled via appropriate specifications for polymorphic form, particle size distribution, and other attributes important to the quality of the resulting drug product. To the extent possible and using compendial monographs where appropriate, sponsors of ANDAs should attempt to duplicate the specifications considered important for the RLD. Where the necessary information is not available, applicants may wish to rely on in vitro release to ensure batch-to-batch consistency. CMC guidances available from the Food and Drug Administration (FDA) are generally applicable to ensure the identity, strength, quality, purity, and potency of the drug substance and drug product for a topical dermatological drug product.

As stated in 21 CFR 320.24, the approaches to document BE in order of preference are (*i*) pharmacokinetic (PK) measurements based on measurement of an active drug and/or metabolite in blood, plasma, and/or urine; (*ii*) pharmacodynamic (PD) measurements; (*iii*) comparative clinical trials; and (*iv*) in vitro studies.

BE is defined in 21 CFR 320.1 as "the absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study." The FDA usually considers that the plasma concentration of a drug is a surrogate for the concentration at the site of action for a systemically acting drug. 21 CFR 320.24 outlines options for BE testing. Therefore, proving equivalence requires integration of several studies, such as PK, PD, controlled-clinical, in vitro studies, and any other specific model or study that may prove useful in proving equivalence.

The concept of BE and the required proof by the regulatory agencies has evolved over the past several decades:

- In the United States, the 1902 federal law for biologics, particularly vaccines, required evaluation for "safety, purity, and potency."
- The 1906 Food and Drugs Act added drugs other than biologics.
- The 1938 FDC Act created FDA and evaluation of new drugs based on data in a filed NDA.

- The 1962 law added effectiveness requirements for the approval of NDA. 1902 Federal law required that biologics (vaccines) be evaluated for "safety, purity, and potency."
- The 1906 Food and Drugs Act added drugs other than biologics.
- The 1938 FDC Act created FDA and required safety evaluation on new drugs before marketing based on data in an NDA.
- The 1962 law added effectiveness requirement for approval of an NDA; in the 1960s, the FDA permitted marketing of "similars," while corresponding pioneer products underwent Drug Efficacy Study Implementation (DESI) reviews. "Similars" came into the market between 1938 and 1962.
- In 1970 the FDA terminates marketing of "similars" unless
  - DESI pioneer showed safety and efficacy.
  - "Similar" manufacturer submits ANDA with formulation and manufacture information; (*The Supreme Court in the United States vs. Generix Drug Corporation* supported FDA requirement for ANDA).
- The 1984 generic law in the United States (Waxman–Hatch) created a generic approval system for all new drugs, including those approved after 1962. The FDA finalized the bioequivalence (BA/BE) regulations (21 CFR 320), wherein the pioneer shows BA in NDA; "similars" to DESI-effective pioneers show BE leading to first United States first generics. Several revisions to 21 CFR 320 were made including the most recent one in April 2006. The Drug Price Competition and Patent Term Restoration Act of 1984 (Pub. L. No. 98-417) (the Hatch–Waxman Amendments) created section 505(j) of the Act, which established the current ANDA approval process. The showing that must be made for an ANDA to be approved is quite different from what is required in an NDA. An NDA applicant must prove that the drug product is safe and effective. An ANDA does not have to prove the safety and effectiveness of the drug product, because an ANDA relies on the finding the FDA has made that the RLD is safe and effective. Instead, an ANDA applicant must demonstrate, among other things, that its drug product is bioequivalent to the RLD [21 U.S.C. 355(j)(2)(A)(iv)]. The scientific premise underlying the Hatch–Waxman amendments is that in most circumstances, bioequivalent drug products may be substituted for each other. The Generic Animal Drug and Patent Term Restoration Act signed into law on November 16, 1988, permits sponsors to submit an ANADA for a generic version of any off-patent approved animal drug (with the certain exceptions noted in the law), regardless of whether the drug was approved prior to 1962 and subject to the National Academy of Sciences/National Research Council/DESI review.

A generic drug is bioequivalent to the listed drug if "the rate and extent of the absorption of the drug do not show a significant difference from the rate and extent of absorption of the listed drug when administered at the same molar dose of the therapeutic ingredient under similar experimental conditions in either a single dose or multiple doses" [21 U.S.C. 355(j)(8)(B)(i)].

The science of BE is still undergoing major changes and final rules are established after years of debate and the validation of protocols. The U.S. FDA has finalized or drafted several guidelines (Table 1).

The FDA has also provided a Therapeutic Classification of drugs and dosage forms for the purpose of BE testing (Table 2).

OVERVIEW OF BE TESTING

Is a BE Study Required?

The submission of an NDA, ANDA, or supplemental application requires that it contains in vivo BA and BE either data by direct measurement of in vivo BA of the drug product that is the subject of the application or information to permit the FDA to waive the submission of evidence measuring in vivo BA. The supplemental application involves a change in the manufacturing site or a change in the manufacturing process, including a change in product formulation or dosage strength, beyond the variations provided for in the approved

TABLE 1 Final and Draft-Stage Biopharmaceutics Guidelines of the U.S. Food and Drug Administration

Guideline	Date finalized/draft issued
Bioanalytical Method Validation—final	May 23, 2001
Bioavailability and Bioequivalence Studies for Orally Administered Drug Products—General Considerations (Revised)—final	March 19, 2003
Cholestyramine Powder In Vitro Bioequivalence—final	July 15, 1993
Clozapine Tablets: In Vivo Bioequivalence and In Vitro Dissolution Testing—final	June 20, 2005
Corticosteroids, Dermatological (topical) In Vivo—final	June 2, 1999
Dissolution Testing of Immediate Release Solid Oral Dosage Forms—final	August 25, 1997
Extended Release Oral Dosage Forms: Development, Evaluation, and Application of In Vitro/In Vivo Correlations—final	September 26, 1997
Metaproterenol Sulfate and Albuterol Metered Dose Inhalers—final	June 27, 1989
Statistical Approach to Establishing Bioequivalence—final	February 2, 2001
Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate Release Solid Oral Dosage Forms on a Biopharmaceutical Classification System—final	August 31, 2000
Potassium Chloride (Slow-Release Tablets and Capsules) In Vivo Bioequivalence and In Vitro Dissolution Testing	June 6, 1994
Food-Effect Bioavailability and Fed Bioequivalence Studies	December 2002
Antifungal (topical)—draft	February 24, 1990
Antifungal (vaginal)—draft	February 24, 1990
Bioavailability and Bioequivalence Studies for Nasal Aerosols and Nasal Sprays for Local Action—draft	April 3, 2003

application, or a change in the labeling to provide for a new indication for use of the drug product, for which a new clinical trial may be required.

The FDA may approve a full NDA, or a supplemental application proposing any of the changes set forth above that does not contain evidence of in vivo BA or information to permit waiver of the requirement for in vivo BA data.

- For certain drug products, the in vivo BA or BE of the drug product may be self-evident. The FDA shall waive the requirement for the submission of evidence obtained in vivo measuring the BA or demonstrating the BE of these drug products. A drug product's in vivo BA or BE may be considered self-evident based on other data in the application.
- If the drug product is a parenteral solution intended solely for administration by injection, or an ophthalmic or otic solution, and contains the same active and inactive ingredients in the same concentration as a drug product that is the subject of an approved full NDA or ANDA or
- If the drug product is administered by inhalation as a gas, e.g., a medicinal or an inhalation anesthetic, and contains an active ingredient in the same dosage form as a drug product that is the subject of an approved full NDA or ANDA or
- If the drug product is a solution for application to the skin, an oral solution, elixir, syrup, tincture, a solution for aerosolization or nebulization, a nasal solution, or similar other solubilized form, and contains an active drug ingredient in the same concentration and dosage form as a drug product that is the subject of an approved full NDA or ANDA, and contains no inactive ingredient or other change in formulation from the drug product that is the subject of an approved full NDA or ANDA that may significantly affect absorption of the active drug ingredient or active moiety for products that are systemically absorbed, or that may significantly affect systemic or local availability for products intended to act locally.

The FDA also waives the requirement for the submission of evidence measuring the in vivo BA or demonstrating the in vivo BE of a solid oral dosage form (other than a delayed- or extended-release dosage form) of a drug product determined to be effective for at least one indication in a DESI notice or which is identical, related, or similar (IRS) to such a drug product, unless the FDA has evaluated the drug product, included the drug product in the approved

(*Text continues on page 7.*)

TABLE 2 Therapeutic Equivalence Code Classifications of the U.S. Food and Drug Administration

Name	Definition	FDA code
Products in conventional dosage forms not presenting bioequivalence problems	Products coded as AA contain active ingredients and dosage forms that are not regarded as presenting either actual or potential bioequivalence problems or drug quality or standards issues. However, all oral dosage forms must, nonetheless, meet an appropriate in vitro test(s) for approval	AA
Products meeting necessary bioequivalence requirements	Products generally will be coded AB if a study is submitted demonstrating bioequivalence. Even though drug products of distributors and/or repackagers are not included in the list, they are considered therapeutically equivalent to the application holder's drug product if the application holder's drug product is rated AB or is single source in the List. The only instance in which a multisource product will be rated AB on the basis of bioavailability rather than bioequivalence is where the innovator product is the only one listed under that drug ingredient heading and has completed an acceptable bioavailability study. However, it does not signify that this product is therapeutically equivalent to the other drugs under the same heading. Drugs coded AB under an ingredient heading are considered therapeutically equivalent only to other drugs coded AB under that heading	AB
Solutions and powders for aerosolization	Uncertainty regarding the therapeutic equivalence of aerosolized products arises primarily because of differences in the drug-delivery system. Solutions and powders intended for aerosolization that are marketed for use in any of several delivery systems are considered to be pharmaceutically and therapeutically equivalent and are coded AN. Those products that are compatible only with a specific delivery system or those products that are packaged in and with a specific delivery system are coded BN, unless they have met an appropriate bioequivalence standard, because drug products in their respective delivery systems are not necessarily pharmaceutically equivalent to each other and, therefore, are not therapeutically equivalent	AN
Injectable oil solutions	The absorption of drugs in injectable (parenteral) oil solutions may vary substantially with the type of oil employed as a vehicle and the concentration of the active ingredient. Injectable oil solutions are therefore considered to be pharmaceutically and therapeutically equivalent only when the active ingredient, its concentration, and the type of oil used as a vehicle are all identical	AO
Injectable aqueous solutions	It should be noted that even though injectable (parenteral) products under a specific listing may be evaluated as therapeutically equivalent, there may be important differences among the products in the general category, Injectable; Injection. For example, some injectable products that are rated therapeutically equivalent are labeled for different routes of administration. In addition, some products evaluated as therapeutically equivalent may have different preservatives or no preservatives at all. Injectable products available as dry powders for reconstitution, concentrated sterile solutions for dilution, or sterile solutions ready for injection are all considered to be pharmaceutically and therapeutically equivalent provided they are designed to produce the same concentration prior to injection and are similarly labeled. Consistent with accepted professional practice, it is the responsibility of the prescriber, dispenser, or individual administering the product to be familiar with a product's labeling to assure that it is given only by the route(s) of administration stated in the labeling Certain commonly used large volume intravenous products in glass containers are not included on the list (e.g., dextrose injection 5%, dextrose injection 10%, and sodium chloride injection 0.9%), since these products are on the market without FDA approval and the FDA has not published conditions for marketing such parenteral products under approved NDAs. When packaged in plastic containers, however, FDA regulations require approved applications prior to marketing. Approval then depends on, among other things, the extent of the available safety data involving the specific plastic component of the product. All large volume parenteral products are manufactured under similar standards, regardless of whether they are packaged in glass or plastic. Thus, the FDA has no reason to believe that the packaging container of large volume parenteral drug products that are pharmaceutically equivalent would have any effect on their therapeutic equivalence	AP

Topical products	There are a variety of topical dosage forms available for dermatologic, ophthalmic, otic, rectal, and vaginal administration, including solutions, creams, ointments, gels, lotions, pastes, sprays, and suppositories. Even though different topical dosage forms may contain the same active ingredient and potency, these dosage forms are not considered pharmaceutically equivalent. Therefore, they are not considered therapeutically equivalent. All solutions and DESI drug products containing the same active ingredient in the same topical dosage form for which a waiver of in vivo bioequivalence has been granted and for which chemistry and manufacturing processes are adequate, are considered therapeutically equivalent and coded AT. Pharmaceutically equivalent topical products that raise questions of bioequivalence including all post-1962 topical drug products are coded AB when supported by adequate bioequivalence data, and BT in the absence of such data	AT
Extended release dosage forms (capsules, injectables, and tablets)	An extended release dosage form is defined by the official compendia as one that allows at least a twofold reduction in dosing frequency when compared with that drug presented as a conventional dosage form (e.g., as a solution or a prompt drug-releasing, conventional solid dosage form) Although bioavailability studies have been conducted on these dosage forms, they are subject to bioavailability differences, primarily because firms developing extended release products for the same active ingredient rarely employ the same formulation approach. The FDA, therefore, does not consider different extended release dosage forms containing the same active ingredient in equal strength to be therapeutically equivalent unless equivalence between individual products in both rate and extent has been specifically demonstrated through appropriate bioequivalence studies. Extended release products for which such bioequivalence data have not been submitted are coded BC, while those for which such data are available have been coded AB	BC
Active ingredients and dosage forms with documented bioequivalence problems	The BD code denotes products containing active ingredients with known bioequivalence problems and for which adequate studies have not been submitted to the FDA demonstrating bioequivalence. Where studies showing bioequivalence have been submitted, the product has been coded AB	BD
Delayed release oral dosage forms	A delayed release dosage form is defined by the official compendia as one that releases a drug (or drugs) at a time other than promptly after administration. Enteric-coated articles are delayed release dosage forms. Drug products in delayed release dosage forms containing the same active ingredients are subject to significant differences in absorption. Unless otherwise specifically noted, the agency considers different delayed release products containing the same active ingredients as presenting a potential bioequivalence problem and codes these products BE in the absence of in vivo studies showing bioequivalence. If adequate in vivo studies have demonstrated the bioequivalence of specific delayed release products, such products are coded AB	BE
Products in aerosol nebulizer drug-delivery systems	This code applies to drug solutions or powders that are marketed only as a component of, or as compatible with, a specific drug-delivery system. There may, for example, be significant differences in the dose of drug and particle size delivered by different products of this type. Therefore, the agency does not consider different metered aerosol dosage forms containing the same active ingredient(s) in equal strengths to be therapeutically equivalent unless the drug products meet an appropriate bioequivalence standard	BN
Active ingredients and dosage forms with potential bioequivalence problems	The FDA's bioequivalence regulations (21 CFR 320.33) contain criteria and procedures for determining whether a specific active ingredient in a specific dosage form has a potential for causing a bioequivalence problem. It is the FDA's policy to consider an ingredient meeting these criteria as having a potential bioequivalence problem even in the absence of positive data demonstrating inequivalence. Pharmaceutically equivalent products containing these ingredients in oral dosage forms are coded BP, until adequate in vivo bioequivalence data are submitted	BP

(Continued)

TABLE 2 Therapeutic Equivalence Code Classifications of the U.S. Food and Drug Administration *(Continued)*

Name	Definition	FDA code
Active ingredients and dosage forms with potential bioequivalence problems (*continued*)	Injectable suspensions containing an active ingredient suspended in an aqueous or oleaginous vehicle have also been coded BP. Injectable suspensions are subject to bioequivalence problems, because differences in particle size, polymorphic structure of the suspended active ingredient, or the suspension formulation can significantly affect the rate of release and absorption. The FDA does not consider pharmaceutical equivalents of these products bioequivalent without adequate evidence of bioequivalence	
Suppositories or enemas that deliver drugs for systemic absorption	The absorption of active ingredients from suppositories or enemas that are intended to have a systemic effect (as distinct from suppositories administered for local effect) can vary significantly from product to product. Therefore, the FDA considers pharmaceutically equivalent systemic suppositories or enemas bioequivalent only if in vivo evidence of bioequivalence is available. In those cases where in vivo evidence is available, the product is coded AB. If such evidence is not available, the products are coded BR	BR
Products having drug standard deficiencies	If the drug standards for an active ingredient in a particular dosage form are found by the FDA to be deficient so as to prevent an FDA evaluation of either pharmaceutical or therapeutic equivalence, all drug products containing that active ingredient in that dosage form are coded BS. For example, if the standards permit a wide variation in pharmacologically active components of the active ingredient such that pharmaceutical equivalence is in question, all products containing that active ingredient in that dosage form is coded BS	BS
Topical products with bioequivalence issues	This code applies mainly to post-1962 dermatologic, ophthalmic, otic, rectal, and vaginal products for topical administration, including creams, ointments, gels, lotions, pastes, and sprays, as well as suppositories not intended for systemic drug absorption. Topical products evaluated as having acceptable clinical performance, but that are not bioequivalent to other pharmaceutically equivalent products or that lack sufficient evidence of bioequivalence will be coded BT	BT
Drug products for which the data are insufficient to determine therapeutic equivalence	The code BX is assigned to specific drug products for which the data that have been reviewed by the agency are insufficient to determine therapeutic equivalence under the policies stated in this document. In these situations, the drug products are presumed to be therapeutically inequivalent until the agency has determined that there is adequate information to make a full evaluation of therapeutic equivalence	BX

Abbreviations: DESI, drug efficacy study implementation; FDA, Food and Drug Administration; NDA, new drug application.

drug products with therapeutic equivalence evaluations list, and rated the drug product as having a known or potential BE problem. A drug product so rated reflects a determination by the FDA that an in vivo BE study is required. {A DESI drug is any drug that lacks substantial evidence of effectiveness [less than effective (LTE)] and is subjected by the FDA to a notice of opportunity for hearing (NOOH). This includes drugs which are IRS to DESI drugs. Valid values: 2, safe and effective or non-DESI drug; 3, drug under review [no NOOH issued]; 4, LTE/IRS drug for SOME indications; 5, LTE/IRS drug for ALL indications; 6, LTE/IRS drug withdrawn from market.}

For certain drug products, BA may be measured or BE may be demonstrated by evidence obtained in vitro in lieu of in vivo data. The FDA shall waive the requirement for the submission of evidence obtained in vivo measuring the BA or demonstrating the BE of the drug product if the drug product meets one of the following criteria:

- The drug product is in the same dosage form, but in a different strength, and is proportionally similar in its active and inactive ingredients to another drug product for which the same manufacturer has obtained approval and the following conditions are met that the BA of this other drug product has been measured and both drug products meet an appropriate in vitro test approved by the FDA; and the applicant submits evidence showing that both drug products are proportionally similar in their active and inactive ingredients. (Except for delayed- or extended-release products.)
- The drug product is, on the basis of scientific evidence submitted in the application, shown to meet an in vitro test that has been correlated with in vivo data.
- The drug product is a reformulated product that is identical, except for a different color, flavor, or preservative that could not affect the BA of the reformulated product, to another drug product for which the same manufacturer has obtained approval and the following conditions are met: the BA of the other product has been measured; and both drug products meet an appropriate in vitro test approved by the FDA.

The FDA, for good cause, may waive a requirement for the submission of evidence of in vivo BA or BE if waiver is compatible with the protection of the public health. For full NDAs, the FDA may defer a requirement for the submission of evidence of in vivo BA if deferral is compatible with the protection of the public health.

The FDA, for good cause, may require evidence of in vivo BA or BE for any drug product, if the agency determines that any difference between the drug product and a listed drug may affect the BA or BE of the drug product.

How to Demonstrate BE?

A list of therapeutic, PK, and physicochemical factors has been compiled to classify which product needs the demonstration of BE by in vivo testing (Table 3). A large number of drugs have been classified in this category (Table 4). All enteric coated and controlled release dosage forms of any solid oral dosage form require in vivo BA testing. It is generally suggested that if there is more than 25% intra-batch or batch-to-batch variability in BA is observed, in vivo tests will be required for batch certification. Any changes in the manufacturing process, including product formulation or dosage strength change, beyond that suggested in the NDA or ANDA and changes in labeling for a new indication or new dosage regimen also require in vivo BA testing.

The pharmacotherapeutic nature of the drug plays an important role in the regulations regarding its BA. Drugs which exhibit narrow therapeutic index, i.e., less than a twofold difference in median lethal dose and median effective dose values (or less than a twofold difference in the minimum effective concentration and minimum toxic concentration in the blood), require careful demonstration of BA and the consistency with which this requirement is met. Further consideration is needed in the type of side effects occurring if a toxic level is reached. For example, the therapeutic index (the U.S. FDA prefers to call this therapeutic range) for salicylates is smaller than cardiac glycosides; it does not mean that cardiac glycosides are less toxic. It merely signifies that the concentration of salicylates for therapeutic response is

TABLE 3 Factors Determining the Establishment of Bioequivalence Requirement by the Food and Drug Administration

Therapeutic factors evidence from
Clinical trials
Controlled observations on patients
Well-controlled bioequivalence studies that
The drug exhibits a low therapeutic ratio
The drug requires careful dosage titration
Bioinequivalence would produce adverse prophylacitc or therapeutic effects
Pharmacokinetic factors evidence that the drug entity
Is absorbed from localized sites in the gastrointestinal tract
Is subject to poor absorption
Is subject to first-pass metabolism
Requires rapid dissolution and absorption for effectiveness
Is unstable in specific portions of the gastrointestinal tract
Is subject to dose-dependent kinetics in or near the therapeutic range
Physiochemical factors evidence that the drug
Possesses low solubility in water or gastric fluids
Is dissolved slowly from one or more of its dosage forms
Particle size and/or surface area affects bioavailability
Exhibits certain physical–structural characteristics, e.g., polymorphism, solvates, etc., which modify its bioavailability
Has a high ratio of excipients to active ingredients as formulated
Has a bioavailability which may be affected by the presence or absence of hydrophilic or hydrophobic excipients and lubricant

closer to the concentration where undesirable side effects start to appear. Another consideration along the same line is the potency of drug in question. Generally, highly potent drugs will require greater control of BA than the one with lesser potency. Because of the logarithmic nature of the response, the curves flatten out at low and high doses. Thus, a highly potent drug used in large doses will show lesser variability in response due to BA factor than a low potency drug used at a dose level where the response is log-linear. Any such comparison, however, should take into account the relative nature of the slope of the response to dose.

The physicochemical evidence needed to establish a BE includes low water solubility, e.g., <5 mg/mL, or if dissolution in the stomach is critical to absorption, the volume of gastric fluids required to dissolve the recommended dose (gastric fluid content is assumed to be 100 mL for adults and is prorated for infants and children). The dissolution rates are also taken into consideration if less than 50% of the drug dissolves in 30 minutes using official methods. Also included under physicochemical evidence are particle size and surface area of the active drug ingredient. Certain physical structural characteristics of the active drug ingredient, e.g., polymorphism, solvation, etc., are also considered. Drug products which have a high ratio of excipients to active ingredients (e.g., greater than 5:1) may also be subjected to bioequivalency demonstration. Other evidence includes specific absorption sites or where the available dose is less than 50% of an administered dose. Drugs which are rapidly biotransformed in the intestinal wall or liver during absorption, and drugs which are unstable in specific portions of the gastrointestinal (GI) tract requiring special coating or formulations are also subjected to bioequivalency requirements, as are drugs which show dose-dependent absorption, distribution, biotransformation, or elimination.

For some dosage forms, bioequivalency requirements can be waived such as with topical products, oral dosage forms not intended for absorption, inhalations, and solutions if there is sufficient evidence that the inactive ingredients do not affect the release and delivery of drugs from the dosage form.

Rationale for Estimation

The BA of a drug is controlled by three factors, namely:

- the rate and extent of release of the drug from the dosage form,
- its subsequent absorption from the solution state, and
- the biotransformation during the process of absorption.

TABLE 4 Drugs with Potential Bioequivalency Problems

Acetazolamide	Perphenazine
Acetyldigitoxin	Phenacemide
Alseroxylon	Phensuximide
Aminophyllin	Phenylaminosalicylate
Aminosalicylic acid	Phenytoin
Bendrolflumethiazide	Pheytonadione
Benzthiazide	Polythiazide
Betamethasone	Prednisolone
Bishydroxycoumarin	Primidone
Chlorambucil	Probenecid
Chlorodiazepoxide	Procainamide
Chloropromazine	Prochlorperazine
Chlorothiazide	Promazine
Cortisone acetate	Promethazine
Deserpidine	Propylthiouracil
Dexamethasone	Pyrimethamine
Dichlorphenamide	Quinethiazide
Dienestrol	Quinidine
Diethylstilbestrol	Rauwolfia serpentina
Dyphylline	Rescinnamine
Ethinyl estradiol	Reserpine
Ethosuxmide	Salicylazosulfapyridine
Ethotoin	Sodium sulfoxone
Ethoxzolamide	Spironolactone
Fludrocortisone	Sulfadiazine
Fluphenazine	Sulfadimethoxine
Fluprednisolone	Sulfamerazine
Hydralazine	Sulfaphenazole
Hydrochlorothiazide	Sulfasomidine
Hydroflumethiazide	Sulfasoxazole
Imipramine	Theophylline
Isoproterenol	Thioridazine
Liothyronine	Tolbutamide
Menadione	Triamcinolone
Mephenytoin	Trichlormethiazide
Methazolamide	Triethyl melamine
Methyclothizaide	Trifluoperazine
Methylprednisolone	Triflupromazine
Methyltestosterone	Trimeprazine
Nitrofurantoin	Trimethadione
Oxtriphylline	Uracil mustand
p-Aminosalicylic acid	Warfarin
Paramethadione	

In all quantitative determinations of BA, concentration is measured in blood, plasma, and urine. Plasma concentrations following the oral administration of a drug assume four sequential phases depending on the magnitude of absorption and elimination:

1. Absorption $>>$ elimination
2. Absorption $=$ elimination
3. Absorption $<$ elimination
4. Absorption $=$ elimination $=0$

The shape of the plasma concentration profile depends on the relative rates of absorption and elimination and thus, the plasma concentration profiles may be quite different with different routes of administration. Intravenous and sometimes intramuscular routes yield an early peak due to fast or almost instantaneous absorption, whereas oral, subcutaneous, rectal,

and other routes may show delayed peaks due to slower rates of absorption. It should be noted that the rate of elimination is considered constant since it depends primarily in the specific nature of the active drug ingredient.

The purpose of BA studies is to demonstrate therapeutic equivalence. However, depending on the mechanism of action, more meaningful comparisons can be made from such parameters as peak plasma concentration or the time to reach peak plasma concentration. For example, in the case of antibiotics, it is important to know how soon the minimum inhibitory concentration is reached and maintained. The choice of the single dose versus multiple dose study depends on the mechanism of drug action. For example, antidepressants like imipramine show delayed action, a characteristic of many psychotropic and antihypertensive agents. In these instances, a new product should be judged for its quality from repeated administration because in these examples, the peak concentration or time for peak concentration is relatively unimportant. Therefore, it is important to isolate the clinically important parameter but in all instances, the area under the curve (AUC) must be monitored since it represents the proportionality to the total amount of drug eliminated from the body and hence absorbed.

The estimation of BA from plasma concentration profiles requires a thorough understanding of the nature of plasma level profiles. For example, a higher or earlier peak does not necessarily mean greater overall absorption than from a product giving a smaller or delayed peak. The total absorption of drugs is, therefore, proportional not only to the plasma concentrations achieved but also to the length of time, these concentrations persist in the blood. One parameter that characterizes this aspect is the area under the plasma concentration versus time profile.

The major contribution to the AUC for a fast absorbed formulation is due to the high peak concentration, whereas for a slowly absorbed formulation, the area is mainly due to sustained or prolonged plasma concentration. It should be noted that the area under the plasma concentration versus time profile (AUC) is only proportional to the total amount of drug absorbed and cannot be used to determine the actual amount of drug administered unless it is compared with a known standard, whereby the extent of absorption is either measured by other methods or assumed to be 100%, as in the case of intravenous administration.

The in vivo BA of a drug product is measured if the product's rate and extent of absorption, as determined by comparison of measured parameters, e.g., concentration of the active drug ingredient in the blood, urinary excretion rates, or pharmacological effects, do not indicate a significant difference from the reference material's rate and extent of absorption. For drug products that are not intended to be absorbed into the bloodstream, BA may be assessed by measurements intended to reflect the rate and extent to which the active ingredient or active moiety becomes available at the site of action.

Statistical techniques used in establishing BE shall be of sufficient sensitivity to detect differences in rate and extent of absorption that are not attributable to subject variability.

A drug product that differs from the reference material in its rate of absorption, but not in its extent of absorption, may be considered to be bioavailable if the difference in the rate of absorption is intentional, appropriately reflected in the labeling, not essential to the attainment of effective body drug concentrations on chronic use, and considered medically insignificant for the drug product.

Two drug products will be considered bioequivalent drug products if they are pharmaceutical equivalents or pharmaceutical alternatives, whose rate and extent of absorption do not show a significant difference when administered at the same molar dose of the active moiety under similar experimental conditions, either single dose or multiple dose. Some pharmaceutical equivalents or pharmaceutical alternatives may be equivalent to the extent of their absorption but not in their rate of absorption and yet may be considered bioequivalent, because such differences in the rate of absorption are intentional and reflected in the labeling, not essential to the attainment of effective body drug concentrations on chronic use, and considered medically insignificant for the particular drug product studied.

Evidence to Measure BE

In vivo BE may be determined by one of several direct or indirect methods. Selection of the method depends upon the purpose of the study, the analytical method available, and the nature of the drug product. BE testing should be conducted using the most appropriate method available for the specific use of the product.

The preferred hierarchy of BE studies (in descending order of sensitivity) is the blood level study, pharmacologic end-point study, and clinical end-point study. When absorption of the drug is sufficient to measure drug concentration directly in the blood (or other appropriate biological fluids or tissues) and systemic absorption is relevant to the drug action, then a blood (or other biological fluid or tissue) level BE study should be conducted. The blood level study is generally preferred above all others as the most sensitive measure of BE. The sponsor should provide justification for choosing either a pharmacologic or a clinical end-point study over a blood-level (or other biological fluids or tissues) study.

When the measurement of the rate and extent of absorption of the drug in biological fluids cannot be achieved or is unrelated to drug action, a pharmacologic end-point (i.e., drug induced physiologic change which is related to the approved indications for use) study may be conducted. Lastly, in order of preference, if drug concentrations in blood (or fluids or tissues) are not measurable or are inappropriate, and there are no appropriate pharmacologic effects that can be monitored, then a clinical end-point study may be conducted, comparing the test (generic) product to the reference (pioneer) product and a placebo (or negative) control.

BA may be measured or BE may be demonstrated by several in vivo and in vitro methods. FDA may require in vivo or in vitro testing, or both, to measure the BA of a drug product or establish the BE of specific drug products. Information on BE requirements for specific products is included in the current edition of the FDA's publication "Approved Drug Products with Therapeutic Equivalence Evaluations" and any current supplement to the publication. The selection of the method used to meet an in vivo or in vitro testing requirement depends upon the purpose of the study, the analytical methods available, and the nature of the drug product. The following in vivo and in vitro approaches, in descending order of accuracy, sensitivity, and reproducibility, are acceptable for determining the BA or BE of a drug product.

- An in vivo test in humans in which the concentration of the active ingredient or active moiety, and, when appropriate, its active metabolite(s), in whole blood, plasma, serum, or other appropriate biological fluid is measured as a function of time. This approach is particularly applicable to dosage forms intended to deliver the active moiety to the bloodstream for systemic distribution within the body or
- An in vitro test that has been correlated with and is predictive of human in vivo BA data or
- An in vivo test in humans in which the urinary excretion of the active moiety, and, when appropriate, its active metabolite(s), are measured as a function of time. The intervals at which measurements are taken should ordinarily be as short as possible so that the measure of the rate of elimination is as accurate as possible. Depending on the nature of the drug product, this approach may be applicable to the category of dosage forms described in paragraph (b)(1)(i) of this section. This method is not appropriate where urinary excretion is not a significant mechanism of elimination.
- An in vivo test in humans in which an appropriate acute pharmacological effect of the active moiety, and, when appropriate, its active metabolite(s), are measured as a function of time if such effect can be measured with sufficient accuracy, sensitivity, and reproducibility. This approach is applicable only when appropriate methods are not available for measurement of the concentration of the moiety, and, when appropriate, its active metabolite(s), in biological fluids or excretory products but a method is available for the measurement of an appropriate acute pharmacological effect. This approach may be particularly applicable to dosage forms that are not intended to deliver the active moiety to the bloodstream for systemic distribution.
- Well-controlled clinical trials that establish the safety and effectiveness of the drug product, for purposes of measuring BA, or appropriately designed comparative clinical trials, for purposes of demonstrating BE. This approach is the least accurate, sensitive, and

reproducible of the general approaches for measuring BA or demonstrating BE. For dosage forms intended to deliver the active moiety to the bloodstream for systemic distribution, this approach may be considered acceptable only when analytical methods cannot be developed to permit use of one of the approaches outlined above are not available. This approach may also be considered sufficiently accurate for measuring BA or demonstrating BE of dosage forms intended to deliver the active moiety locally, e.g., topical preparations for the skin, eye, and mucous membranes; oral dosage forms not intended to be absorbed, e.g., an antacid or radiopaque medium; and bronchodilators administered by inhalation if the onset and duration of pharmacological activity are defined.

- A currently available in vitro test acceptable to FDA (usually a dissolution rate test) that ensures human in vivo BA.
- Any other approach deemed adequate by the FDA to measure BA or establish BE.

The FDA may require in vivo testing in humans of a product at any time if the agency has evidence that the product:

- May not produce therapeutic effects comparable with a pharmaceutical equivalent or alternative with which it is intended to be used interchangeably;
- May not be bioequivalent to a pharmaceutical equivalent or alternative with which it is intended to be used interchangeably or
- Has greater than anticipated potential toxicity related to PK or other characteristics?

MEASUREMENT INDICES

Whenever comparison of the test product and the reference material is to be based on blood concentration–time curves or cumulative urinary excretion–time curves at steady state, appropriate dosage administration and sampling should be carried out to document attainment of steady state. A more complete characterization of the blood concentration or urinary excretion rate during the absorption and elimination phases of a single dose administered at steady state is encouraged to permit estimation of the total area under concentration–time curves or cumulative urinary excretion–time curves and to obtain PK information, e.g., half-life or blood clearance, that is essential in preparing adequate labeling for the drug product.

When comparison of the test product and the reference material is to be based on acute pharmacological effect–time curves, measurements of this effect should be made with sufficient frequency to demonstrate a maximum effect and a lack of significant difference between the test product and reference material.

Dose Selection

Dose selection will depend on the label claims, consideration of assay sensitivity, and relevance to the practical use conditions of the reference product. A blood-level BE study should generally be conducted at the highest dose approved for the pioneer product.

However, the FDA will consider a BE study conducted at a higher than approved dose in certain cases. Such a study may be appropriate when a multiple of the highest approved dose achieves measurable blood levels, but the highest approved dose does not. In general, the study would be limited to two to three times the highest dose approved for the pioneer product. The pioneer product should have an adequate margin of safety at the higher than approved dose level. The generic sponsor should also confirm (e.g., through literature) that the drug follows linear kinetics. A higher than approved dose BE study in food animal species would be accompanied by a tissue residue withdrawal study conducted at the highest approved dose for the pioneer product.

For products labeled for multiple claims involving different pharmacologic actions at a broad dose range (e.g., therapeutic and production claims), a single BE study at the highest approved dose will usually be adequate. However, multiple BE studies at different doses may

be needed if the drug is known to follow nonlinear kinetics. The sponsor should consult with the FDA to discuss the BE study or studies appropriate to a particular drug.

Multiple Strengths of Solid Oral Dosage Forms

The generic sponsor should discuss with the FDA the appropriate in vivo BE testing and in vitro dissolution testing to obtain approval for multiple strengths (or concentrations) of solid oral dosage forms. The FDA will consider the ratio of active to inactive ingredients and the in vitro dissolution profiles of the different strengths, the water solubility of the drug, and the range of strengths for which approval is sought. One in vivo BE study with highest strength product may suffice if the multiple strength products have the same ratio of active to inactive ingredients and are otherwise identical in formulation. In vitro dissolution testing should be conducted using an FDA approved method, to compare each strength of the generic product to the corresponding strength of the reference product.

Manufacturing of Pilot Batch ("Biobatch")

A pilot batch or "biobatch" should be the source of the finished drug product used in the pivotal studies (i.e., BE studies and tissue residue studies), stability studies, and the validation studies for the proposed analytical and stability indicating methods. Batch testing, individual batch testing is necessary to assure that all batches of the same drug product meet an appropriate in vitro test. The commissioner will ordinarily terminate a requirement for a manufacturer to submit samples for batch testing on a finding that the manufacturer has produced four consecutive batches that were tested by the FDA and found to meet the BE requirement, unless the public health requires that batch testing be extended to additional batches.

If a BE requirement specifies a currently available in vitro test or an in vitro BE standard comparing the drug product to a reference standard, the manufacturer shall conduct the test on a sample of each batch of the drug product to assure batch-to-batch uniformity.

Dosing by Labeled Concentration

The potency of the pioneer and generic products should be assayed prior to conducting the BE study to ensure that the FDA or compendial specifications are met. The Center recommends that the potency of the pioneer and generic lots should differ by no more than $\pm 5\%$ for dosage form products.

The animals should be dosed according to the labeled concentration or strength of the product, rather than the assayed potency of the individual batch (i.e., the dose should not be corrected for the assayed potency of the product). The BE data or derived parameters should not be normalized to account for any potency differences between the pioneer and generic product lots.

Single-Dose vs. Multiple-Dose Studies

A single-dose study at the highest approved dose will generally be adequate for the demonstration of BE. A single-dose study at a higher than approved dose may be appropriate for certain drugs.

A multiple-dose study may be appropriate when there are concerns regarding poorly predictable drug accumulation (e.g., a drug with nonlinear kinetics) or a drug with a narrow therapeutic window. A multiple-dose study may also be needed when assay sensitivity is inadequate to permit drug quantification out to three terminal elimination half-lives beyond the time when maximum blood concentrations (C_{max}) are achieved, or in cases where prolonged or delayed absorption exist. The determination of prolonged or delayed absorption (i.e., flip-flop kinetics) may be made from pilot data, from the literature, or from information contained with Freedom of Information summaries pertaining to the particular drug or family of drugs.

Guidelines on the Design of a Single-Dose Study

A BE study should be a single dose comparison of the drug product to be tested and the appropriate reference material conducted in normal adults. The test product and the reference material should be administered to subjects in the fasting state, unless some other approach is more appropriate for valid scientific reasons. A single-dose study should be crossover in design, unless a parallel design or other design is more appropriate for valid scientific reasons, and should provide for a drug elimination period. Unless some other approach is appropriate for valid scientific reasons, the drug elimination period should be either: at least three times the half-life of the active drug ingredient or therapeutic moiety, or its metabolite(s), measured in the blood or urine; or at least three times the half-life of decay of the acute pharmacological effect.

When comparison of the test product and the reference material is to be based on blood concentration–time curves, unless some other approach is more appropriate for valid scientific reasons, blood samples should be taken with sufficient frequency to permit an estimate of both the peak concentration in the blood of the active drug ingredient or therapeutic moiety, or its metabolite(s), measured; and the total AUC for a time period at least three times the half-life of the active drug ingredient or therapeutic moiety, or its metabolite(s), measured.

In a study comparing oral dosage forms, the sampling times should be identical. In a study comparing an intravenous dosage form and an oral dosage form, the sampling times should be those needed to describe both the distribution and the elimination phase of the intravenous dosage form; and the absorption and elimination phase of the oral dosage form.

In a study comparing drug-delivery systems other than oral or intravenous dosage forms with an appropriate reference standard, the sampling times should be based on valid scientific reasons.

When comparison of the test product and the reference material is to be based on cumulative urinary excretion–time curves, unless some other approach is more appropriate for valid scientific reasons, samples of the urine should be collected with sufficient frequency to permit an estimate of the rate and extent of urinary excretion of the active drug ingredient or therapeutic moiety, or its metabolite(s), measured.

When comparison of the test product and the reference material is to be based on acute pharmacological effect–time curves, measurements of this effect should be made with sufficient frequency to permit a reasonable estimate of the total AUC for a time period at least three times the half-life of decay of the pharmacological effect, unless some other approach is more appropriate for valid scientific reasons.

The use of an acute pharmacological effect to determine BA may further require demonstration of dose-related response. In such a case, BA may be determined by comparison of the dose–response curves as well as the total area under the acute pharmacological effect–time curves for any given dose.

Guidelines for Multiple-Dose Study

In selected circumstances, it may be necessary for the test product and the reference material to be compared after repeated administration to determine steady-state levels of the active drug ingredient or therapeutic moiety in the body. The test product and the reference material should be administered to subjects in the fasting or nonfasting state, depending upon the conditions reflected in the proposed labeling of the test product.

A multiple-dose study may be required to determine the BA of a drug product in the following circumstances that there is a difference in the rate of absorption but not in the extent of absorption, there is excessive variability in BA from subject to subject; the concentration of the active drug ingredient or therapeutic moiety, or its metabolite(s), in the blood resulting from a single dose is very low for accurate determination by the analytical method; the drug product is an extended release dosage form.

A multiple-dose study should be crossover in design, unless a parallel design or other design is more appropriate for valid scientific reasons, and should provide for a drug elimination period if steady-state conditions are not achieved. A multiple-dose study is not

required to be of crossover design if the study is to establish dose proportionality under a multiple-dose regimen or to establish the PK profile of a new drug product, a new drug-delivery system, or an extended release dosage form.

If a drug elimination period is required, unless some other approach is more appropriate for valid scientific reasons, the drug elimination period should be either at least five times the half-life of the active drug ingredient or therapeutic moiety, or its active metabolite(s), measured in the blood or urine; or at least five times the half-life of decay of the acute pharmacological effect.

Whenever a multiple-dose study is conducted, unless some other approach is more appropriate for valid scientific reasons, sufficient doses of the test product and reference material should be administered in accordance with the labeling to achieve steady-state conditions.

Fed vs. Fasted State

Feeding may either enhance or interfere with drug absorption, depending upon the characteristics of the drug and the formulation. Feeding may also increase the inter- and intrasubject variability in the rate and extent of drug absorption. The rationale for conducting each BE study under fasting or fed conditions should be provided in the protocol. Fasting conditions, if used, should be fully described, giving careful consideration to the PKss of the drug and the humane treatment of the test animals. The protocol should describe the diet and feeding regime which will be used in the study.

If a pioneer product label indicates that the product is limited to administration either in the fed or fasted state, then the BE study should be conducted accordingly. If the BE study parameters pass the agreed upon confidence intervals, then the single study is acceptable as the basis for approval of the generic drug.

However, for certain product classifications or drug entities, such as enteric coated and oral-sustained release products, demonstration of BE in both the fasted and the fed states may be necessary, if drug BA is highly variable under feeding conditions, as determined from the literature or from pilot data. A BE study conducted under fasted conditions may be necessary to pass the confidence intervals. A second smaller study may be necessary to examine meal effects. The FDA will evaluate the smaller study with respect to the means of the pivotal parameters (AUC and C_{max}). The sponsors should consult with the FDA prior to conducting the studies.

Pharmacological End-Point Studies

Where the direct measurement of the rate and extent of absorption of the new animal drug in biological fluids is inappropriate or impractical, the evaluation of a pharmacologic end-point related to the labeled indications for use will be acceptable.

Typically, the design of a pharmacologic end-point study should follow the same general considerations as the blood-level studies. However, specifics such as the number of subjects or sampling times will depend on the pharmacologic end point monitored. The parameters to be measured will also depend upon the pharmacologic end points and may differ from those used in blood-level studies. As with blood-level studies, when pharmacologic end-point studies are used to demonstrate BE, a tissue residue study will also be required in food-producing animals.

For parameters which can be measured over time, a time versus effect profile is generated, and equivalence is determined with the method of statistical analysis essentially the same as for the blood level BE study.

For pharmacologic effects, for which effect versus time curves cannot be generated, then alternative procedures for statistical analysis should be discussed with the FDA prior to conducting the study.

Clinical End-Point Studies

If measurement of the drug or its metabolites in blood, biological fluids, or tissues is inappropriate or impractical, and there are no appropriate pharmacologic end-points to

monitor (e.g., most production drugs and some coccidiostats and anthelmintics), then well-controlled clinical end-point studies are acceptable for the demonstration of BE.

Generally, a parallel group design with three treatment groups should be used. The groups should be a placebo (or negative) control, a positive control (reference/pioneer product), and the test (generic) product. The purpose of the placebo (or negative) control is to confirm the sensitivity or validity of the study. Dosage(s) approved for the pioneer product should be used in the study. Dosage(s) should be selected following consultation with the FDA and should reflect consideration for experimental sensitivity and relevance to the common use of the pioneer product.

Studies should generally be conducted using the target animal species, with consideration for the sex, class, body weight, age, health status, and feeding and husbandry conditions, as described on the pioneer product labeling. In general, the length of time that the study is conducted should be consistent with the duration of use on the pioneer product labeling.

In general, the response(s) to be measured in a clinical end-point study should be based upon the labeling claims of the pioneer product and selected in consultation with Center for Veterinary Medicine (CVM). It may not be necessary to collect data on some overlapping claims (e.g., for a production drug which is added at the same amount per ton of feed for both growth rate and feed efficiency, data from only one of the two responses need be collected).

When considering sample size, it is important to note that the pen, not the individual animal, is often the experimental unit. As with blood-level BE studies, the FDA is advocating the use of 90% confidence intervals as the best method for evaluating clinical end-point studies. The bounds for confidence limits [e.g., $\pm 20\%$ of the improvement over placebo (or negative) control] for the particular drug should be agreed upon with the FDA prior to initiation of the study.

The analysis should be used to compare the test product and the reference product. In addition, a traditional hypothesis test should be performed comparing both the test and reference products separately to the placebo (or negative) control. The hypothesis test is conducted to ensure that the study has adequate sensitivity to detect differences when they actually occur. If no significant improvement ($\alpha = 0.05$) is seen in the parameter [i.e., the mean of the test and the mean of the reference products are each not significantly better than the mean of the placebo (or negative) control], generally, the study will be considered inadequate to evaluate BE.

Assuming that the test and reference products have been shown to be superior to the placebo (or negative) control, the determination of BE is based upon the confidence interval of the difference between the two products.

Some clinical end-point studies may not include a placebo (or negative) control for ethical and/or practical considerations. If the placebo is omitted, then the response(s) to the test and reference products should each provide a statistically significant improvement over baseline.

If the results are ordered categorical data (e.g., excellent, good, fair, or poor), a non-parametric hypothesis test of no difference between the test product and placebo (or negative) control, and between the reference product and placebo (or negative) control should be performed. As above, if these tests result in significant differences between the test product and control and reference product and control, then a non-parametric confidence interval on the difference between the test and reference products is calculated.

Another acceptable approach for categorical data is to calculate the confidence interval on the odds ratio between the test and reference products after showing that the test and reference products are significantly better than the control.

ANALYTICAL METHODS

The analytical method used in an in vivo BA or BE study to measure the concentration of the active drug ingredient or therapeutic moiety, or its active metabolite(s), in body fluids or excretory products, or the method used to measure an acute pharmacological effect shall be demonstrated to be accurate and of sufficient sensitivity to measure, with appropriate

precision, the actual concentration of the active drug ingredient or therapeutic moiety, or its active metabolite(s), achieved in the body. When the analytical method is not sensitive enough to measure accurately the concentration of the active drug ingredient or therapeutic moiety, or its active metabolite(s), in body fluids or excretory products produced by a single dose of the test product, two or more single doses may be given together to produce higher concentration.

Assay Consideration

A properly validated assay method is pivotal to the acceptability of any PK study. Sponsors should discuss any questions or problems concerning the analytical methodology with CVM before undertaking the BE studies. The ANADA submission should contain adequate information necessary for the CVM reviewer to determine the validity of the analytical method used to quantitate the level of drug in the biological matrix (e.g., blood).

The following aspects should be addressed in assessing method performance.

Concentration Range and Linearity

The quantitative relationship between concentration and response should be adequately characterized over the entire range of expected sample concentrations. For linear relationships, a standard curve should be defined by at least five concentrations. If the concentration response function is nonlinear, additional points would be necessary to define the nonlinear portions of the curve. Extrapolation beyond a standard curve is not acceptable.

Limit of Detection

The standard deviation of the background signal and limit of detection (LOD) should be determined. The LOD is estimated as the response value calculated by adding three times the standard deviation of the background response to the average background response.

Limit of Quantitation

The initial determination of limit of quantitation (LOQ) should involve the addition of 10 times the standard deviation of the background response to the average background response. The second step in determining LOQ is assessing the precision (reproducibility) and accuracy (recovery) of the method at the LOQ. The LOQ will generally be the lowest concentration on the standard curve that can be quantified with acceptable accuracy and precision.

Specificity

The absence of matrix interferences should be demonstrated by the analysis of six independent sources of control matrix. The effect of environmental, physiological, or procedural variables on the matrix should be assessed. Each independent control matrix will be used to produce a standard curve, which will be compared with a standard curve produced under chemically defined conditions. The comparison of curves should exhibit parallelism and superimposability within the limits of analytical variation established for the chemically defined standard curve.

Accuracy (Recovery)

This parameter should be evaluated using at least three known concentrations of analyte freshly spiked in control matrix, one being at a point two standard deviations above the LOQ, one in the middle of the range of the standard curve ("mid-range") and one at a point two standard deviations below the upper quantitative limit of the standard curve. The accuracy of the method, based upon the mean value of six replicate injections, at each concentration level, should be within 80% to 120% of the nominal concentration at each level (high, mid-range, and LOQ).

Precision

This parameter should be evaluated using at least three known concentrations of analyte freshly spiked in control matrix, at the same points used for determination of accuracy. The coefficient of variation (CV) of six replicates should be $\pm 10\%$ for concentrations at or above 0.1 ppm (0.1 μg/mL). A CV of $\pm 20\%$ is acceptable for concentrations below 0.1 ppm.

Analyte Stability

Stability of the analyte in the biological matrix under the conditions of the experiment (including any period for which samples are stored before analyses) should be established. It is recommended that the stability be determined with incurred analyte in the matrix of dosed animals in addition to, or instead of, control matrix spiked with pure analyte. In addition, the influence of three freeze–thaw cycles at two concentrations should be determined.

Stability samples at three concentrations should be stored with the study samples and analyzed through the period of time in which study samples are analyzed. These analyses will establish whether or not analyte levels have decreased during the time of analysis.

Analytical System Stability

To assure that the analytical system remains stable over the time course of the assay, the reproducibility of the standard curve should be monitored during the assay. A minimal design would be to run analytical standards at the beginning and at the end of the analytical run.

Quality Control Samples

The purpose of quality control (QC) samples is to assure that the complete analytical method, sample preparation, extraction, clean-up, and instrumental analysis perform according to acceptable criteria. The stability of the drug in the text matrix for the QC samples should be known and any tendency for the drug to bind to tissue or serum components over time should also be known.

Drug free control matrix, e.g., tissue, serum, etc., that is freshly spiked known quantities of test drug, should be analyzed contemporaneously with test samples, evenly dispersed throughout each analytical run. This can be met by the determination of accuracy and precision of each analytical run.

Replicate and Repeat Analyses

Single rather than replicate analyses are recommended, unless the reproducibility and/or accuracy of the method are borderline. Criteria for repeat analyses should be determined prior to running the study and recorded in the method standard operating procedure (SOP).

Summary of Samples to Be Run with Each Analysis

1. Accuracy estimate
2. Precision estimate
3. Analytical system stability
4. Analyte stability samples

PRIOR REVIEW

The commissioner of food and drugs strongly recommends that, to avoid the conduct of an improper study and unnecessary human research, any person planning to conduct a BA or BE study submit the proposed protocol for the study to the FDA for review prior to the initiation of the study. The FDA may review a proposed protocol for a BE study and will offer advice with

respect to whether the conditions an appropriate design, the choice of reference product, and the proposed chemical and statistical analysis methods are met.

The commissioner of food and drugs shall consider the following factors, when supported by well-documented evidence, to identify specific pharmaceutical equivalents and pharmaceutical alternatives that are not or may not be bioequivalent drug products.

- Evidence from well-controlled clinical trials or controlled observations in patients that such drug products do not give comparable therapeutic effects or
- Evidence from well-controlled BE studies that such products are not bioequivalent drug products or
- Evidence that the drug products exhibit a narrow therapeutic ratio, e.g., there is less than a twofold difference in median lethal dose (LD50) and median effective dose (ED50) values, or have less than a twofold difference in the minimum toxic concentrations and minimum effective concentrations in the blood, and safe and effective use of the drug products requires careful dosage titration and patient monitoring or
- Competent medical determination that a lack of BE would have a serious adverse effect in the treatment or prevention of a serious disease or condition.
- The physicochemical evidence that the active drug ingredient has a low solubility in water, e.g., <5 mg/1 mL, or if dissolution in the stomach is critical to absorption, the volume of gastric fluids required to dissolve the recommended dose far exceeds the volume of fluids present in the stomach (taken to be 100 mL for adults and prorated for infants and children); or the dissolution rate of one or more such products is slow, e.g., $<50\%$ in 30 minutes when tested using either a general method specified in an official compendium or a paddle method at 50 rpm in 900 mL of distilled or deionized water at 37°C, or differs significantly from that of an appropriate reference material such as an identical drug product that is the subject of an approved full NDA; or the particle size and/or surface area of the active drug ingredient is critical in determining its BA; or certain physical structural characteristics of the active drug ingredient, e.g., polymorphic forms, conforms, solvates, complexes, and crystal modifications, dissolve poorly and this poor dissolution may affect absorption; or such drug products have a high ratio of excipients to active ingredients, e.g., greater than 5:1; or specific inactive ingredients, e.g., hydrophilic or hydrophobic excipients and lubricants, either may be required for absorption of the active drug ingredient or therapeutic moiety or alternatively, if present, may interfere with such absorption.
- The PK evidence that the active drug ingredient, therapeutic moiety, or its precursor is absorbed in large part in a particular segment of the GI tract or is absorbed from a localized site; or the degree of absorption of the active drug ingredient, therapeutic moiety, or its precursor is poor, e.g., $<50\%$, ordinarily in comparison to an intravenous dose, even when it is administered in pure form, e.g., in solution; or there is rapid metabolism of the therapeutic moiety in the intestinal wall or liver during the process of absorption (first-class metabolism), therefore the therapeutic effect and/or toxicity of such drug product is determined by the rate as well as the degree of absorption; or the therapeutic moiety is rapidly metabolized or excreted so that rapid dissolution and absorption are required for effectiveness; or the active drug ingredient or therapeutic moiety is unstable in specific portions of the GI tract and requires special coatings or formulations, e.g., buffers, enteric coatings, and film coatings, to assure adequate absorption; or the drug product is subject to dose-dependent kinetics in or near the therapeutic range, and the rate and extent of absorption are important to BE.

RECORD MAINTENANCE

All records of in vivo or in vitro tests conducted on any marketed batch of a drug product to assure that the product meets a BE requirement shall be maintained by the manufacturer for at least two years after the approval of the application submitted and would available to the FDA on request.

- If the formulation of the test article is the same as the formulation(s) used in the clinical studies demonstrating substantial evidence of safety and effectiveness for the test article's claimed indications, a reserve sample of the test article used to conduct an in vivo BA study comparing the test article to a reference oral solution, suspension, or injection.
- If the formulation of the test article differs from the formulation(s) used in the clinical studies demonstrating substantial evidence of safety and effectiveness for the test article's claimed indications, a reserve sample of the test article and of the reference standard used to conduct an in vivo BE study comparing the test article to the formulation(s) (reference standard) used in the clinical studies.
- For a new formulation, new dosage form, or a new salt or ester of an active drug ingredient or therapeutic moiety that has been approved for marketing, a reserve sample of the test article and of the reference standard used to conduct an in vivo BE study comparing the test article to a marketed product (reference standard) that contains the same active drug ingredient or therapeutic moiety.

Each reserve sample shall consist of a sufficient quantity to permit the FDA to perform five times all of the release tests required in the application or supplemental application. Each reserve sample shall be adequately identified so that the reserve sample can be positively identified as having come from the same sample as used in the specific BA study. Each reserve sample shall be stored under conditions consistent with product labeling and in an area segregated from the area where testing is conducted and with access limited to authorized personnel. Each reserve sample shall be retained for a period of at least five years following the date on which the application or supplemental application is approved, or if such application or supplemental application is not approved, at least five years following the date of completion of the BA study in which the sample from which the reserve sample was obtained was used.

Authorized FDA personnel will ordinarily collect reserve samples directly from the applicant or contract research organization at the storage site during a preapproval inspection. If authorized FDA personnel are unable to collect samples, the FDA may require the applicant or contract research organization to submit the reserve samples to the place identified in the agency's request. If the FDA has not collected or requested delivery of a reserve sample, or if the FDA has not collected or requested delivery of any portion of a reserve sample, the applicant or contract research organization shall retain the sample or remaining sample for the five-year period.

Upon release of the reserve samples to the FDA, the applicant or contract research organization shall provide a written assurance that, to the best knowledge and belief of the individual executing the assurance, the reserve samples came from the same samples as used in the specific BA or BE study identified by the agency. The assurance shall be executed by an individual authorized to act for the applicant or contract research organization in releasing the reserve samples to the FDA.

A contract research organization may contract with an appropriate, independent third party to provide storage of reserve samples provided that the sponsor of the study has been notified in writing of the name and address of the facility at which the reserve samples will be stored. If a contract research organization conducting a BA or BE study that requires reserve sample retention goes out of business, it shall transfer its reserve samples to an appropriate, independent third party, and shall notify in writing the sponsor of the study of the transfer and provide the study sponsor with the name and address of the facility to which the reserve samples have been transferred.

The applicant of an abbreviated application or a supplemental application submitted under section 505 of the federal FDC Act, or if BE testing was performed under contract, the contract research organization shall retain reserve samples of any test article and reference standard used in conducting an in vivo or in vitro BE study required for approval of the abbreviated application or supplemental application and beyond as required.

Pharmacokinetic and Statistical Considerations in Study Design

Sampling Time Considerations

The total number of sampling times necessary to characterize the blood level profiles will depend upon the curvature of the profiles and the magnitude of variability associated with the BA data (including PK variability, assay error, and interproduct differences in absorption kinetics).

The sampling times should adequately define peak concentration(s) and the extent of absorption. The sampling times should extend to at least three terminal elimination half-lives beyond T_{max}. The sponsor should consult with FDA prior to conducting the pivotal BE study if the assay is unable to quantify samples to three half-lives.

Maximum sampling time efficiency may be achieved by conducting a pilot investigation. The pilot study should identify the general shapes of the test and reference curves, the magnitude of the difference in product profiles, and the noise associated with each blood sampling time (e.g., variability attributable to assay error and the variability between subjects, for parallel study designs, or within subjects, for crossover study designs). This information should be applied to the determination of an optimum blood sampling schedule. Depending upon these variability estimates, it may be more efficient to cluster several blood samples rather than to have samples which are periodically dispersed throughout the duration of blood sampling.

Protein Binding

In general, product BE should be based upon total (free plus protein bound) concentrations of the parent drug (or metabolite, when applicable). However, if nonlinear protein binding is known to occur within the therapeutic dosing range (as determined from literature or pilot data), then sponsors may need to submit data on both the free and the total drug concentrations for the generic and pioneer products.

Similarly, if the drug is known to enter blood erythrocytes, then the protocol should address the issue of potential nonlinearity in erythrocyte uptake of the drug administered within the labeled therapeutic dosing range.

The BE protocol or completed study report should provide any information available from the literature regarding erythrocyte uptake and protein-binding characteristics of the drug or drug class, including the magnitude of protein binding and the type of blood protein to which it binds.

Subject Number

Pilot studies are recommended as a means of estimating the appropriate sample size for the pivotal BE study. Estimated sample size will vary depending upon whether the data are analyzed on a log or linear scale. Useful references for sample size estimates include Westlake, Hauschke, and Steinijans.

Crossover and Parallel Design Considerations

A two-period crossover design is commonly used in blood-level studies. The use of crossover designs eliminates a major source of study variability: between-subject differences in the rates of drug absorption, drug clearance, and the volume of drug distribution.

In a typical two-period crossover design, subjects are randomly assigned to either sequence A or sequence B with the restriction that equal number of subjects are initially assigned to each sequence. The design is as follows:

	Sequence A	Sequence B
Period 1	Test	Reference
Period 2	Reference	Test

A crucial assumption in the two-period crossover design is that of equal residual effects. Unequal residual effects may result, for example, from an inadequate washout period. Another assumption of the crossover (or extended period) design is that there is no subject

by formulation interaction. In other words, the assumption is that all subjects are from a relatively homogeneous population and will exhibit similar relative BA of the test and reference products. If there are subpopulations of subjects, such that the relationship between product BA is a function of the subpopulation within which they are being tested, then a subject by formulation interaction is said to exist.

A one-period parallel design may be preferable in the following situations:

1. The drug induces physiological changes in the animal (e.g., liver microsomal enzyme induction) which persist after total drug clearance and alter the BA of the product administered in the second period.
2. The drug has a very long terminal elimination half-life, creating a risk of residual drug present in the animal at the time of the second period dosing.
3. The duration of the washout time for the two-period crossover study is so long as to result in significant maturational changes in the study subjects.
4. The drug follows delayed or prolonged absorption (flip-flop kinetics[2]), where the slope of the [[β]]-elimination phase is dictated by the rate of drug absorption rather than the rate of drug elimination from one or both products.

Other designs, such as the two-period design with four treatment sequences (test/test, reference/reference, test/reference, and reference/test) or the extended period design may be appropriate depending on the circumstances. The use of alternative study designs should be discussed with the FDA prior to conducting the BE study. Pilot data or literature may be used in support of alternative study designs.

Duration of Washout Time for Crossover Study

For drugs which follow a one or two compartment open body model, the duration of the washout time should be approximately 10 times the plasma apparent terminal elimination half-life, to provide for 99.9% of the administered dose to be eliminated from the body. If more highly complex kinetic models are anticipated (e.g., drugs for which long withdrawal times have been assigned due to prolonged tissue binding), or for drugs with the potential for physiologic carryover effects, the washout time should be adjusted accordingly. The washout period should be sufficiently long to allow the second period of the crossover study to be applicable in the statistical analysis. However, if sequence effects are noted, the data from the first period may be evaluated as a parallel design study.

Pivotal Parameters for Blood Level BE

The sponsor is encouraged to calculate parameters using formulas which involve only the raw data (i.e., so-called model independent methods).

AREA UNDER THE CURVE ESTIMATES

The extent of product BA is estimated by the area under the blood concentration versus time curve (AUC). The AUC is most frequently estimated using the linear trapezoidal rule. Other methods for AUC estimation may be proposed by the sponsor and should be accompanied by appropriate literature references during protocol development. For a single dose BE study, AUC should be calculated from time 0 (predose) to the last sampling time associated with quantifiable drug concentration AUC(0-LOQ). The comparison of the test and reference product value for this noninfinity estimate provides the closest approximation of the measure of uncertainty (variance) and the relative BA estimate associated with AUC(0-INF), the full extent of product BA. The relative AUC values generally change very little once the absorption of both products has been completed. However, because of the possibility of multifunctional absorption kinetics, it cannot always be determined when the available drug has been completely absorbed. Therefore, the FDA recommends extending the duration of sampling until such time that AUC(0-LOQ)/AUC(0-INF) $\geq$ 0.80. Generally, the sampling times

should extend to at least three multiples of the drug's apparent terminal elimination half-life, beyond the time when maximum blood concentrations are achieved.

AUC(0-INF) should be used to demonstrate that the concentration–time curve can be quantitated such that AUC(0-LOQ)/AUC(0-INF) ≥ 0.80. The method for estimating the terminal elimination phase should be described in the protocol and the final study report. The AUC(0-LOQ)/AUC(0-INF) is calculated to determine whether AUC(0-LOQ) adequately reflects the extent of absorption.

The sponsor should consult with FDA if AUC(0-LOQ)/AUC(0-INF) is determined to be <0.80. If AUC(0-LOQ)/AUC(0-INF) is <0.80, then a multiple-dose study to steady state may be needed to allow an accurate assessment of AUC(0-INF) [where AUC(0-INF) = AUC(0-t) at steady state and t is the dosing interval].

In a multiple-dose study, the AUC should be calculated over one complete dosing interval AUC(0-t). Under steady-state conditions, AUC(0-t) equals the full extent of BA of the individual dose AUC(0-INF) assuming linear kinetics. For drugs which are known to follow nonlinear kinetics, the sponsor should consult with the FDA to determine the appropriate parameters for the BE determination.

AUC Measurements

The Trapezoidal Rule

This is the simplest of all the methods and involves the breaking up of the plasma concentration versus time profile into several trapezoids, calculating the areas of individual trapezoids, and then adding up these areas to arrive at a cumulative AUC:

$$\left(\frac{C_0 + C_1}{2}\right)(t_1 - t_0) + \left(\frac{C_1 + C_2}{2}\right)(t_2 - t_1) + \cdots + \left(\frac{C_{n-1} + C_n}{2}\right)(t_n - t_{n-1}) \quad (1)$$

The units for the AUC are: concentration $\times$ time, e.g., μg hr/mL or mg min/L. As a general rule, the larger the number of segments or trapezoids formed, the greater is the accuracy achieved. In other words, the closer the interval between each plasma concentration reading taken, the more accurate will be the results. If the plasma concentration values are quite far apart, a smooth curve may be drawn which then can be broken up into a large number of trapezoids.

The AUC is proportional to the dose absorbed only when the calculations are extended to the point where the plasma concentration approaches zero. This may not be possible in some instances, in which case the comparisons can either be made up to a given time or the plasma concentrations can be extended to follow the shape of the curve, both of these approaches adding to the errors in the BA estimations.

Integration Method

The rate of change of plasma concentration (C) is described as:

$$\frac{dC}{dt} = \text{rate of absorption} - \text{rate of elimination} \quad (2)$$

$$= K_a X_a - KX \quad (3)$$

where k_a and K are absorption and elimination rate constants, respectively, and X_a and X are the amounts of drug in the gatrointestinal tract and the body, respectively. An integration of this equation between limits of time for which the drug remains in the body, as reflected by the plasma concentration, gives:

$$C = A(e^{-Kt} - e^{-K_a t}) \quad (4)$$

and the total AUC, for which the total integral between time zero and infinity is given by:

$$\text{AUC} = A\left(\frac{1}{K} - \frac{1}{K_a}\right) \quad (5)$$

Thus, if C can be fitted to an equation which will allow calculation of the absorption and elimination rate constants, the exact calculation of AUC can be made very easily. This approach is identical to the trapezoidal rule method described earlier, except that it uses trapezoids whose time differential is approaching zero.

Computer Applications

Whereas in the past, a variety of physical methods were used for area comparison, most of these including those described earlier have now been replaced with sophisticated computer programs which often combine the statistical evaluation of differences using a variety of linear and nonlinear approaches. These will be described in detail in another chapter.

Rate of Absorption

The rate of absorption will be estimated by the maximum observed drug concentration (C_{max}) and the corresponding time to reach this maximum concentration (T_{max}). When conducting a steady-state investigation, data on the minimum drug concentrations (trough values) observed during a single dosing interval (C_{min}) should also be collected. Generally, three successive C_{min} values should be provided to verify that steady-state conditions have been achieved. Although C_{min} most frequently occurs immediately prior to the next successive dose, situations do occur with C_{min} observed subsequent to dosing. To determine a steady-state concentration, the C_{min} values should be regressed over time and the resultant slope should be tested for its difference from zero.

Determination of Product BE

Unless otherwise indicated by the FDA during the protocol development for a given application, the pivotal BE parameters will be C_{max} and AUC (0-LOQ) (for a single dose study) or AUC(0-t) (for a multiple-dose study). To be indicative of product BE, the pivotal metrics should be associated with confidence intervals which fall within a set of acceptability limits.

The sponsor and the FDA should agree to the acceptable bounds for the confidence limits for the particular drug and formulation during protocol development. If studies or literature demonstrate that the pioneer drug product exhibits highly variable kinetics, then the generic drug sponsor may propose alternatives to the generally acceptable bounds for the confidence limits. T_{max} in single dose studies and C_{min} in multiple-dose studies will be assessed by clinical judgment.

Errors in BE Studies

Erroneous conclusions can easily be made if the logic behind BA studies is not clearly understood. The following are the important highlights of the most common errors:

1. When concentrations are monitored in the biologic fluids, the specificity of the assay methods is of utmost importance. This is especially applicable to single dose studies in which small concentrations should be monitored in order to allow study the complete elimination of the drug from the body.
2. It is generally assumed that the absorption rates of drugs are higher than the rates of elimination; but there can be exceptions, in which case the terminal plasma concentration profiles would represent both the absorption and elimination processes and the mathematical/statistical models used should take this into account.
3. The extrapolation of plasma or urinary concentration data to compensate for missing experimental points always introduces some error in the calculations; it is desirable to extend the study to at least three elimination half-lives when plasma concentration is monitored, and for at least seven half-lives when monitoring urinary excretion of drugs to estimate their BA.
4. There is often lack of sufficient data points to characterize the plasma concentration profiles. Significant area can be lost if sufficient points are not collected during the peak of the

concentration. In general, there should be at least three data points before the peak occurs and at least four or five values after the peak, if possible.

5. The variation among individuals in the elimination rates of a drug should be considered. The proportionality between AUC and BA is based on the assumption that the elimination rates are invariant; any deviation from the norm will result in significant error. Correction of this error can be made if the elimination rate constants are calculated for each subject and the AUC is corrected as follows:

$$\mathrm{AUC}_{\mathrm{corrected}} = \mathrm{AUC}_{\mathrm{apparent}}(K) \tag{6}$$

If a drug is eliminated fast, K will be large, accounting for possible under-estimation of the AUC.

6. Comparison of data for different studies which may not be well matched in terms of the characteristics of the subject population, study conditions, or routes of drug administration should be made with due consideration to these factors. It is ironic that such cross-study comparisons are both very common and very misleading.
7. When identical drug concentrations are obtained in the plasma following administration of equimolar doses from different formulations, these formulations are considered bioequivalent and the principle is referred to as the superimposition principle. In using this principle, one must choose a number of subjects in accordance with the statistical criteria which will demonstrate at least 20% differences in the means of values in order to make them clinically significant. This criterion can be applied to the concentration at each sampling time, to the peak concentration, and to the time of the peak concentrations and the AUCs.
8. It should be noted that just because a drug product meets compendial standards of purity and other criteria, its BA is not assured. In fact, compendial requirements fall far short of assuring the efficiency of dosage forms in releasing drugs. The latest edition of United States Pharmacopoeia and National Formulary requires the demonstration of sufficient dissolution for many drugs where evidence of dissolution affecting BA has been suggested. A large number of drugs remain to be included in this list and it is hoped that eventually demonstration of BA will become a compendial requirement. The costs of performing BA studies make such requirements impractical for some drugs. However, without such requirements, it is difficult to justify the rejection of a product on the grounds that its chemical equivalence varies by more than 10%, when its biologic equivalent is allowed to vary to any degree.

Statistical Analysis

The statistical models used in the evaluation of BE data have been evolving over the past few decades. The standard statistical method of null hypothesis was the first to be used where no difference is proved and rejection of null indicates statistically significant different ($p<0.05$). A problem arises since small differences with $p<0.05$ may be unimportant and large differences with $p>0.05$ may be important. This prompted the FDA to solve the problem by requesting power analysis confidence interval test of Schuirman, where two one-sided comparisons are made; this also evolved in the use of the famous 75-125 rule to deal with individual effects.

The FDA advocates the use of 90% confidence intervals, as the best available method for evaluating BE study data. The confidence interval approach should be applied to the individual parameters of interest (e.g., AUC and $C_{\max}$). The sponsor may use untransformed or log-transformed data. However, the choice of untransformed or log-transformed data should be made by the sponsor with concurrence by the FDA prior to conducting the study.

Untransformed Data

If we let $\bar{X}_{T1}$ be the mean for the test drug in period 1, $\bar{X}_{T2}$ be the mean for the test drug in period 2, and $\bar{X}_{R1}$ and $\bar{X}_{R2}$ are the respective means for the reference drug, then the estimates for the drugs averaged over both periods are $\bar{X}_T = (1/2)(\bar{X}_{T1} + \bar{X}_{T2})$ for the test drug and $\bar{X}_R = (1/2) \times (\bar{X}_{R1} + \bar{X}_{R2})$ for the reference drug. Although both sequence groups usually start with the same

number of animals, the number of animals in each sequence group (n_A and n_B) that successfully finish the study may not be equal. The formulas above utilize the marginal or least squares estimates of μT and μR, the corresponding means in the target population. These means are not a function of the sample size in each sequence.

An analysis of variance is needed to obtain the estimate of σ^2, the error variance. The estimator, s^2, which will be used in the calculation of the 90% confidence interval should be obtained from the "error" mean square term found in the following ANOVA table.

Source	Degrees-of-freedom
Sequence	1
Animal (sequence)	nA + nB − 2
Period	1
Formulation	1
Error	nA + nB − 2
Total	2nA + 2nB − 1

Lower and upper 90% confidence intervals are then found by formulas based on Student's t-distribution.

$$L = (\bar{X}_T - \bar{X}_R) - t_{nA} + nB^{-2;0.05} s \sqrt{\frac{1}{2}\left(\frac{1}{n_A} + \frac{1}{n_B}\right)} \tag{7}$$

$$U = (\bar{X}_T - \bar{X}_R) - t_n + n^{-2;0.05} s \sqrt{\frac{1}{2}\left(\frac{1}{n_A} + \frac{1}{n_B}\right)} \tag{8}$$

The procedure of declaring two formulations bioequivalent if the 90% confidence interval is completely contained in some fixed interval, is statistically equivalent to performing two one-sided statistical tests ($\alpha = 0.05$) at the end-points of the interval.

Consider the following example with $L = 3$, $U = 17$, $\bar{X}_T = 110$, and $\bar{X}_R = 100$. By the traditional hypothesis testing approach, the result would be considered statistically significant since the confidence interval does not include zero. Using the confidence interval approach, the entire confidence interval lies within 17% of $\bar{X}_R$. (The lower end of the confidence interval lies within $L/\bar{X}_R = 3/100 = 3\%$ of $\bar{X}_R$, while the upper end of the confidence interval lies within $U/\bar{X}_R = 17/100 = 17\%$ of $\bar{X}_R$.) If it were determined by FDA that only differences larger than 20% were biomedically important, then using the confidence interval approach, the results of this study would be considered adequate to demonstrate BE.

Now consider an example with $L = -4$, $U = 24$, $\bar{X}_T = 110$, and $\bar{X}_R = 100$. In this case, by the traditional hypothesis testing approach, the result would not be considered statistically significant since the confidence interval includes zero. However, the confidence interval extends as far as 24% from $\bar{X}_R$. (The lower end of the confidence interval lies within $L/\bar{X}_R = -4/100 = -4\%$ of $\bar{X}_R$, while the upper end of the confidence interval extends to $U/\bar{X}_R = -24/100 = -24\%$ of $\bar{X}_R$.) If it were determined by FDA that only differences larger than 20% were biomedically important, then the results of this study would be considered inadequate to demonstrate BE, since the entire confidence interval is not within 20% of $\bar{X}_R$.

Logarithmically Transformed Data

This section discusses how the 90% confidence interval approach should be applied to log-transformed data. In this situation, the individual animal AUC and C_{max} values are log-transformed and the analysis is done on the transformed data. For a two-period crossover study, the ANOVA model used to calculate estimates of the error variance and the least square means are identical for both transformed and untransformed data. The procedural difference comes after the lower and upper 90% confidence intervals are found by formulas based on Student's t-distribution.

The lower and upper confidence bounds of the log-transformed data will then need to be back-transformed in order to be expressed on the original scale of the measurement. One thing to keep in mind when moving between the logarithm scale and the original scale is that the back-transformed mean of a set of data that has been transformed to the logarithm scale is not strictly equivalent to the mean that would be calculated from the data on the original scale of measurement. This back-transformed mean is known instead as the geometric mean.

It may help to see the calculations involved. If the AUC from each animal has been transformed to the logarithm scale, we can express the transformed AUC as LnAUC. Then the mean on the logarithm scale is as follows:

$$\bar{Ln}\text{AUC}_t = \sum_{i=1}^{n} \frac{Ln\text{AUC}_t}{n} \tag{9}$$

where the subscript t represents the AUC determinations for the test article, i is the AUC of the ith animal, and n is the total number of animals receiving the text article. When this mean is back-transformed, it becomes the geometric mean: $e^{(Ln\text{AUC}_t)}$. This geometric mean will be on the original scale of the measurement. It will be close to but not exactly equal to the mean obtained on the original scale of the measurement. The back-transformation of the confidence bounds is accomplished in the following way:

$$\text{Lower bound (expressed as a percentage)} = (e^{L} - 1) \times 100$$

$$\text{Upper bound (expressed as a percentage)} = (e^{U} - 1) \times 100$$

where L is the lower 90% confidence interval and calculated on the log-transformed data; U is the upper 90% confidence interval and calculated on the log-transformed data.

As an example, consider the data for AUC from a hypothetical crossover study in the following table:

		Reference article		Test article	
Animal	**Crossover sequence**	**AUC**	**LogAUC**	**AUC**	**LogAUC**
1	1	518.0	6.25	317.8	5.76
2	1	454.9	6.12	465.0	6.14
3	1	232.8	5.45	548.4	6.31
4	1	311.1	5.74	334.8	5.81
5	2	340.4	5.83	224.7	5.41
6	2	497.7	6.21	249.2	5.52
7	2	652.0	6.48	625.4	6.44
8	2	464.1	6.14	848.7	6.74
	Mean	433.8	6.03	451.7	8602
	Standard deviation	133.3	0.33	214.3	047
	Geometric mean		414.7		

The statistics for AUC will be calculated from the log-transformed data. In this example, L, the lower 90% confidence interval calculated on the log scale is -0.395. U, the upper 90% confidence interval calculated on the log scale is 0.372. To back-transform these intervals and express them as percentages, we do the following:

Back-transformed lower bound:

$$(e^{-0.395} - 1) \times 100 = (0.674 - 1) \times 100 = (-0.326) \times 100 = -32.6\%$$

Back-transformed upper bound:

$$(e^{0.372} - 1) \times 100 = (1.451 - 1) \times 100 = (0.451) \times 100 = 45.1\%$$

Therefore, the lower end of the confidence bound lies within -32.6% of the geometric mean of the reference article, while the upper end of the confidence interval lies within 45.1% of the geometric mean of the reference article. If it were determined by FDA that the acceptable

confidence bound was 80% to 125% of the geometric mean of the reference article in order to demonstrate BE, then the back-transformed lower bound can be as low as −20% and the back-transformed upper bound can be as high as 25%. In this example, we would determine that the study had not demonstrated an acceptable level of BE between the test article and reference article.

The width of the confidence interval is determined by the within-subject variance (between-subject variance for parallel group studies) and the number of subjects in the study. In general, the confidence interval for untransformed data should be 80% to 120% (the confidence interval should lie within ±20% of the mean of the reference product). For logarithmically transformed data, the confidence interval is generally 80% to 125% (the confidence interval should lie within −20% to +25% of the mean of the reference product). The sponsor and FDA should determine the acceptable bounds for confidence limits for the particular drug and formulation during protocol development.

Animal Drug BE Testing

A BE study may also be part of a NADA or supplemental NADA for approval of an alternative dosage form, new route of administration, or a significant manufacturing change which may affect drug BA. Many requirements described above for human studies also apply to animal studies; various descriptions of experimental design and data handling are common to both. FDA has concluded that the tissue residue depletion of the generic product is not adequately addressed through BE studies. Therefore, sponsors of ANADAs for drug products for food-producing animals will generally be asked to include BE and tissue residue studies [21 USC 360 b (n) (1) (E)]. A tissue residue study should generally accompany clinical end-point and pharmacologic end-point BE studies, and blood-level BE studies that cannot quantify the concentration of the drug in blood throughout the established withdrawal period [21 USC 360 b (n) (1) (A) (ii)]. BE studies (i.e., blood level, pharmacologic end-point, and clinical end-point studies) and tissue residue depletion studies should be conducted in accordance with good laboratory practice regulations (21 CFR Part 58). Whereas the focus of the guidance is BE testing for ANADA approval, the general principles also apply to relative BA studies conducted for NADAs.

Reference Product

As a general rule, the proposed generic product should be tested against the original pioneer product. If the original pioneer product is no longer marketed, but remains eligible to be copied, then the first approved and available generic copy of the pioneer should be used as the reference product for BE testing against the proposed new generic product.

If several approved NADAs exist for the same drug product, and each approved product is labeled differently (i.e., different species and/or claims), then the generic sponsor must clearly identify which product label is the intended pioneer. BE testing should be conducted against the single approved product which bears the labeling that the generic sponsor intends to copy. The generic sponsor should consult with CVM (FDA) regarding selection of the appropriate reference product before conducting the BE study.

Waiver of In Vivo BE Study

The requirement for the in vivo BE study may be waived for certain generic products [21 USC 360 b (n) (1) (E)]. Categories of products which may be eligible for waivers include, but are not limited to, the following:

- Parenteral solutions intended for injection by the intravenous, subcutaneous, or intramuscular routes of administration.
- Oral solutions or other solubilized forms.
- Topically applied solutions intended for local therapeutic effects. Other topically applied dosage forms intended for local therapeutic effects for non-food animals only.
- Inhalant volatile anesthetic solutions.

In general, the generic product being considered for a waiver contains the same active and inactive ingredients in the same dosage form and concentration and has the same pH and physicochemical characteristics as an approved pioneer product.

However, the CVM will consider BE waivers for non-food animal topical products with certain differences in the inactive ingredients of the pioneer and generic products.

If a waiver of the in vivo BE and/or the tissue residue study/studies is granted for a food animal drug product, then the withdrawal period established for the pioneer product will be assigned to the generic product. Sponsors may apply for waivers of in vivo BE studies prior to submission of the ANADAs.

Species Selection

A BE study generally should be conducted for each species for which the pioneer product is approved on the label, with the exception of "minor" species [as defined in section 514.1 (d) (1) of Title 21 of the Code of Federal Regulations] on the label.

Subject Characteristics

Ordinarily, studies should be conducted with healthy animals representative of the species, class, gender, and physiological maturity for which the drug is approved. The BE study may be conducted with a single gender for which the pioneer product is approved, unless there is a known interaction of formulation with gender. An attempt should be made to restrict the weight of the test animals to a narrow range in order to maintain the same total dose across study subjects. The animals should not receive any medication prior to testing for a period of two weeks or more, depending upon the biological half-life of the ancillary drug.

Human Food Safety Considerations

The toxicology and tolerance developed for the pioneer animal drug are applied to generic copies of the drug. The CVM has concluded that in addition to a BE study, a tissue residue depletion study should be conducted for the approval of a generic animal drug product in a food-producing species. Two drug products may have the same plasma disposition profile at the concentrations used to assess product BE, but may have very different tissue disposition kinetics when followed out to the withdrawal time for the pioneer product. Therefore, to show the withdrawal period at which residues of the generic product will be consistent with the tolerance for the pioneer product, a tissue residue depletion study is necessary.

The results of a BE study or tissue residue depletion study in one animal species cannot generally be extrapolated to another species. Possible species differences in drug partitioning or binding in tissues could magnify a small difference in the rate or extent of drug absorbed into a large difference in marker residue concentrations in the target tissue. Therefore, for a pioneer product labeled for more than one food-producing species, a BE study and a tissue residue depletion study will generally be requested for each major food-producing species on the label.

A traditional withdrawal study, as described in CVM's guidance number 3, "General Principles for Evaluating the Safety of Compounds Used in Food-Producing Animals," is considered the best design for collecting data useful for the calculation of a preslaughter withdrawal period for drugs used in food-producing animals. In the traditional withdrawal study, 20 animals are divided into four or five groups of four to five animals each. Groups of animals are slaughtered at carefully preselected time points following the last administration of the test product and the edible tissues are collected for residue analysis. A statistical tolerance limit approach is used to determine when, with 95% confidence, 99% of treated animals would have tissue residues below the codified limits.

For purposes of calculating a withdrawal period for a generic animal drug, only the generic product would be tested (i.e., not the pioneer product), and only the marker residue in the target tissue would be analyzed. Other study designs will be considered on a case-by-case basis. Sponsors are encouraged to submit the proposed tissue residue depletion protocol for CVM concurrence before proceeding with the withdrawal study.

The generic animal drug will be assigned the withdrawal time supported by the residue depletion data, or the withdrawal time currently assigned to the pioneer product, whichever is the longer.

The generic animal drug sponsor may request a shorter withdrawal period for the generic product by supplementing the ANADA and providing tissue residue data necessary to support the shorter withdrawal period request. Such a supplement will be reviewed under the agency's policy for Category II supplements. For a Category II supplement, a reevaluation of the safety (or effectiveness) data in the parent application (i.e., the pioneer NADA) may be required [21 CFR 514.106 (b) (2)]. The CVM will ordinarily approve a request for a shorter withdrawal period when the residue data are adequate and when no other human food safety concerns for the drug are evident.

Under 21 CFR 514.1(b)(7), applications are required to include a description of practicable methods for determining the quantity, if any, of the new animal drug in or on food, and any substance formed in or on food because of its use, and the proposed tolerance or withdrawal period or other use restrictions to ensure that the proposed use of the drug will be safe. For certain drug products, a tissue residue depletion study is not needed to ensure that residues of the test product will be consistent with the codified drug tolerance at the withdrawal time assigned to the reference product. These include but may not be limited to products for which a waiver of in vivo BE testing is granted, and products for which the assay method used in the blood-level BE study is sensitive enough to measure blood levels of the drug for the entire withdrawal period assigned to the reference product. Other requests for waiver of the tissue residue study will be considered on a case-by-case basis.

CVM will not request that the assay methodology used to determine the withdrawal period for the generic product be more rigorous than the approved methodology used to determine the existing withdrawal period for the pioneer product. If an analytical method other than the approved method of analysis is used, the generic sponsor should provide data comparing the alternate method to the approved method.

LOCALLY ACTING GI DRUGS

For drugs whose site of action is the GI tract, determination of BE is more complicated as local drug concentrations cannot be measured directly requiring evaluation of PKss, its relationship in vitro tests including dissolution and binding assays and correlation with clinical studies.

The PK studies for locally acting drugs provide safety data, whereas PK studies may not correlate with therapeutic effectiveness, the relationship with BE is not so straightforward. If a drug is acting locally and also absorbed in the systemic circulation, the PK studies would still reflect the dosage form factors even though the site of action is also local. The premise here remains same any differences noted in the C_{max} of AUC is due to differences in absorption rates and extent attributable to dosage form differences, such as release of drug. However, if plasma levels can be connected to product effectiveness, then we can determine the significance of differences in product performance. When the connection to efficacy is broken, we do not have a simple way to say what difference in PK is significant. In this sense, downstream PK is similar to a PD endpoint for which a dose–response curve needs to be established. Another concern about PK studies on locally acting drugs is that drug may be able to reach the plasma without passing the site of action. An example is an inhalation product for which some of the dose is swallowed and potentially absorbed orally. An important distinction is between parallel and sequential absorption paths. In the inhalation example, drug either goes to the lung or to the stomach or could appear in plasma at the same time by either path. In a locally acting GI drug, the absorption process is sequential so drug absorbed from the intestine appears before drug absorbed in the colon and thus can be distinguished.

The PK studies often fail for locally acting drugs because of the very low concentration observed in plasma and even at the site of local action. For example, mesalamine must reach the mucosal surface lining the GI tract in order to exert its pharmacological effect, which is dependent on the dissolution rate; for other dosage forms which dissolve instantly, the rate limiting factors would be transit rate in the GI tract. The use of dissolution thus becomes an

important tool to demonstrate BE. Some GI-acting drugs are formulated to target different regions of the GI tract, often via coatings that lead to pH-dependent dissolution. Comparative dissolution testing at different pH could demonstrate that test and reference products are targeting the same region of the GI tract. Biowaivers for Biopharmaceutics Classification System (BCS) Class I drugs formulated in rapidly dissolving immediate release solid oral dosage forms are well established. Since a GI-acting drug does not need to be absorbed, application of the scientific basis of the BCS would suggest that a high solubility drug in a rapidly dissolving formulation with no excipients that affect product performance may be eligible for a biowaiver.

Generally, studies that measure the concentration of drug in the small intestinal mucosa could provide more direct evidence of equivalent tissue concentration at the site of action. But those studies are difficult to conduct and interspecies correlations often add a lot of variability; as a result, there is a consensus developing that comparative clinical trails be conducted to demonstrate BE but only in those situations where other methods fail since not only are these expensive to conduct, but also these can often be insensitive to formulation differences—the purpose the study.

Topical Drugs

For topical dermatological drug products, PK measurements in blood, plasma, and/or urine are usually not feasible to document BE, because topical dermatologic products generally do not produce measurable concentrations in extra cutaneous biological fluids. The BE determination for these products is thus often based on PD or clinical studies. An additional approach is to document BE through reliance on measurement of the active moiety(ies) in the stratum corneum. This approach is termed dermatopharmacokinetics (DPKs). Although measurement of the active moiety(ies) in blood or urine is not regarded as an acceptable measurement of BE for dermatological drug products, it may be used to measure systemic exposure.

Inactive Ingredients

During the investigational new drug (IND) process for an NDA, the safety of inactive ingredients in a topical drug product should be documented by specific studies or may be based on a prior history of successful use in the same amount administered via the same route of administration in an approved product. The requisite safety studies to establish the safety of a new excipient during the IND process should be discussed with appropriate review staff at the FDA. For an ANDA, the safety of inactive ingredients in an ANDA can be based on a prior history of successful use in an NDA or ANDA. If the inactive ingredients in an ANDA are not the same as the RLD, the applicant should demonstrate to the Agency that the changes(s) do not affect the safety and/or efficacy of the proposed drug product. In some instances, a comparative BA study will satisfy this recommendation. If preclinical or clinical studies are needed to demonstrate the safety of inactive ingredients(s) in the generic drug product, the ANDA may not be approved. In this circumstance, the applicant may wish to resubmit their application as an NDA under the provisions of 505(b)(1) or (b)(2) of the Act.

Waiver of BE

In accordance with 21 CFR 314.94 (a) (9) (v), generally, the test (generic) product intended for topical use must contain the same inactive ingredients as the RLD. For all topical drug products intended for marketing under an abbreviated application, documentation of in vivo BE is required under 21 CFR 320.21 (b). For a topical solution drug product, in vivo BE may be waived if the inactive ingredients in the product are qualitatively identical and quantitatively essentially the same when compared with the listed drug. In this setting, quantitatively *essentially the same* means that the amount/concentration of the inactive ingredient(s) in the test product cannot differ by more than +5% of the amount/concentration of the listed drug. Where a test solution differs qualitatively or quantitatively from the listed drug, in vivo BE may be waived, provided the sponsor submits evidence that the difference does not affect safety and/or efficacy of the product at the time a waiver is requested.

BE Approaches

Comparative clinical trials are generally difficult to perform, highly variable, and insensitive. For these reasons, other approaches, such as DPK or PD may be used for BE determination.

DPK Approaches

The DPK approach is comparable to a blood, plasma, urine PK approach applied to the stratum corneum. DPK encompasses drug concentration measurements with respect to time and provides information on drug uptake, apparent steady-state levels, and drug elimination from the stratum corneum based on a stratum corneum concentration–time curve.

When applied to diseased skin, topical drug products induce one or more therapeutic responses, where onset, duration, and magnitude depend on the relative efficiency of three sequential processes, namely, (*i*) the release of the drug from the dosage form, (*ii*) penetration of the drug through the skin barrier, and (*iii*) generation of the desired pharmacological effect. Because topical products deliver the drug directly to or near the intended site of action, measurement of the drug uptake into and drug elimination from the stratum corneum can provide a DPK means of assessing the BE of two topical drug products. Presumably, two formulations that produce comparable stratum corneum concentration–time curves may be BE, just as two oral formulations are judged BE if they produce comparable plasma concentration–time curves. Even though the target site for topical dermatologic drug products in some instances may not be the stratum corneum, the topical drug must still pass through the stratum corneum, except in instances of damage, to reach deeper sites of action. In certain instances, the stratum corneum itself is the site of action. For example, in fungal infections of the skin, fungi reside in the stratum corneum and therefore, DPK measurement of an antifungal drug in the stratum corneum represents direct measurement of drug concentration at the site of action. In instances where the stratum corneum is disrupted or damaged, in vitro drug release may provide additional information toward the BE assessment. In this context, the drug release rate may reflect drug delivery directly to the dermal skin site without passage through the stratum corneum. For antiacne drug products, target sites are the hair follicles and sebaceous glands. In this setting, the drug diffuses through the stratum corneum, epidermis, and dermis to reach the site of action. The drug may also follow follicular pathways to reach the sites of action. The extent of follicular penetration depends on the particle size of the active ingredient if it is in the form of a suspension. Under these circumstances, the DPK approach is still expected to be applicable because studies indicate a positive correlation between the stratum corneum and follicular concentrations. Although the exact mechanism of action for some dermatological drugs is unclear, the DPK approach may still be useful as a measure of BE because it has been demonstrated that the stratum corneum functions as a reservoir, and stratum corneum concentration is a predictor of the amount of drug absorbed.

For reasons thus cited, DPK principles should be generally applicable to all topical dermatological drug products, including antifungal, antiviral, antiacne, antibiotic, corticosteroid, and vaginally applied drug products. The DPK approach can thus be the primary means to document BA/BE. Additional information, such as comparative in vitro release data and particle size distribution of the active ingredient between the RLD and the test product, may provide additional supportive information. Generally, BE determinations using DPK studies are performed in healthy subjects because skin where disease is present demonstrates high variability and changes over time. Use of healthy subjects is consistent with similar use in BE studies for oral drug products.

A DPK approach is not generally applicable (*i*) when a single application of the dermatological preparation damages the stratum corneum, (*ii*) for otic preparations except when the product is intended for otic inflammation of the skin, and (*iii*) for ophthalmic preparations because the cornea is structurally different from the stratum corneum. The following three sections of the guidance provide general procedures for conducting a BA/BE study using DPK methodology.

PERFORMANCE AND VALIDATION OF THE SKIN STRIPPING TECHNIQUE

DPK studies should include validation of both analytical methods and the technique of skin stripping. Since the DPK approach involves two components of validation (sampling and analytical method), overall DPK variability may be greater than with other methodologies. For analytical methods, levels of accuracy, precision, sensitivity, specificity, and reproducibility should be documented according to established procedures. Although the forearm, back, thigh, or other part of the body can be used for skin-stripping studies, most studies are conducted on the forearm, for reasons of convenience. Care should be taken to avoid any damage with physical, mechanical, or chemical irritants (e.g., soaps, detergents, agents). Usual hydration and environmental conditions should be maintained. After washing prior to treatment, sufficient time, preferably two hours, should be allowed to normalize the skin surface. Detailed and workable SOPs for area and amount of drug application, excess drug removal, and skin stripping methodology should be developed. The product's stability during the course of the study should be established. If the product is unstable, the rate and extent of degradation in situ over the period should be determined accurately so that a correction factor may be applied. Skin on both left and right arms of healthy subjects may be used to provide eight or more sites per arm. The size of the skin stripping area is important to allow collection of a sufficient drug in a sample to achieve adequate analytical detectability. Inter- and intra-arm variability should be assessed, and the treatment sites should be randomized appropriately. If a sponsor or applicant is using multiple investigators to conduct a single study, the reproducibility of skin stripping data between the investigators should be established. Either of the following approaches is recommended:

- A dose–response relationship between the drug concentration in the applied dosage form and the drug concentration in the stratum corneum should be established using the skin-stripping method. A DPK dose–response relationship is analogous to a dose proportionality study performed with solid oral dosage forms. This type of study can be readily performed using three different strengths of the formulations. These can be marketed or specially manufactured products. Alternatively, a solution of the active drug representing three concentrations can be prepared for this purpose. Amount of drug in the stratum corneum at the end of a specified time interval, such as three hours, can provide a dose–response relationship.
- The skin stripping method should be capable of detecting differences of $\pm 25\%$ in the strength of a product. This can be determined by applying different concentrations (e.g., 75%, 100%, 125%) of a test dosage form such as a simple solution to the skin surface for a specified exposure time such as three hours, executing the skin stripping method, and performing the appropriate statistical tests comparing the strength applied to the measured drug concentration in the stratum corneum.

Using the reference product, the approximate minimum time required for drug to reach saturation level in the stratum corneum should be determined. This study establishes the time point at which the elimination phase of the study may be initiated.

The drug concentration–time profile may vary with the drug, the drug potency class, formulation, subject, sites of application, circadian rhythm, ambient temperature, and humidity. These factors should be considered and controlled as necessary.

Circadian rhythms may be present and may affect the measurement of skin stripping drug concentration if the drug is also an endogenous chemical (e.g., corticosteroid or retinoic acid). In such circumstances, the baseline concentration of the endogenous compound should be measured over time from sites where no drug product has been applied.

Sample Pilot Study

The reference drug product is randomly applied to eight sites on one forearm, with skin stripping performed at incremental times after application (e.g., 15, 30, 60, and 180 minutes) (Fig. 1). One site is used for each time point. Four additional sites at 180 minutes on the same

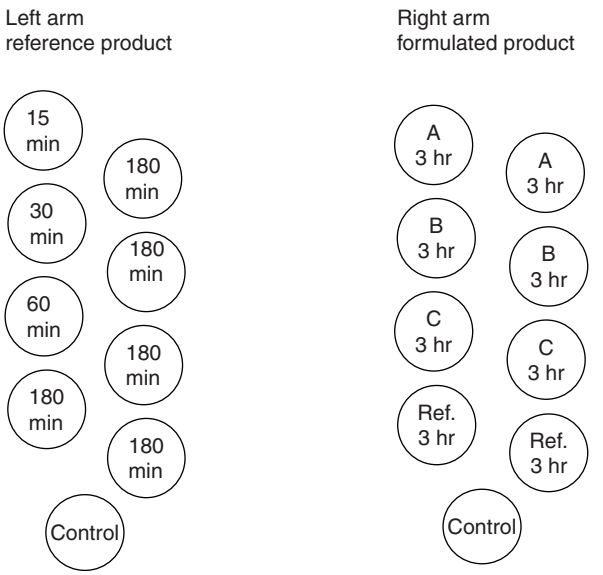

FIGURE 1 Schematic for drug application and removal sites for pilot study. A, B, and C represent three concentrations of the drug product or drug solution.

arm should be assessed to provide a total of five replicates for the same time point. An additional site with no application of a drug product should be sampled as a control, yielding a total of nine sampling sites. The contra-lateral forearm may be used to assess dose–response and sensitivity relationships by applying at least three concentrations of the drug product or simple drug solution for 180 minutes in duplicates. Two additional applications of the reference drug product on the same arm should be tested for 180 minutes as well to provide additional information about inter- and intra-arm variability and reproducibility. A control site with no drug application should also be included for a total of nine sites on the contralateral arm. The pilot study should be carried out in at least six subjects. Stratum corneum samples are removed according to procedures described below and analyzed for drug concentration. Standard procedures should be followed in all elements of the study and should be carried through all subsequent studies.

DPK BE Study Protocol

Protocol and Subject Selection

Healthy volunteers with no history of previous skin disease or atopic dermatitis and with a healthy, homogeneous forearm (or other) skin areas sufficient to accommodate at least eight treatment and measurement sites (time points) should be recruited. The number of subjects to be entered may be obtained from power calculations using intra- and intersubject variability from the pilot study. Because skin stripping is highly sensitive to specific study site factors, care should be taken to perfecting the technique and enrolling a sufficient number of subjects. The following study design is based on a crossover study design, where the crossover occurs at the same time using both arms of a single subject. A crossover design in which subjects are studied on two different occasions may also be employed. If this design is employed, at least 28 days should be allowed to rejuvenate the harvested stratum corneum.

Application and Removal of Test and Reference Products

The treatment areas are marked using a template without disturbing or injuring the stratum corneum/skin. The size of the treatment area will depend on multiple factors, including drug strength, analytical sensitivity, the extent of drug diffusion, and exposure time. The stratum corneum is highly sensitive to certain environmental factors. To avoid bias and to remain within the limits of experimental convenience and accuracy, the treatment sites and arms should be randomized. Uptake, steady-state, and elimination phases, as described

in more detail below, may be randomized between the right and left arms in a subject. Exposure time points in each phase may be randomized among various sites on each arm. The test and reference products for a particular exposure time point may be applied on adjacent sites to minimize differences. Test and reference products should be applied concurrently on the same subjects according to a SOP that has been previously developed and validated. The premarked sites are treated with predetermined amounts of the products (e.g., 5 mg/cm^2) and covered with a nonocclusive guard. Occlusion is used only if recommended in product labeling. Removal of the drug product is performed according to SOPs at the designated time points, using multiple cotton swabs or Q-tips with care to avoid stratum corneum damage. In case of certain oily preparations, such as ointments, washing the area with a mild soap may be needed before skin stripping. If washing is carried out, it should be part of an SOP.

Sites and Duration of Application

The BE study should include measurements of drug uptake into the stratum corneum and drug elimination from skin. Each of these elements is important to establish BA and/or BE of two products, and each may be affected by the excipients present in the product. A minimum of eight sites should be employed to assess uptake/elimination from each product. The time to reach steady state in the stratum corneum should be used to determine timing of samples. For example, if the drug reaches steady state in three hours, 0.25, 0.5, 1, and 3 hours post-treatment may be selected to determine uptake and 4, 6, 8, and 24 hours may be used to assess elimination. A zero time point (control site away from test sites) on each subject should be selected to provide baseline data. If the test/reference drug products are studied on both forearms, randomly selected sites on one arm may be designated to measure drug uptake/ steady state. Sites on the contralateral arm may then be designated to measure drug elimination. During drug uptake, both the excess drug removal and stratum corneum stripping times are the same so that the stratum corneum stripping immediately follows the removal of the excess drug. In the elimination phase, the excess drug is removed from the sites at the steady-state time point, and the stratum corneum is harvested at succeeding times over 24 hours to provide an estimate of an elimination phase (Fig. 2).

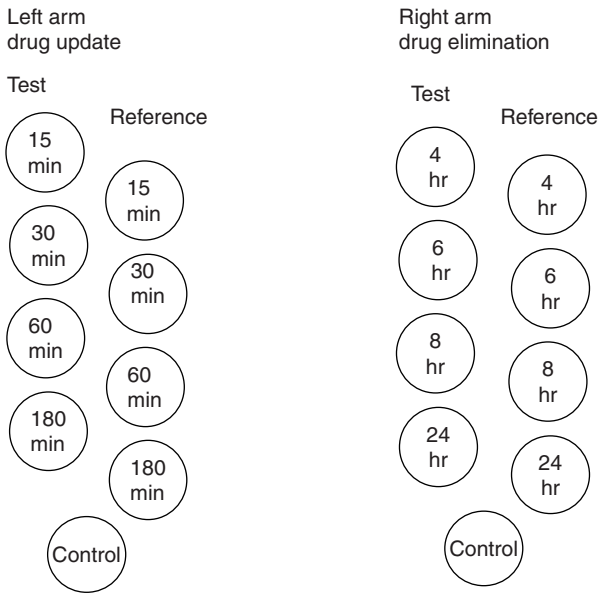

FIGURE 2 Schematic for drug uptake and drug elimination for bioequivalence study.

Collection of Sample

Skin-stripping proceeds first with the removal of the first one to two layers of stratum corneum with two adhesive tapes strip/disc applications, using a commercially available product (e.g., D-Squame, Transpore). These first two tape strip(s) contain the generally unabsorbed, as opposed to penetrated or absorbed, drug, and therefore should be analyzed separately from the rest of the tape-strips. The remaining stratum corneum layers from each site are stripped at the designated time intervals. This is achieved by stripping the site with an additional 10 adhesive tape strips. All 10 tape strips obtained from a given time point are combined and extracted, with drug content determined using a validated analytical method. The values are generally expressed as amounts/area (e.g., ng/cm) to maintain uniformity in reported values. Data may be computed to obtain full drug concentration–time profiles, $C_{max\text{-}ss}$, $T_{max\text{-}ss}$, and AUCs for the test and reference products.

Procedure for Skin Stripping

The general test procedures in either the pilot study or the pivotal BA/BE study are summarized below.

To assess drug uptake:

- Apply the test and/or reference drug products concurrently at multiple sites.
- After an appropriate interval, remove the excess drug from a specific site by wiping three times lightly with a tissue or cotton swab.
- Using information from the pilot study, determine the appropriate times of sample collection to assess drug uptake.
- Repeat the application of adhesive tape two times, using uniform pressure, discarding these first two tape strips.
- Continue stripping at the same site to collect 10 more stratum corneum samples.
- Care should be taken to avoid contamination with other sites.
- Repeat the procedure for each site at other designated time points.
- Extract the drug from the combined 10 skin strippings and determine the concentration using a validated analytical method.
- Express the results as amount of drug per square centimeter treatment area of the adhesive tape.

To assess drug elimination:

- Apply the test and reference drug product concurrently at multiple sites chosen based on the results of the pilot study. Allow sufficient exposure period to reach apparent steady-state level.
- Remove any excess drug from the skin surface as described previously, including the first two skin strippings.
- Collect skin stripping samples using 10 successive tape strips at time intervals based on the pilot study and analyze them for drug content.

Metrics and Statistical Analyses

A plot of stratum corneum drug concentration versus a time profile should be constructed to yield stratum corneum metrics of C_{max}, T_{max}, and AUC. The two one-sided hypotheses at the $p=0.05$ level of significance should be tested for AUC and C_{max} by constructing the 90% confidence interval (CI) for the ratio between the test and reference averages. Individual subject parameters, as well as summary statistics (average, standard deviation, and CV, 90% CI) should be reported. For the test product to be BE, the 90% CI for the ratio of means (population geometric means based on log-transformed data) of test and reference treatments should fall within 80% to 125% for AUC and 70% to 143% for C_{max}. Alternate approaches in the calculation of metrics and statistics are acceptable with justification.

Pharmacodynamic Approaches

Sometimes topically applied dermatological drug products produce direct/indirect PD responses that may be useful to measure BE. For example, topically applied corticosteroids produce a vasoconstrictor effect that results in skin blanching. This PD response has been correlated with corticosteroid potency and efficacy. Based on this PD response, the FDA issued a guidance entitled *Topical Dermatological Corticosteroids: In Vivo Bioequivalence* (June 1995). The guidance recommends that a pilot study be conducted to assess the dose–response characteristics of the corticosteroid followed by a formal study to assess/BE. Topically applied retinoid produces transepidermal water loss that may be used as a PD measure to assess BE.

In Vitro Release Approaches (Lower Strength)

Usually only one strength of a topical dermatological drug product is available although sometimes two or rarely, three strengths may be marketed. When multiple strengths are available, a standard practice is to create lower strengths by altering the percentage of active ingredients without otherwise changing the formulation or its manufacturing process. Topical dermatological drug products usually contain relatively small amounts of the active drug substance, usually $\leq 5\%$ and frequently $\leq 1\%$. In this setting, changes in the active ingredient may have little impact on the overall formulation.

Safety and efficacy should be documented for all strengths of topical drug products in the NDA submissions. Using some of the approaches suggested in this guidance, BA may also be documented for the highest strength. For lower strengths, where documentation of BA is considered important, this guidance suggests that in vitro release may be performed. Similarly, for an ANDA, when BE has been documented for the highest strength, in vitro release may also be used to waive in vivo studies to assess BE between these lower strengths and the corresponding strengths of the RLD. If this approach suggests bioinequivalence, further studies may be important.

To support the BE of lower strengths in an ANDA, the following conditions are important.

- Formulations of the two strengths should differ only in the concentration of the active ingredient and equivalent amount of the diluent.
- No differences should exist in manufacturing process and equipment between the two strengths.
- For an ANDA, the RLD should be marketed at both higher and lower strengths.
- For an ANDA, the higher strength of the test product should be BE to the higher strength of RLD.

In vitro drug release rate studies should be measured under the same test conditions for all strengths of both the test and the RLD products. The in vitro release rate should be compared between (*i*) the RLD at both the higher (RHS) and lower strengths (RLS); and (*ii*) the test (generic) products at both higher (THS) and lower strengths (TLS). Using the in vitro release rate, the following ratios and comparisons should be made:

$$\text{Release rate of RHS/Release rate of RLS} \approx \text{Release rate of THS/Release rate of TLS}$$

The ratio of the release rates of the two strengths of the test products should be about the same as the ratio of the release rate of reference products, that is:

$$\frac{\text{Release rate of RHS} \times \text{Release rate of TLS}}{\text{Release rate of RLS} \times \text{Release rate of THS}} \approx 1$$

Using appropriate statistical methods, the standard BE interval (80–120) for a lower strength comparison of test and reference products should be used.

After approval, a sponsor may wish to develop an intermediate strength of a topical dermatological drug product when two strengths have been approved and are in the marketplace. In this case, the in vitro release rate of the intermediate strength should fall between the in vitro release rates of the upper and lower strengths. Modifications of the approach described

in this section of the guidance can thus be applied, providing all strengths differ only in the amount of active ingredient and do not differ in manufacturing processes and equipment.

In Vitro Release: Extension of the Methodology

Drug release from semisolid formulations is a property of the dosage form. Current scientific consensus is that in vitro release is an acceptable regulatory measure to signal inequivalence in the presence of certain formulation and manufacturing changes. With suitable validation, in vitro release may be used to assess batch-to-batch quality, replacing a series of tests that in the aggregate assess product quality and drug release (e.g., particle size determination, viscosity, and rheology). Because topical dosage forms are complex dosage forms, manufacturers should optimize the in vitro release test procedure for their product in a manner analogous to the use of in vitro dissolution to assess the quality of extended release products from batch-to-batch. In addition, in vitro release might be used in a sponsor-specific comparability protocol to allow more extensive postapproval changes in formulation and/or manufacturing, provided that BE between two products representing the extremes of the formulation and manufacturing changes have been shown to be bioequivalent, using approaches recommended earlier in this document.

Systemic Exposure Studies

To ensure safety, and, when appropriate, comparable safety, information on systemic exposure is important for certain types of topical dermatological drug products, such as retinoid and high-potency corticosteroids. The degree of systemic exposure for the majority of topical dermatological drug products may be determined via standard in vivo blood, plasma, or urine PK techniques. For corticosteroids, an in vivo assessment of the HPA axis suppression test may provide the information. For other topical dermatological drug products, such tests may not be needed.

FED BE STUDIES

Food effect BA studies are usually conducted for new drugs and drug products during the IND period to assess the effects of food on the rate and extent of absorption of a drug when the drug product is administered shortly after a meal (fed conditions), as compared with administration under fasting conditions. Fed BE studies, on the other hand, are conducted for ANDAs to demonstrate their BE to the RLD under fed conditions. Food can influence the BE between the test and reference products. Food effects on BA can have clinically significant consequences. Food can alter BA by various means, including:

- Delay gastric emptying
- Stimulate bile flow
- Change GI pH
- Increase splanchnic blood flow
- Change luminal metabolism of a drug substance
- Physically or chemically interact with a dosage form or a drug substance

Food effects on BA are generally greatest when the drug product is administered shortly after a meal is ingested. The nutrient and caloric contents of the meal, the meal volume, and the meal temperature can cause physiological changes in the GI tract in a way that affects drug product transit time, luminal dissolution, drug permeability, and systemic availability. In general, meals that are high in total calories and fat content are more likely to affect the GI physiology and thereby result in a larger effect on the BA of a drug substance or drug product. It is recommended to use of high-calorie and high-fat meals during food-effect fed BE studies.

Food Effects on Drug Products

Administration of a drug product with food may change the BA by affecting either the drug substance or the drug product. In practice, it is difficult to determine the exact mechanism by which food changes the BA of a drug product without performing specific mechanistic studies.

Important food effects on BA are least likely to occur with many rapidly dissolving, immediate release drug products containing highly soluble and highly permeable drug substances (BCS Class I) because absorption of the drug substances in Class I is usually pH- and site-independent and thus insensitive to differences in dissolution. However, for some drugs in this class, food can influence BA when there is a high first-pass effect, extensive adsorption, complexation, or instability of the drug substance in the GI tract. In some cases, excipients or interactions between excipients and the food-induced changes in gut physiology can contribute to these food effects and influence the demonstration of BE. For rapidly dissolving formulations of BCS Class I drug substances, food can affect C_{max} and the time at which this occurs (T_{max}) by delaying gastric emptying and prolonging intestinal transit time. However, we expect the food effect on these measures to be similar for test and reference products in fed BE studies.

For other immediate release drug products (BCS Class II, III, and IV) and for all modified release drug products, food effects are most likely to result from a more complex combination of factors that influence the in vivo dissolution of the drug product and/or the absorption of the drug substance. In these cases, the relative direction and magnitude of food effects on formulation BA and the effects on the demonstration of BE are difficult, if not impossible, to predict without conducting a fed BE study.

Recommendations

For Immediate Release Drugs

- For uncomplicated drugs in immediate release dosage forms, BE must be demonstrated under fasted conditions. In addition to a BE study under fasting conditions, we recommend a BE study under fed conditions for all orally administered immediate release drug products, with the following exceptions.
- When both test product and RLD are rapidly dissolving, have similar dissolution profiles, and contain a drug substance with high solubility and high permeability (BCS Class I) or
- When the Dosage and Administration section of the RLD label states that the product should be taken only on an empty stomach or
- When the RLD label does not make any statements about the effect of food on absorption or administration.
- When the reference listed product label does not make any statements about the effect of food on absorption or administration.
- For complicated drugs in immediate release dosage forms, e.g., narrow therapeutic range drugs (drugs with a steep dose–response curve, critical drugs), highly toxic drugs and drugs known to have nonlinear PKss. BE must be demonstrated under both fasted and fed conditions.
- Nonlinear drugs. BE must be demonstrated under both fasted and fed conditions unless the nonlinearity occurs after the drug enters the systemic circulation and there is no evidence that the product exhibits a food effect.
- Drugs in modified release dosage forms. BE must be demonstrated under both fasted and fed conditions.

For Modified Release Products

In addition to a BE study under fasting conditions, a BE study under fed conditions should be conducted for all orally administered modified release drug products. It is recommended that food-effect BA and fed BE studies be conducted using meal conditions that are expected to provide the greatest effects on GI physiology so that systemic drug availability is maximally affected. A high-fat (approximately 50% of total caloric content of the meal) and high-calorie (approx. 800–1000 cal) meal is recommended as a test meal for food-effect BA and fed BE studies. This test meal should derive approximately 150, 250, and 500 to 600 cal from protein,

carbohydrate, and fat, respectively. The caloric breakdown of the test meal should be provided in the study report.

For fasting administration, following an overnight fast of at least 10 hours, subjects should be administered the drug product with 240 mL (8 oz of fluid) of water. No food should be allowed for at least four hours post-dose. Water may be allowed as desired, except one hour before and after drug administration. Subjects should receive standardized meals scheduled at the same time in each period of the study.

For fed administration, following an overnight fast of at least 10 hours, subjects should start the recommended meal 30 minutes prior to the administration of the drug product. Study subjects should eat this meal in 30 minutes or less; however, the drug product should be administered 30 minutes after start of the meal. The drug product should be administered with 240 mL (8 oz of fluid) of water. No food should be allowed for at least four hours post-dose. Water may be allowed as desired, except one hour before and after drug administration. Subjects should receive standardized meals scheduled at the same time in each period of the study.

Study Design

A sponsor may propose any study designs and data analyses. The scientific rationale and justification for these study designs and analyses should be provided in the study protocol. Sponsors may choose to conduct additional studies for a better understanding of the drug product and to provide optimal labeling statements for dosage and administration (e.g., different meals and different times of drug intake in relation to meals). In studying modified release dosage forms, consideration should be given to the possibility that coadministration with food can result in *dose dumping*, in which the complete dose may be more rapidly released from the dosage form than intended, creating a potential safety risk for the study subjects.

General Design

A randomized, balanced, singledose, two-treatment (fed vs. fasting), two-period, two-sequence crossover design is recommended for studying the effects of food on the BE of either an immediate or a modified release drug product. The formulation to be tested should be administered following a test meal (fed condition). The treatments should consist of both test and reference formulations administered following a test meal (fed condition). An adequate washout period should separate the two treatments in food-effect BE studies.

Subject Selection

Fed BE studies can be carried out in healthy volunteers drawn from the general population. Studies in the patient population are also appropriate if safety concerns preclude the enrollment of healthy subjects. A sufficient number of subjects should complete the study to achieve adequate power for a statistical assessment of food effects. A minimum of 12 subjects should complete the fed BE studies.

Dosage Strength

In general, the highest strength of a drug product intended to be marketed should be tested in fed BE studies. In some cases, clinical safety concerns can prevent the use of the highest strength and warrant the use of lower strengths of the dosage form. For ANDAs, the same lot and strength used in the fasting BE study should be tested in the fed BE study. For products with multiple strengths in ANDAs, if a fed BE study has been performed on the highest strength, BE determination of one or more lower strengths can be waived based on dissolution profile comparisons.

Test Meal

The fed BE studies be conducted using meal conditions that are expected to provide the greatest effects on GI physiology so that systemic drug availability is maximally affected. A high-fat (approx. 50% of total caloric content of the meal) and high-calorie (approx. 800–1000 cal) meal is

recommended as a test meal for food-effect BA and fed BE studies. This test meal should derive approximately 150, 250, and 500 to 600 cal from protein, carbohydrate, and fat, respectively. (An example test meal would be two eggs fried in butter, two strips of bacon, two slices of toast with butter, four ounces of hash brown potatoes, and eight ounces of whole milk.) Substitutions in this test meal can be made as long as the meal provides a similar amount of calories from protein, carbohydrate, and fat and has comparable meal volume and viscosity. The caloric breakdown of the test meal should be provided in the study report. If the caloric breakdown of the meal is significantly different from the one described above, the sponsor should provide a scientific rationale for this difference.

Administration

Fed Treatments

Following an overnight fast of at least 10 hours, subjects should start the recommended meal 30 minutes prior to administration of the drug product. Study subjects should eat this meal in 30 minutes or less; however, the drug product should be administered 30 minutes after start of the meal. The drug product should be administered with 240 mL (8 oz of fluid) of water. No food should be allowed for at least four hours post-dose. Water can be allowed as desired except for one hour before and after drug administration. Subjects should receive standardized meals scheduled at the same time in each period of the study.

Sample Collection

Timed samples in biological fluid, usually plasma, should be collected from the subjects to permit characterization of the complete shape of the plasma concentration–time profile for the parent drug. It may be advisable to measure other moieties in the plasma, such as active metabolites. Consideration should be given to the possibility that coadministration of a dosage form with food can alter the time course of plasma drug concentrations so that fasted and fed treatments can have different sample collection times.

Data Analysis and Labeling

The following exposure measures and PK parameters should be obtained from the resulting concentration–time curves for the test and reference products:

- Total exposure, or area under the concentration–time curve ($AUC_{0\text{-inf}}$, $AUC_{0\text{-}t}$)
- Peak exposure (C_{max})
- Time-to-peak exposure (T_{max})
- Lag-time (t_{lag}) for modified release products, if present
- Terminal elimination half-life
- Other relevant PK parameters

Individual subject measurements, as well as summary statistics (e.g., group averages, standard deviations, coefficients of variation) should be reported. An equivalence approach is recommended analyzing data using an average criterion. Log-transformation of exposure measurements (AUC and C_{max}) prior to analysis is recommended. The 90% CI for the ratio of population geometric means between test and reference products should be provided for $AUC_{0\text{-inf}}$, $AUC_{0\text{-}t}$, and C_{max}. For ANDA fed BE studies, the RLD administered under fed condition serves as the reference treatment.

For an ANDA, BE of a test product to the RLD product under fed conditions is concluded when the 90% CI for the ratio of population geometric means between test and RLD product, based on log-transformed data, is contained in the BE limits of 80% to 125% for AUC and C_{max}, respectively. Although no criterion applies to T_{max}, the T_{max} values for the test and reference products are expected to be comparable based on clinical relevance. The conclusion of BE under fed conditions indicates that with regard to food, the language in the package insert of the test product can be the same as the reference product.

Other Considerations

Sprinkles

In ANDAs, BE of the test to the RLD is demonstrated in a single dose crossover study. Both treatments should be sprinkled on one of the soft foods mentioned in the labeling, usually applesauce. The BE data should be analyzed using average BE and the 90% CI criteria should be used to declare BE. If there are questions about other foods, the design, or the analysis of such BE studies, the sponsors and/or applicants should contact the Office of Generic Drugs.

Special Vehicles

In ANDAs, BE of the test to the RLD is demonstrated in a single dose crossover study. Both treatments should be mixed with one of the beverages mentioned in the labeling. Sponsors should provide evidence that BE differences would not be expected from the use of other listed vehicles. The BE data should be analyzed using average BE, and the 90% CI criteria should be used to declare BE.

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2 Regulatory Aspects of Bioequivalence Testing

BACKGROUND

In vivo and/or in vitro bioequivalence (BE) testing is required for most generic drug products submitted for marketing approval. A proposed generic drug product must be compared in vivo and/or in vitro to the officially designated reference drug product. Harmonized BE criteria for the interchangeability of pharmaceutical products address the issue of waivers for in vivo trials, which are expensive and as recently concluded, not always discriminating enough to form the sole basis of approval of interchangeability. As discussed below, the worldwide requirements to demonstrate BE vary widely, mostly as a result of the ability of the regulatory authorities to enforce such requirements, both from an economic as well as ethical perspective. Waiver for BE testing therefore becomes a topic of great interest worldwide. Several consortiums have debated this topic for years and a consensus has begun to develop on this topic. A large number of policy documents address this topic and include the published Food and Drug Administration (FDA) and International Conference on Harmonization (ICH) guidelines; Health Canada's Guideline on Preparation of Drug Identification Number (DIN) Submissions; the World Health Organization (WHO) document (1999) entitled "Marketing Authorization of Pharmaceutical Products with Special Reference to Multisource (Generic) Products: A Manual for Drug Regulatory Authorities; Multisource (Generic) Pharmaceutical Products: Guidelines on Registration Requirements to Establish Interchangeability"; Note for Guidance on the Investigation of Bioavailability and Bioequivalence, Committee for Proprietary Medicinal Products (CPMP) (http://www.emea.eu.int/pdfs/human/ewp/140198en.pdf), 26 July 2001 (CPMP/EWP/QWP/98), Pan-American Network on Regulatory Harmonization: Bioavailability and Bioequivalence Working Group 2004.

Drug regulatory authorities must ensure that all pharmaceutical products, including generic drug products, conform to the same standards of quality, efficacy, and safety required of innovator drug products. Therefore, regulatory frameworks must be able to respond to varied and emerging drugs and dosage forms where BE demonstration is required; issues such as BE of topical products, products acting locally, endogenous therapeutic proteins, and, more recently, botanical products now need regulatory pathways, besides streamlining and reducing cost of evaluation of more traditional dosage forms where cost considerations, especially in the Third World, and often a lack of good correlation between in vivo studies and clinical response is observed. This chapter addresses these issues and provides a pathway for the prospective filers of marketing approval applications worldwide.

REGULATORY ASPECTS

The regulation of drug quality involves three arrangements in this country. First, the U.S. Congress gave the United States Pharmacopoeia (USP) and the National Formulary revision committees the authority to set standards of strength, quality, and purity of drugs and their finished preparations. The FDA, also authorized by the U.S. Congress, establishes regulations for the development and manufacture of safe and effective drugs. Finally, in house good manufacturing practices of the manufacturer, mostly dictated by the FDA regulations, assure the quality of drug products. The FDA has also decreed on the bioavailability and BE of drug products. All new drug applications (NDA) and amended new drug applications (ANDA)

TABLE 1 Data Requirement for Drug Approval in the United States

Application	FD&C 505(b)(1) NDA	FD&C 505(b)(2) NDA	FD&C 505(j) ANDA	PHS BLA
Preclinical	Yes	Yes/No	No	Yes
Clinical	Yes	Yes/No	No	Yes
CMC	Yes	Yes	Yes (PE)	Yes
PK & BE	Yes	Yes		Yes
Labelling	Yes	Yes	Yes	Yes

Abbreviations: ANDA, amended new drug applications; BE, bioequivalance; BLA, biological license application; NDA, new drug application; PHS, public health service; PK, pharmacokinetics.

must demonstrate in vivo bioavailability of the drug product that is followed by an in vitro test, usually a dissolution test, of individual batches to assure the quality. Table 1 shows a comparison of regulatory filing requirements under various applications.

EQUIVALENCE DOCUMENTATION FOR MARKETING AUTHORIZATION

Pharmaceutically equivalent multisource pharmaceutical products must be verified to be therapeutically equivalent to one another in order to be considered interchangeable. Several test methods are available to assess equivalence, including:

- Comparative bioavailability (BE) studies, in which the active drug substance or one or more metabolites are measured in an accessible biologic fluid such as plasma, blood, or urine
- Comparative pharmacodynamic (PD) studies in humans
- Comparative clinical trials
- In vitro dissolution tests in combination with the biopharmaceutics classification system (BCS) (see below).

Acceptance of any test procedure in the equivalence documentation between two pharmaceutical products by a drug regulatory authority depends on many factors, including characteristics of the active drug substance and the drug product and the availability of resources to carry out a specific type of study. Wherever a drug produces meaningful concentrations in an accessible biologic fluid, such as plasma, BE studies are preferred. Wherever a drug does not produce measurable concentrations in an accessible biologic fluid, comparative clinical trials or PD studies may be necessary to document equivalence. In vitro testing, preferably based on a documented in vitro/in vivo correlation or on consideration based on the BCS, may sometimes provide an indication of equivalence between two pharmaceutical products.

Oral Drugs/Drug Products for which In Vivo Equivalence Documentation Is Important: Regulatory authorities require equivalence documentation for multisource pharmaceutical products in which the product is compared to the reference pharmaceutical product. Studies must be carried out using the formulation proposed for marketing. For certain drugs and dosage forms, in vivo equivalence documentation, through either a BE study, a comparative clinical PD study, or a comparative clinical trial, is considered especially important. The following are the factors for oral drug products that should be considered when requiring in vivo equivalence documentation.

Immediate-release oral pharmaceutical products with systemic action when one or more of the following criteria apply:

1. Indicated for serious conditions requiring definite therapeutic response
2. Narrow therapeutic window/safety margin, steep dose–response curve
3. Pharmacokinetics (PK) complicated by variable or incomplete absorption or absorption window, nonlinear PK, presystemic elimination/high first-pass metabolism $>70\%$
4. Unfavorable physicochemical properties, e.g., low solubility, instability, metastable modifications, poor permeability

5. Documented evidence of bioavailability problems related to the drug or drugs of similar chemical structure or formulations
6. Where there is a high ratio of excipients to active ingredients

Nonoral and nonparenteral pharmaceutical products designed to act through systemic absorption (such as transdermal patches, suppositories, etc.): Plasma concentration measurements over time (BE) are normally sufficient proof for efficacy and safety.

Sustained or otherwise modified release pharmaceutical products designed to act through systemic absorption: Plasma concentration measurements over time (BE) are normally sufficient proof for efficacy and safety.

Fixed combination products (see WHO Technical Report Series No. 825, 1992) with systemic action: Plasma concentration measurements over time (BE) are normally sufficient proof for efficacy and safety.

Nonsolution pharmaceutical products for nonsystemic use (oral, nasal, ocular, dermal, rectal, vaginal, etc., application) and intended to act without systemic absorption: In these cases, the BE concept is not suitable and comparative clinical or PD studies are required to prove equivalence. This does not, however, exclude the potential need for drug concentration measurements in order to assess unintended partial absorption.

Clarification on Requirements

After the revision of the Note for Guidance (NfG) on the Investigation on Bioavailability and Bioequivalence in 2002 (http://www.emea.eu.int/pdfs/human/ewp/140198en.pdf), it appears that some harmonization in the interpretation of critical parts of the guideline is needed.

In Which Cases Is the Use of a Wider Acceptance Range for the Ratio of C_{max} Allowed?

The NfG states under 3.6.2 that "[w]ith respect to the ratio of C_{max} the 90% CI for this measure of relative bioavailability should lie within an acceptance range of 0.80 to 1.25. In specific cases, such as a narrow therapeutic range, the acceptance interval may need to be tightened."

The NfG also states that "[i]n certain cases a wider interval may be acceptable. The interval must be *prospectively defined*, e.g., 0.75 to 1.33, *and justified* addressing in particular any safety or efficacy concerns for patients switched between formulations."

The possibility offered here by the guideline to widen the acceptance range of 0.80 to 1.25 for the ratio of C_{max}, not for area under the curve values (AUC), should be considered exceptional and limited to a small widening (0.75–1.33). Furthermore, this possibility is restricted to those products for which at least one of the following criteria applies:

1. Data regarding *PK/PD relationships* for safety and efficacy are adequate to demonstrate that the proposed wider acceptance range for C_{max} does not affect PD in a clinically significant way.
2. If PK/PD data are either inconclusive or not available, clinical safety and efficacy data may still be used for the same purpose, but these data should be specific for the compound to be studied and persuasive.
3. The reference product has a highly variable within-subject bioavailability. Please refer to the question on highly variable drug or drug products for guidance on how to address this issue at the planning stage of the BE trial.

A post hoc justification of an acceptance range wider than defined in the protocol cannot be accepted. Information that would be required to justify results lying outside the conventional acceptance range at the post hoc stage should be utilized at the planning stage, either for a scientific justification of a wider acceptance range for C_{max}, or for selecting an experimental approach that allows the assessment of different sources of variability.

When Can Subjects Classified as Outliers Be Excluded from the Analysis in BE Studies?

Under 3.6.3, the NfG states that "[p]ost-hoc exclusion of outliers is generally not accepted," but at the same time acknowledges that "the protocol should also specify methods for identifying biologically implausible outliers."

Unbiased assessment of results from randomized studies requires that all subjects are observed and treated according to the same rules that should be independent from treatment or outcome. In consequence, PK data can only be excluded based on nonstatistical reasons that have been either defined previously in the protocol or, at the very least, established before reviewing the data. Acceptable explanations to exclude PK data or to exclude a subject would be protocol violations like vomiting, diarrhea, analytical failure, etc. The search for such explanations must apply to all subjects in all groups independently of the size of the observed PK parameters or its outlying position. Exclusion of data can never be accepted on the basis of statistical analysis or for PK reasons alone because it is impossible to distinguish between formulation effects and PK effects.

Exceptional reasons may justify post hoc data exclusion but this should be considered with utmost care. In such a case, the applicant must demonstrate that the condition stated to cause the deviation is present in the outlier(s) only and absence of this condition has been investigated using the same criteria for all other subjects.

Results of statistical analyses with and without the group of excluded subjects should be provided.

If One Side of the 90% CI of a PK Variable for Testing BE Lies on Either 0.80 or 1.25, Can We Conclude that the Products Are Bioequivalent?

For establishing BE, the 90% CI should lie *within* the acceptance interval (in most cases, 0.80–1.25), the borders being included. The conclusion that products are bioequivalent is based on the overall scientific assessment of the PK studies, not only on meeting the acceptance range.

In Which Cases May a Nonparametric Statistical Model Be Used?

The NfG states under 3.6.1—Statistical Analysis: "AUC and C_{max} should be analysed using ANOVA after log transformation."

The reasons for this request are the following:

1. The AUC and C_{max} values as biological parameters are usually not normally distributed.
2. A multiplicative model may be plausible.
3. After log transformation the distribution may allow a parametric analysis.

However, the true distribution in a PK data set usually cannot be characterized due to the small sample size, so it is *not recommended* to have the analysis strategy depend on a pretest for normality. Parametric testing using analysis of variance (ANOVA) on log-transformed data should be the rule. Results from nonparametric statistical methods or other statistical approaches are nevertheless welcome as sensitivity analyses. Such analyses can provide reassurance that conclusions from the experiment are robust against violations of the assumptions underlying the analysis strategy.

For t_{max}, the use of nonparametric methods on the original data set is recommended.

When Should Metabolite Data Be Used to Establish BE?

According to the guideline, the only situations where metabolite data *can be used* to establish BE are:

- *If the concentration of the active substance is too low to be accurately measured in the biological matrix, thus giving rise to significant variability.* Comments: Metabolite data can only be used if the applicant presents convincing, state-of-the art arguments that measurements of the parent compound are unreliable. Even so, it is important to point out that C_{max} of the metabolite is less sensitive to differences in the rate of absorption than C_{max} of the parent

drug. Therefore, when the rate of absorption is considered of clinical importance, BE should, if possible, be determined for C_{max} of the parent compound, if necessary at a higher dose. Furthermore, when using metabolite data as a substitute for parent drug concentrations, the applicant should present data supporting the view that the parent drug exposure will be reflected by metabolite exposure.

- *If metabolites significantly contribute to the net activity of an active substance* and *the pharmacokinetic system is non-linear*. Comments: To evaluate the significance of the contribution of metabolites, relative AUCs and nonclinical or clinical PD activities should be compared with those of the parent drug. PK/PD modeling may be useful. If criteria for significant contribution to activity and PK nonlinearity are met, then "it is necessary to measure both parent drug and active metabolite plasma concentrations and evaluate them separately." Any discrepancy between the results obtained with the parent compound and the metabolites should be discussed based on relative activities and AUCs. If the discrepancy lies in C_{max}, the results of the parent compound should usually prevail. Pooling of the plasma concentrations or PK parameters of the parent drug and its metabolite for calculation of BE is not acceptable.

When Using Metabolite Data to Establish BE, May One Use the Same Justification for Widening the C_{max} Acceptance Criteria as in the Case of the Parent Compound?

In principle, the same criteria apply as for the parent drug (see question on widening the acceptance range for C_{max}). However, as stated above (see question regarding when metabolite data can be used), C_{max} of the metabolite is less sensitive to differences in the rate of absorption than C_{max} of the parent drug. Therefore, widening the C_{max} acceptance range when using metabolites instead of the parent compound is generally not accepted. When the metabolite has a major contribution to, or is completely responsible for, the therapeutic effect, and if it can be demonstrated that a widened acceptance range would not lead to any safety or efficacy concerns, which will usually prove more difficult than for the parent compound (see question on widening the acceptance range for C_{max}), then a widened acceptance range for C_{max} of metabolite may be accepted.

What Is a "Highly Variable Drug or Drug Product"?

The standard approach to the analysis of a two-treatment, two-sequence, two-period crossover trial is ANOVA for the log-transformed PK parameters, where the factors formulation, period, sequence, and subject nested within sequence are used to explain overall variability in the observations. The residual coefficient of variation (CV) is a measure of the variability that is unexplained by the aforementioned factors. Among others, within-subject variability, formulation variability, analytical errors, and subject by formulation interaction can contribute to this residual variance.

A drug product is called highly variable if its intraindividual (i.e., within-subject) variability is greater than 30%. A high CV as estimated from the ANOVA model is thus an indicator for high within-subject variability. However, a replicate design is needed to assess within-subject variability.

When Testing for BE of a Product with a Nonlinear PK, How Should One Select the Strengths with the Largest Sensitivity to Detect Differences in the Two Products?

Section 5.4 of the Guideline states: "If a new application concerns several strengths of the active substance a bioequivalence study investigating only one strength may be acceptable," provided that five conditions are fulfilled, among which, when PK is not linear over the therapeutic dose range, "the strengths where the sensitivity is largest to identify differences in the two products should be used." Nonlinear PK, in this case, should reflect a nonlinear drug input rate as stated in the guideline.

Generally, it is the studied dose and not the studied formulation strength that is of importance when considering BE for drugs with nonlinear PK characteristics. An exception

is when bioavailability is governed by the solubility of the active ingredient. Then BE studies should include the highest formulation strength.

When studies are warranted at the high dose range, they should be performed at the highest commonly recommended dose. If this dose cannot be administered to volunteers, the study may need to be performed in patients. If the study is conducted at the highest acceptable dose in volunteers, the applicant should justify this and discuss how BE determined at this dose can be extrapolated to the highest commonly recommended dose.

When proof of linear absorption or elimination kinetics is lacking, or if evidence of nonlinearity is available, BE between test and reference formulations should be established with both the lowest and the highest doses unless adequately justified by the applicant. This approach is the most sensitive for detecting differences in rate and extent of absorption for substances with dose-dependent PK. On the other hand, if only one dose is chosen in the BE studies, which dose to choose depends on the cause of nonlinearity. For instance, single-strength studies may be conducted

- On the highest dose for drugs with a demonstrated greater than proportional increase in AUC or C_{max} with increasing dose during single or multiple dose studies. In this case, an additional steady state study may be needed if the drug accumulates (steady state concentrations are higher than those reached after single dose administration).
- On the lowest dose (or a dose in the linear range) for drugs with a demonstrated less than proportional increase in AUC or C_{max} with increasing dose, e.g., if this phenomenon is due to saturable absorption.

When bioavailability of a substance with nonlinear PK is governed by the solubility of the active substance, resulting in a less than proportional increase in AUC with increasing dose, BE should be established with both the lowest and the highest dose (which may exceed the recommended initial dose) and should include the highest formulation strength.

It is worth mentioning that in case of linear kinetics but low or critical solubility there is a similar need to test the highest strength and dose.

What Are the Conditions for Using Urinary PK Data for BE Assessment?

Section 3.3 of the Guideline states: "The use of urinary excretion data may be advantageous in determining the extent of drug input in case of products predominantly excreted renally, but has to be justified when used to estimate the rate of absorption."

The extent of drug input may be determined by the use of urinary excretion data provided elimination is dose-linear and is predominantly renal as intact drug. However, the use of urinary data has to be carefully justified when used to estimate the *rate of absorption*. If a reliable plasma C_{max} can be determined, this should be combined with urinary data on the extent of absorption for assessing BE.

Standardization of BE Studies with Regard to Food Intake: How Strictly Should the Guideline Be Interpreted?

Section 3.2.2 of the Guideline states: "If the Summary of Product Characteristics (SPC) of the reference product contains specific recommendations in relation to food intake related to food interaction the study should be designed accordingly."

The recommendations concerning food intake in the SPC are not sufficient for regulatory decisions on the adequacy of BE studies. Preferably, the following conditions should be considered separately when the SPC recommends administration of the substance together with food intake:

- If the recommendation of food intake in the SPC is based on PK properties such as higher bioavailability, then a BE study under fed conditions is generally required.
- If the recommendation of food intake is intended to decrease adverse events or to improve tolerability, a BE study under fasting conditions is considered acceptable although it would be advisable to perform the study under fed conditions.

- If the SPC leaves a choice between fasting and fed conditions, then BE should preferably be tested under fasting conditions as this situation will be more sensitive to differences in PK.

The composition of the meal should be described and taken into account, since a light meal might sometimes be preferable to mimic clinical conditions, especially when the fed state is expected to be less sensitive to differences in PK. However, for modified release products, a high-fat meal is required.

For products with release characteristics differing from conventional immediate release (e.g., improved release, dissolution, or absorption), even if they cannot be classified as modified release products with prolonged or delayed release, BE studies may be necessary in both the fasted and the fed states.

Worldwide Considerations

Although there is a general consensus among the West European, North American, and Japanese regulatory authorities on the BE requirements for marketing authorization of generic products, such is not the case in the rest of the world. For example, the varied nature of the requirement in South America perhaps typifies the heterogeneity in other continents. For example, an examination of the regulatory systems of the 10 South American agencies showed that out of the 96 active ingredients, only 4 active ingredients commonly require BE studies in all 10 countries: valproic acid, carbamazepine, cyclosporine, and phenytoin. All of them are considered high health risks. The countries with least number of active ingredient with BE study requirements are Colombia (only five) followed by Costa Rica (only seven) and the countries with the highest number of requirements remain the United States and Canada. Chile is in the process of establishing that requirement for all active ingredients that require BE studies. Although the WHO has established certain guidelines, these are not widely followed in much of the Third World countries and BE studies remain haphazardly managed. Following are some of the common occurrences in the marketing approvals of generic products in the Third World countries:

- Nonvalidated test methods
- Statistically incorrect experimental designs
- Lack of authenticity of study
- Lack of assurance that the study is conducted on the manufactured batches; the MNCs routinely submitting studies from their filings in the West in support of products to be manufactured locally

Risk-Based BE

Health Risk Categories

The selection of active ingredients for which BE studies should be required is a public health decision and as such should take into account the benefit/risk ratio of the same. This situation leads to the health risk concept, that is, which active ingredients require rigorous handling to prevent public health problems? One way of doing this is to take into account which active ingredients, because of their pharmacological characteristics, should be controlled through blood determinations.

Definition

As operational definition, the health risk concept should be established in the context of the problems of BE. For this purpose it would be reasonable to establish what are the health consequences when the drug is outside (under or above) the therapeutic window (the margin determined by the nontoxic maximum concentration and the effective minimum concentration). Thus, in relating the therapeutic window (the margin whose limits are the nontoxic maximum and effective minimum concentrations) and adverse effects of the drugs, three risk levels can be established, as described below.

High Health Risk
This is the probability of the appearance of threatening complications of the disease for the life or the psychophysical integrity of the person and/or serious adverse reactions (death, patient hospitalization, extension of the hospitalization, significant or persistent disability, disability, or threat of death), when the blood concentration of the active ingredient is not within the therapeutic window. For purposes of the selection, this risk level was assigned a score of 3 (three).

Intermediate Health Risk
This is the probability of the appearance of nonthreatening complications of the disease for the life or the psychophysical integrity of the person and/or adverse reactions, not necessarily serious, when the blood concentration of the active ingredient is not found within the therapeutic window. For purposes of the selection, this risk level was assigned a score of 2 (two).

Low Health Risk
This is the probability of the appearance of a minor complication of the disease and/or mild adverse reactions, when the blood concentration of the active ingredient is not within the therapeutic window. For purposes of the selection, this risk level was assigned a score of 1 (one).

While there are other factors to be considered such as the physicochemical and PK parameters, from the standpoint of public health the most important element to take into account is the health risk. Table 2 lists the active ingredients classified in accordance with their health risk and the established scores.

Typical Examples of Complex BE

Digoxin
Digoxin in tablet form is not listed in the Orange Book, since this is a "grandfathered" dosage form of digoxin. Since the tablet formulation of digoxin was established in clinical use before passage of the federal Food, Drug, and Cosmetic Act of 1938, generic versions of digoxin tablets may be marketed without an approved ANDA. Data showing BE of generic digoxin tablet products to the innovator product Lanoxin® are generally not available or forthcoming, so that comparable rate and extent of absorption between generic products and Lanoxin brand tablets, or between different generic products, is not ensured. Seventeen generic digoxin tablets (0.25 mg) have been listed as currently marketed, though some of these may be marketed by suppliers or distributors of another manufacturer's product. Without PK data to verify the BE of these products to Lanoxin, the clinical responses (both therapeutic and toxic) from these generic products compared with Lanoxin are unpredictable. This inability to guarantee therapeutic equivalence to a reference product opposes the entire premise of generic substitution: the practitioner should expect the same responses (no more, no less) from a therapeutically equivalent generic product. Consequently, generic substitution is not advised. Use of a generic digoxin product as initial therapy may result in lower or higher than expected bioavailability, requiring additional monitoring and dosage adjustment, and ultimately increasing costs of therapy far above the cost savings from a less expensive generic product.

Levothyroxine
Levothyroxine sodium tablets are also currently not listed in the Orange Book. In the words of the FDA, levothyroxine sodium was first introduced into the market before 1962 without an approved NDA, apparently in the belief that it was not a new drug. The lack of BE data of generic preparations to the two major brand name products Synthroid® and Levothroid® has been noted, along with the adoption in 1984 of USP guidelines for potency of levothyroxine sodium tablets. However, between 1987 and 1994, a total of 58 adverse drug experience reports with levothyroxine sodium tablets were received by the FDA, with 47 of the incidences apparently related to subpotency and nine incidences related to superpotency. These adverse events were caused by not only switching product brands but also inconsistencies

TABLE 2 Classification of Active Ingredients According to Their Health Risk

Active ingredient	Health risk	Active ingredient	Health risk
Acetazolamide	1	Haloperidol	2
Allopurinol	1	Hydrochlorothiazide	2
Calcium folinate	1	Indometacin	2
Captopril	1	Isoniazid	2
Clomifene	1	Ketoconazole	2
Cloxacillin	1	Levodopa + Inhib. DOPA decarboxylase (DDC)	2
Dexamethasone	1	Levonorgestrel	2
Diazepam	1	Levotiroxina	2
Folic acid + ferrous sulfate	1	6-Mercaptopurine	2
Ibuprofen	1	Methotrexate	2
Isosorbide dinitrate	1	Methyldopa	2
Levamisole	1	Metoclopramide	2
Mebendazole	1	Metronidazole	2
Mefloquine	1	Nitrofurantoin	2
Nalidixic acid	1	Norestisterona	2
Niclosamide	1	Oxamniquine	2
Nifedipine	1	Paracetamol	2
Nystatin	1	Penicillamine	2
Phenoxymethylpenicillin	1	Piperazine	2
Phytomenadione	1	Piridostigmina	2
Pirantelo	1	Procarbazine	2
Praziquantel	1	Promethazine	2
Pyrazinamide	1	Propranolol	2
Sulfasalazine	1	Propylthiouracil	2
Amiloride	2	Pyrimethamine	2
Amitriptyline	2	Quinine	2
Amoxicillin	2	Rifampicin	2
Atenolol	2	Salbutamol, sulfate	2
Azathioprine	2	Spironolactone	2
Biperiden	2	Tamoxifen	2
Chloramphenicol	2	Tetracycline	2
Cimetidine	2	Carbamazepine	3
Ciprofloxacin	2	Cyclosporine	3
Clofazimine	2	Digoxin	3
Clomipramine	2	Ethambutol	3
Clorpromazine	2	Ethosuximide	3
Co-Trimoxazole	2	Griseofulvin	3
Cyclophosphamide	2	Lithium carbonate	3
Dapsone	2	Oxcarbazepine	3
Diethylcarbamazine	2	Phenytoin	3
Doxycycline	2	Procainamide	3
Erythromycin	2	Quinidine	3
Ethinylestradiol	2	Theophylline	3
Etoposide	2	Tolbutamide	3
Flucytosine	2	Valproic acid	3
Fludrocortisone	2	Verapamil	3
Furosemide	2	Warfarin	3

in bioavailability between different lots from the same source. BE issues regarding levothyroxine sodium tablets were highlighted when the results of a BE study comparing the innovator product Synthroid with several generic brands finally appeared in the literature. The study sponsor (the marketer of Synthroid) attempted to prevent publication of these results, which claimed BE of Synthroid to three other levothyroxine sodium products. After publication of these study results, advertisements appeared in journals and trade magazines advocating the substitution of other brand-name levothyroxine sodium products (e.g., Levothroid, Levoxyl®) for Synthroid. In addition, statements were made such as, "Feel comfortable using Levothroid, Levoxyl, or Synthroid in hypothyroid patients. These three are bioequivalent … even though they're not AB-rated."

Several points should be considered before routinely switching marketed brands of levothyroxine sodium tablets (at least 24 products for the 0.1 mg tablet are listed). First, although the conclusions stated in the peer-reviewed BE study cited appear to be generally accepted, the results of this study were not subjected to the scrutiny of the FDA review process. In view of significant stability and potency problems, the FDA has issued a Federal Register notice stating that (*i*) orally administered levothyroxine sodium products are now considered new drugs, and (*ii*) manufacturers who intend to continue marketing these products must submit an NDA within three years to obtain approval. Recently, the FDA extended this deadline for an additional year. Second, the impression that all levothyroxine sodium tablet formulations are likely to be bioequivalent is not currently supported with FDA-substantiated BE data; routine substitution of these products for refills of existing prescriptions is not advisable until FDA review is complete. Third, practitioners must always comply with the substitution laws in their individual states. If a statute mandates substitution of a therapeutically equivalent or bioequivalent product, reliance upon data reported in the scientific literature may not always guarantee these requirements will be satisfied.

Warfarin Sodium

Three approved generic versions of warfarin sodium tablets (seven strengths) are currently listed in the Orange Book. Before approval of these generic warfarin sodium products, several states either enacted or were considering legislation to require pharmacists to obtain prescriber and patient approval for generic substitution of drugs with a narrow therapeutic index (NTI). In response, the FDA issued a position statement. The FDA's position is clear with regard to the issue of tightening confidence intervals (CIs) and changing study designs for BE determinations of NTI drugs: the present requirements to prove BE, at least in the United States and Canada, are already so difficult and constrained that there is no possibility, even for narrow therapeutic index drugs, that dosage forms meeting the criteria could lead to therapeutic problems. Drugs approved through the NDA process with NTIs, by definition, must have low intrasubject variability. Otherwise, patients would have cycles of toxicity and lack of efficacy, and therapeutic drug monitoring would be useless. The low intrasubject variability associated with NTI drugs ensures that patient response to a specific drug should be consistent, and the statistical criteria required by the FDA for BE appear more than adequate for confidence in generic substitution. This is especially true in light of the notable absence of data that prove otherwise. For the most part, the arguments against generic substitution of NTI drugs appear to be based on economic considerations. Commentaries debating the suitability of generic warfarin products have focused on the results from reports of clinical studies with generic warfarin and the content uniformity requirements for warfarin sodium tablets. As indicated in a letter addressing these issues, no convincing and substantiated scientific data have been published showing bioinequivalence of generic warfarin products or product failure of these products in clinical studies. Recently, an evidence-based medicine approach was used to compare the results reported with Coumadin® and a generic warfarin product in clinical studies. No significant differences were found in the international normalized ratio (INR), number of dosage changes to adjust INR in range, or number of hospitalizations or incidences of bleeding between the reference and generic warfarin products. Physicians may sometimes encounter difficulties in maintaining stabilized INR in patients anticoagulated with warfarin since multiple drug interactions and patient variables affect warfarin levels and create difficulty in achieving consistently therapeutic INR values. However, factors such as diet, concurrent illnesses, interacting drugs, and noncompliance are *intersubject* variables that are unrelated to the BE issue. For crossover studies using log-transformed data, it is largely the within-subject distribution of values (*intrasubject* variability) that determines the validity and efficiency of the standard parametric methods of analysis. For NTI drugs such as warfarin, intrasubject variability, by definition, is low and the available clinical data indicate that lack of BE does not appear to be the explanation for problems experienced during warfarin therapy. Another article introduces the concept of "switchability," that is, the substitution of one approved generic product for another generic product. BE studies submitted to the FDA through an ANDA are conducted by comparing data from the proposed generic product and a reference product. The reference product is selected by the FDA and is typically either the innovator

or pioneer product that was originally introduced into the market. Suppose approved generic product A differed from the reference product in at least one parameter (e.g., mean AUC values) by +4%, and that approved generic product B differed from the reference product by −4%. The net difference of generic products A and B would then be 8%; could this magnitude of difference result in bioinequivalence and lack of equivalent therapeutic response for an NTI drug? No data were presented from any clinical studies that could support the contention that switchability for NTI drugs is problematic. Rather, phrases such as "… with NTI drugs, small variations in bioavailability can potentially pose problems," and conceptual arguments are used to suggest the need for special BE criteria to be applied to NTI drugs. Reference is made to the FDA's draft guidance for population and individual BE studies, which proposes the use of reference scaling (essentially, modifying the BE criteria to account for the variability of the reference product) for NTI drugs, regardless of the intrasubject variability of the reference product. Since NTI drugs have low intrasubject variability as discussed, this approach would likely result in narrower CI requirements. Finally, a recent report further confirms the BE of generic warfarin to the innovator product. More than 100 subjects anticoagulated with Coumadin were switched to a generic warfarin product for eight weeks in a nonrandomized comparative clinical observational study. The overall conclusion was that the variability in INR in patients receiving generic warfarin was not statistically significant from that seen in the control group receiving Coumadin. These investigators identified associated factors not related to the product change in subjects whose INR varied by >1.0 from baseline. This further emphasizes the critical role of interpatient factors (physical activity, dietary vitamin K, noncompliance, drug interactions, congestive heart failure, diarrhea, alcohol consumption) affecting the anticoagulant response with warfarin.

Albuterol Metered-Dose Inhalers

Four approved generic versions of albuterol metered-dose inhalers are currently listed in the Orange Book as therapeutically equivalent (AB-rated) to the reference product Ventolin®. The Proventil® product is rated BN, or not therapeutically equivalent to Ventolin or the four generic products. For products administered by metered-dose inhalation and intended for local therapeutic effects, the typical PK methods for evaluating BE cannot be used. Rather, an approach based on acute PD response is recommended with asthmatic patients as subjects. The statistical criteria and appropriate CIs for BE determination are not as rigidly defined for PD methods as for PK methods. Consequently, variability in patient response may be of slightly greater concern, since albuterol metered-dose inhalers are used as "rescue inhalers" for nocturnal asthma attacks (even though they are not considered NTI drugs). However, the FDA is satisfied that these products will produce equivalent therapeutic responses.

BE SURROGATES

It is not always possible or necessary to utilize in vivo human data in the evaluation of drug bioavailability. The large number of physicochemical, physiologic, and pharmaceutic factors that affect absorption of drugs need to be studied in detail and thus models have to be designed to study the effect of many of these factors with aim to optimize absorption. These models are primarily classified as in vitro, in situ, and in vivo.

In Vitro Systems

Several systems have been developed to simulate absorption of drugs across biologic membranes, especially gastrointestinal membranes in vitro. These models pertain to either the membrane permeability aspect or the release of drug from dosage forms.

Disintegration of Dosage Form

It is one of the most widely used tests for the release of drugs from dosage forms. In most situations, poor correlations are found between disintegration and absorption of drugs. Since there are several independent steps involved in the absorption of drugs, this test alone does not

assure proper release and absorption characteristics. The official disintegration test is performed by placing one tablet each in the six tubes placed in a basket which is moved up and down in the immersion fluid at frequency rate between 28 and 32 cycles per minutes through a distance of not less than 5 cm and not more than 6 cm. For uncoated, buccal, and sublingual tablets the immersion fluid is water at 37°C. However, for the latter two types of tablets, the disc placed on top of the tablets is omitted. For coated tablets, the immersion fluid is gastric fluid for 30 minutes followed by intestinal fluid. If one or two tablets fail to disintegrate within the specified time then the test is repeated on 12 additional tablets; not less than 16 out of the total of 18 tablets must disintegrate completely. The most common disintegration requirement is 30 minutes for uncoated tablets.

Dissolution Tests

Dissolution testing is performed not only on the finished products but also on the pure drug and in combination with various excipients to ascertain individual contributions of the components to overall dissolution. Basically, the dissolution test systems are of two types: the stirred-vessel type and the flow-through column. In the stirred-type, agitation is provided by some kind of paddle whereas in the column type the solvent flows over the drug. A large number of variations of these systems are currently used. However, the USP apparatus is used for official certification of batches. The monographs describe the specific temperature, the dissolution medium (distilled water, simulated gastric fluid, or simulated intestinal fluid), the rotation speed of the basket (60–150 rpm), and the percentage of drug to be dissolved as an endpoint. These conditions are determined by the intrinsic properties of the drug and its dissolution behavior. Some key examples include: acetohexamide, nitrofurantoin, digoxin, phenylbutazone, ergotamine tartarate and caffeine tablets, prednisolone, hydrochlorothiazide, prednisone, lithium carbonate, sulfamethoxazole, meprobamate, sulfisoxazole, methaqualone, theophyllin, ephedrine hydrochloride and phenobarbital, methylprednisolone tablets, and tolbutamide.

In the official dissolution tests, 6 or 12 tablets or capsules are tested individually for their dissolution properties. In the first stage, 6 U are tested and each unit must fall to within less than 5% of the specified limit (e.g., 60% dissolved in 30 minutes). If one or more units fail then another 6 U are tested and the average of 12 U (six from first test) should be equal to or greater than the specified percentage and no unit should be less than 15% of the specified limit. If this stage also fails then additional 12 U are tested and the average of all (now 24) should be equal to or greater than the specified limit and not more than 2 U can be less than 15% off the limit.

An inherent problem in this type of testing is that it requires the use of labeled amount of drug for calculation purposes and any content variability is not considered. Conceivably, large variations in the dissolution rates are possible due to these differences, e.g., tablets containing 80% to 120% of the labeled amount will require 75% to 50% dissolution if the requirement is 60%. In addition, the statistical design of the dissolution testing allows a batch with 20% defective tablets to pass 58% of the time. There are also serious problems in the reproducibility of dissolution data since the dissolution is dependent on human errors and such subtle factors as the vibrations in the room. Despite these drawbacks, the FDA considers dissolution testing to be the most discriminating in vitro test with which to establish in vivo correlations.

Everted Intestinal Sac

Although disintegration and dissolution tests characterize the release characteristics of the products, the transport across biologic membrane is studied by everted intestine method. The procedure involves isolating a small segment of the intestine of a laboratory animal such as hamster, guinea pig, rat, etc., everting the segment, filling the sac, ligating it at both ends after filling it with a small volume of buffer. The ligated intestinal sac is incubated in oxygenated buffer solution containing drug at 37°C. The eversion of intestine helps expose the mucosal side to oxygenation, whereas the small volume on the serosal side allows analysis of low concentration of drugs. Several modifications of this concept are in use include the one which allows multiple sample of the serosal side or replacement of membrane during experiment. The permeability characteristic is expressed in terms of the lag time before any

drug appears on the serosal side and the cumulative amount of drug transferred in 60 min/U concentration of the drug in mucosal solution. A large lag time or small transport characterizes drugs where absorption may be limited by the transport process itself. When these data are coupled with release characteristics such as dissolution test, more useful information can be obtained regarding possible hindrance in the absorption of drugs.

The everted sac method can also be utilized to study the effect of various formulation additives on absorption of the active drug. Several such examples have been reported including reduced absorption of chloramphenicol by adjuvants, increase absorption rates of drugs due to *N*-methyl glucamin, effect of surfactants on the permeability of soluble corticosteroids, effect of physiologic surfactants on the permeability of salicylates and the effects of complex formation on the permeability of salicylamide. Other factors studied include differentiation between active and passive transport, potential binding of drugs to the intestinal wall, gasrointestinal metabolism, and the effects of electrolytes and sugars on membrane permeability of drugs. The gastrointestinal metabolism of drugs has also been studied by using everted intestinal ring and slices.

Isolated Perfused Liver

Many drugs undergo metabolism in liver before reaching the general circulation and thus the bioavailability is reduced. In order, to estimate the extent of the loss of drug through this first-pass effect, drug solution is perfused through the liver which is maintained in physiologically active state by oxygenation and providing nutrients. Excellent in vitro–in vivo correlation for drug metabolism has been obtained using this technique. Some examples of drugs where such experiments have been of great value include aspirin, salicylamide, propranolol, acetaminophen, and phencetin.

In Situ Methods

The in situ system better represents the in vivo systems since the blood supply to the absorption organs is maintained. It involves ligating segments of the gastrointestinal tracts and perfusing drug solutions through the segment and recording the amount of drug lost as a function of time. Several procedures including that described by Levine, Schanker, Dolusio et al. are widely used. These models are useful in characterizing the transport characteristics.

In Vivo Systems

LD-50 Comparisons

Comparison of LD-50 with intravenous route of administration or within a group of formulations provides a useful tool in determining bioavailability. The advantages include quick results and no analysis of blood samples. This technique can be used to monitor both the rate and the extent of absorption.

Thiry-Vella Loop

For chronic studies, a loop of small intestine is isolated and exteriorized with intact blood, nerve, and lymph supplies. With access to the proximal and distal ends of the intestinal segment, repeated use of the loop can be made for bioavailability studies. This procedure, however, requires larger species such as dogs and offers a distinct advantage of running intravenous and gastrointestinal absorption studies in same animals.

Hepatobiliary Cannulation

Catheterization of hepatoportal vein to the outside allows direct administration of drug into the hepatic system, assuring 100% passage through the liver prior to entrance into the general circulation. This allows quick estimation whether the bioavailability problem is due to gastrointestinal factors or to postabsorption factors. For example, in the case of drugs like lidocaine, the blood levels following portal administration are significantly lower than when administered intravenously indicating a higher degree of metabolism in the first pass through the liver. Cannulation of bile duct in vivo is of value in determining

the extent of drug excreted in the gastrointestinal tract and thus allows evaluation of degree of recirculation of the drug. A large number of polar compounds and compounds of high molecular weight are excreted in bile including digoxin, diazepam, pivampicillin, ampicillin, nitrofurantion, dicotyl sodium sulfosuccinate, erythyromycin, tetracyclines, fluphenazine, etc.

Choice of Animal Species

In the in vivo models suggested above, the choice of animal species depends on the factors including similarity of gastrointestinal anatomy and physiology to humans. For example, cattle have a very different system of food and drug digestion and transport and thus it will not be a good choice. A good model is a dog wherein, like humans, no continuous secretion of hydrochloric acid and bile is recorded. It should be possible to administer the given dosage form. Many small species such as rats, mice, and hamsters cannot be used since it will not be possible to administer a full-size capsule or tablet to them. It should also be possible to obtain period biologic samples such as blood or urine. Again, small species offer significant problem in this respect. Miniature pig seems to be an ideal species for this purpose.

Disposition kinetic characteristics should be as close to humans as possible. This is probably the most difficult factor to control. However, some species can be totally ruled out depending on the specific drug example if they metabolize, excrete or distribute the drug differently. Also, if a species shows selective absorption of a drug in contrast to humans, it should also be ruled out. Monkey and other primates seem to meet many of these requirements. However, it is generally difficult to obtain primates for studies and cost a lot more too, a factor of great importance in the initial screening of the drugs and dosage forms.

Absorption Profiling

The following are factors and oral drugs/drug products that should be considered when requesting a waiver of evidence of in vivo bioavailability or BE documentation. Generally, both in vivo and in vitro testing are necessary for orally administered drug products. In vivo testing is required for all generic drug products with certain exceptions. Based on scientific information regulatory authorities may waive the requirement for bioavailability or BE.

1. For certain formulations and under certain circumstances, equivalence between two pharmaceutical products may be considered self-evident and no further documentation is required. For example
   a. When multisource pharmaceutical or generic products are to be administered parenterally (e.g., intravenous, intramuscular, subcutaneous, intrathecal administration) as aqueous solutions and contain the same active substance(s) in the same concentration and the same excipients in comparable concentrations.
   b. When multisource pharmaceutical or generic products are solutions for oral use, contain the active substance in the same concentration, and do not contain an excipient that is known or suspected to affect gastro-intestinal transit or absorption of the active substance.
   c. Gas-based multisource pharmaceutical or generic products.
   d. When the multisource pharmaceutical or generic products are powders for reconstitution as a solution and the solution meets either criterion (*i*) or criterion (*ii*) above.
   e. When multisource pharmaceutical or generic products are otic or ophthalmic products prepared as aqueous solutions, containing the same active substance(s) in the same concentration and essentially the same excipients in comparable concentrations.
   f. When multisource pharmaceutical or generic products are topical products prepared as aqueous solutions, containing the same active substance(s) in the same concentration and essentially the same excipients in comparable concentrations.
   g. When multisource pharmaceutical or generic products are inhalation or nasal spray products, tested to be administered with or without essentially the same device, prepared as aqueous solutions, and containing the same active substance(s) in the same concentration and essentially the same excipients in comparable concentrations.

Special in vitro testing should be required to document comparable device performance of the multisource inhalation product.

h. For elements (e), (f) and (g) above, it is incumbent upon the applicant to demonstrate that the excipients in the multisource product are essentially the same and in comparable concentrations as those in the reference product.

2. In the event the applicant cannot provide this information about the reference product and the drug regulatory authority does not have access to these data or the data is protected under data exclusivity rights according to local regulations, in vivo studies should be performed.
3. For certain drug products, bioavailability or BE may be demonstrated by evidence obtained in vitro in lieu of in vivo data. Regulatory authorities should waive the requirement for the submission of evidence obtained in vivo demonstrating the bioavailability of the drug product if the drug product meets one of the following criteria:
   a. The drug product is in the same dosage form, but in a different strength, and is proportionally similar in its active and inactive ingredients to another drug product manufactured at the same site for which the same manufacturer has obtained approval and the following conditions are met:
   b. The bioavailability of this other drug product has been demonstrated;
   c. Both drug products meet an appropriate in vitro test approved by a drug regulatory authority and/or accepted reference pharmacopeias, or has demonstrated in vivo–in vitro correlation (e.g., correlation level A).
   d. The applicant submits evidence showing that both drug products are proportionally similar in their active and inactive ingredients. That is, the ratio of active ingredients and excipients between strengths is essentially the same.
   e. The drug product is a reformulated product that is identical, except for a different color, flavor, or preservative that could not affect the bioavailability of the reformulated product, to another drug product for which the same manufacturer has obtained approval and the following conditions are met:
   f. The bioavailability of the other product has been demonstrated;
   g. Both drug products meet an appropriate in vitro test approved by the regulatory authority.
   h. Regulatory authorities, for good cause, may require evidence of in vivo bioavailability or BE for any drug product if the agency determines that any difference between the drug product and a listed drug may affect the bioavailability or BE of the drug product. The Bioavailability and BE Working Group strongly recommends that in the case of anti-retroviral drug products proof of pharmaceutical equivalence and BE be required to infer therapeutic equivalence.

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3 Pharmacokinetic and Pharmacodynamic Modeling

BACKGROUND

In the process of pharmacokinetic and pharmacodynamic (PK/PD) modeling, it is important to describe, prospectively, the objectives of the modeling, the study design, and the available PK and PD data. The assumptions of the model can be related to dose–response, PK, PD, or one or more of the assumptions listed in Table 1.

The assumptions can be based on previous data or on the results of any available current analysis. What constitutes an appropriate model depends on the mechanism of the drug's action, the assumptions made, and the intended use of the model in decision-making. If the assumptions do not lead to a mechanistic model, an empirical model can be selected, in which case, validating the model's predictability becomes especially important. [Note that nonmechanistic models do not get good reviews from the Food and Drug Administration (FDA).] The model selection process comprises a series of trial-and-error steps in which different model structures or newly added or dropped components to an existing model can be assessed by visual inspection and can be tested using one of several objective criteria. New assumptions can be added when emerging data justify it.

PK MODELING STUDIES

Compartment PK Modeling

PK is the study of the movement of drug molecules in the body, requiring appropriate differential calculus equations to study various rates and processes. The rate of elimination of a drug is described as being dependent on, or proportional to, the amount of drug remaining to be eliminated, a process that obeys first-order kinetics. The rate of elimination can, therefore, be described as:

$$\frac{dX}{dt} = -kX \tag{1}$$

where k is a mere proportionality constant or a rate constant and X the amount remaining to be eliminated (and therefore X_0 is the initial amount or the dose administered). Integration allows converting equation (1) to:

$$X = X_0 e^{k_{el}t} \tag{2}$$

As the amount X is proportional to the concentration, a similar equation describes the time-decay profile of the drug concentration instead of the amount:

$$Cp^t = Cp^0 e^{-k_{el}t} \tag{3}$$

This simple, first-order relationship allows a linear association between the log (more appropriately, the natural logarithm) of concentration and time. It is noteworthy that this concentration is the "effective" concentration, not necessarily the measured concentration. "Effective" refers to a thermodynamic activity rather than the physical concentration. Drugs

TABLE 1 Assumptions in PK and PD Modeling

The mechanism of the drug actions for efficacy and adverse effects	Presence or absence of active metabolites and their contribution to clinical effects
Development of tolerance or absence of tolerance	Immediate or cumulative clinical effects
Disease state progression	Drug-induced inhibition or induction of PK processes
Circadian variations in basal conditions	Response in a placebo group
Absence or presence of an effect compartment	Influential covariates
The PK model of absorption and disposition and the parameters to be estimated	The PD model of effect and the parameters to be estimated
Inclusion or exclusion of specific patient data	Distributions of intra- and interindividual variability in parameters
Distribution of PK and PD measures and parameters	

Abbreviations: PK, pharmacokinetics; PD, pharmacodynamics.

decay in proportion to the concentration of "free" drug molecules, and whatever is bound to proteins may not be available for disposition. This extrapolation becomes more complex when we take into account other factors that might alter the "activity" (in a thermodynamic sense) of the drug in a biologic fluid. For example, structuring of water inside protoplasm imparts lipophilic characteristics, which create significant differences in available concentration gradients. This is a primary reason why it is not always possible to correlate measured concentrations with pharmacologic responses because the level of drug at the site of action or at the receptors depends highly on the thermodynamic activity of the drug, which is difficult to assess.

The relationship between the amount of drug and its concentration is classically represented by the following equation, which functions as if there was a physical space (called distribution volume) throughout, which the drug distributes evenly:

$$V = \frac{\text{amount of drug in the body}}{\text{concentration measured in plasma}} \tag{4}$$

The above relationship is an oversimplification of the distribution characteristics of drug molecules in the body and can provide results in volumes often much larger than the body weight. For example, if a drug, like digoxin or diazepam, was selectively stored in different parts of body, the apparent distribution volumes, using equation (4), would be several multiples of the body's weight. As the distribution of a drug is a time-dependent process, even within the same "compartment," the author suggested that this parameter be treated as a time-dependent variable; treating a "bolus" dose as a short-term infusion, which improves the results of the deconvolution of integrated equations. That assumption allows a more accurate physical representation of the PK models because an "instantaneous" IV injection is treated as a very short duration, zero-order input function. As we shall see, this consideration is more important as we integrate PD models where the action and effect of the drug is delayed for several reasons, including the input and distribution variables.

The area under the plasma concentration time curve (AUC) is a useful parameter in defining the overall body exposure to a drug; that parameter integrates the concentration-over-time function:

$$\text{AUC} = \int_{t=0}^{t=\infty} \text{Cp}^t \, \text{d}t \tag{5}$$

As the time function of drug concentration is dependent on the rate at which the drug is cleared from the hypothetical "volume," the AUC function is dependent on total body clearance, CL:

$$V = \frac{\text{dose}}{\text{AUC}\, k_{\text{el}}} \tag{6}$$

CL is a product of volume, V, and the elimination rate constant, k_{el}, when the drug is removed in the urine or metabolized or removed from the sampled compartment by another means. This description of CL often confuses students of PK. CL is an inherent phenomenon in which distribution volumes are high, and rate constants are small to compensate for the distribution. Both volume and the rate constant are derived phenomenon and do not determine CL. Note that total body CL is a composite of all pathways that clear or remove the drug from the sampled compartment or the compartment from which the drug is cleared; this is based on the mathematical relationship between the observed elimination rate constant and its components: each of the pathways involved in the turnover of the drug within the body. Using the parameters described above, it is possible to "simulate" a sampled compartment (of fluid) concentration as a function of time in a single or multiple dose application using simple, iterative programs. Numerous computer programs are now available that are drug and model specific, which allow simulations of steady-state blood levels that depend on various body functions and body characteristics that affect the CL of the drug. Mixed models, involving a zero-order infusion, a bolus, or other similar combinations, can be made to estimate blood concentrations under different circumstances related to drug administration.

When drugs are received by routes other than IV injection, input is not "instantaneous" or a short-order zero, and the function must often be represented as a mixed-order, primarily a first-order, process, which must then be taken into account in simulating drug concentration. Drug CL, however, is not always a constant parameter, especially when an organ like the liver is involved in the removal of the drug from the body:

$$\text{Organ clearance} = \frac{Q(C_a - C_v)}{C_a} = QE \tag{7}$$

where Q is the blood flow rate to the organ, C_a the concentration of the drug in the blood when entering the organ (in the arterial blood), and C_v the concentration of drug in the blood when leaving the organ (in the venous blood). The term E is the steady-state extraction ratio. High E values mean high CL by the liver and, thus, extensive metabolism. The liver blood flow rate is a physiologic parameter that can be altered in disease states. The extraction ratio depends not only on the function of liver but also on the nature of the drug. Both the hepatic CL and the extraction ratio are empirical parameters and depend on the total hepatic blood flow, the unbound fraction of the drug, and the intrinsic CL rate. Intrinsic CL is differentiated from total CL; the former is the ability to transform when other factors are not present. In other words, the intrinsic CL is the property of a body organ that clears the drug such as the liver or kidney; for example, the maximum CL in kidneys cannot exceed the total blood rate to the kidneys and the hepatic CL cannot exceed the total blood flow to liver. The actual CL of a drug from the body depends on the intrinsic CL as well as its concentration in the fluid that is being cleared; a lower concentration resulting from distribution to body tissues will reduce the total CL but will have no effect on the intrinsic CL:

$$\text{CL} = Q\frac{\text{fu CL}_{int}}{Q + (\text{fu CL}_{int})} = \frac{Q\,\text{CL}_{int}^{total}}{Q + \text{CL}_{int}^{total}} \tag{8}$$

which makes the extraction ratio:

$$\text{with } E = \frac{\text{fu CL}_{int}}{Q + (\text{fu CL}_{int})} \tag{9}$$

High-CL drugs are those for which there is no saturation of the reaction that converts the drug, and therefore, the CL rate approaches the blood flow rate. For capacity-limited drugs, flow rate is irrelevant, and CL is a simple product of the unbound fraction and the intrinsic CL.

The traditional method of PK data analysis uses a two-stage approach: estimation of PK parameters through nonlinear regression using an individual's extensive concentration–time data, and using these data parameters as input data for the second-stage calculation of descriptive summary statistics on the sample. Those statistics typically include the mean parameter estimates, the variance, and the covariance of the individual parameter estimates. Analysis of dependencies between parameters and covariates using classical, statistical

approaches (linear stepwise regression, covariance analysis, and cluster analysis) can be included in the second stage. The two-stage approach yields adequate estimates of population characteristics. Mean estimates of parameters are usually unbiased, but the random effects (variance and covariance) are likely to be overestimated in all realistic situations. Refinements have been proposed (such as the global, two-stage approach) to improve the traditional approach through bias correction for the random effects of covariance and differential weighting of individual data according to the data's quality and quantity.

Physiologically Based PK Studies

Physiologically based PK studies take a different perspective in modeling drug disposition in human body—a mechanistic physiologic distribution model. This approach had been in use in other disciplines long before the compartment kinetic modeling was applied to studying drugs. In 1937, the mathematical basis for physiologic PK modeling was established by Teorell, but the solution to the equations was too difficult to obtain before the invention of the digital computer. An automatic solution of a physiologically realistic, mathematical description of the uptake, distribution, and CL of a chemical agent was proposed by Kenneth Bischoff in the early 1960s. At that time, computation limitations forced several simplifications to the models, including the assumption that the distribution of the drug between tissues and blood is instantaneously at equilibrium, which led to physiologic models with blood flow–limited delivery of chemicals to tissues. The inhalation PK models using instantaneous distribution are well known. Physiologic PK studies progressed no further until the early 1970s, when the physiologic parameters of human organ system became better known, and digital computers became more widely available. Today, physiologic PK modeling is critical to the understanding of the behavior of a drug at the site of action.

Exposure modeling studies are often based on the physiologic functions that determine uptake, distribution, and elimination of drugs from the body. This approach was pioneered using anesthetics in which physical distribution determines both the onset and termination of action. Similar results have been reported for other compounds like D_2O and ethanol, propranolol, and inulin and protein-bound antibiotics. The modeling is based on a quantitative description of distribution process using standardized organ weights and blood flow rates. A simpler model assumes no solute binding and a tissue/plasma equilibrium coefficient of 10 for all tissues, except for muscles where this value is 3.62 and for fat where the value is 2.42 as used for propranolol. Also in the simple model, there is no first-pass effect, and kidney excretion is the only mechanism of drug removal from the body; thus, the input function is equal to systemic availability. In more complex models, tissue binding and other factors that produce nonequilibrium of the tissue: plasma ratios are introduced. The simple model, when used to determine bolus-response function, is well described by a simple two-exponential function; in the more complicated models, three exponents generally provide good fit, and often, going to higher exponents does not improve the predictability. More important is the timing of the first data point obtained in the bolus-response function. This should, ideally, be obtained at or before the end of the constant infusion. (Note that better estimates are obtained from infusion studies than from single-bolus doses because there is always an inevitable delay in the dispersion of the drug in the bolus dosing, but the model assumes no delay.) When a deconvolution method is used (see below), the robustness of analysis depends on the accuracy of venous concentration data because the response function $r(t)$ is established from these data; therefore, any error in this function reduces the reliability of the analysis, particularly when a later time sample, taken after 10 minutes, is used as the first data point.

Bioequivalence and Systemic Exposure Models

Screening drug molecules for suitability for use in humans is often subjected to certain basic toxicity or workability solutions to reduce the cost of screening. The human body must be able to remove the drug in a reasonable time. Drug CL is an intrinsic parameter; however, body CL (extent of drug removal) is dependent on cardiac output and the overall extraction ratio.

$$\text{Body clearance (plasma, blood)} = \dot{Q} \times \text{ER} \tag{10}$$

The ER is the extraction ratio that ranges from 0 to 1, and the cardiac output is proportional to body size:

$$\dot{Q}(\text{mL/kg/min}) = 180\ \text{BW (kg)}^{-0.19} \tag{11}$$

Cross-species comparisons can be made for crude estimates and, generally, for drugs that have CL of less than 4 mL/min/kg can be evaluated only if there are special reasons that require the mechanisms of actions to be evaluated.

In addition to the removal potential of a drug, the entry potential is also a good screening parameter; for drugs that are poorly bioavailable, further development should proceed only if proper modification to the molecular structure or to the drug delivery system is made to provide a reasonable possibility of entry. When evaluating bioavailability, it is important to first establish a PK basis because of the large variation in bioavailability as a result of the differences in population PK. Population models are most appropriate for this type of evaluation. Obviously, the consideration of bioequivalence in establishing compliance of generic products is important, and the guidelines for these measurements are defined in the United States Pharmacopoeia and other guidelines provided by the FDA. It should be noted that the purpose of these studies is to compare the systemic exposure of the body to the drug molecules; this requires measurement of both the extent of absorption and the rate of absorption. Traditionally, parameters like AUC, T_{max}, and C_{max} are studied using specified statistical models. For drugs given orally, these studies cannot be substituted with PD studies, which may be required for some drugs in which the plasma or sample tissue concentration is not available.

Deconvolution Techniques

The bolus-response function $r(t)$ is generally described using a multiexponential function:

$$r(t) = \sum_{i=1}^{\rho} a_i(\mathrm{e}^{-t/T_i}) \tag{12}$$

The optimized values for a_i and T_i are determined by using mathematical approach without any significance attached to it for physiologic reasons. Generally, the resorting required to use a three-exponential term takes the estimates out of the population parameters or global minimum.

The three parameters in γ-distribution are chosen by minimizing the error function:

$$\text{Error function} = \sum_i \frac{|(\text{ygam})_i - (\text{ydat})_i|}{(\text{ydat})_i + \text{noise}} \tag{13}$$

in which $(\text{ydat})_i$ is the sum of overall data points for the experimental venous concentration and $(\text{ygam})_i$ is the venous concentration–determined y convoluting the γ-distribution input using a polyexponential equation as described above for $r(t)$. The *noise* factor in equation (13) determines the weighting of each data point used. When there is no error of *noise*, then the error is simplified for each point. When the error is large, the term $(\text{ydat})_i$ drops out in the denominator, and the error is proportional to the numerator of the error term. As the γ-distribution function is a highly nonlinear process, it is important to use a global annealing procedure such as that used in PKQuest [Minneapolis, Minnesota, U.S.A. (www.pkquest.com), requiring Maple software, Maplesoft, Ontario, Canada (www.maplesoft.com)], and then follow it with nonlinear minimization. The venous concentration is fitted by using interpolation, which means it goes through each data point or uses a smoothing cubic-spline function and then, performing the deconvolution. The B-spline function defines the number and position of "breakpoints" and the order of the spline function. Highly sophisticated models have been used for this purpose.

The course of systemic exposure to a drug is studied by comparing IV administration studies using deconvulation approach in which the systemic concentration, $r(t)$ produced from IV administration (also called bolus function) and $I(t)$ is the systemic input rate (in units such as

g/min) from the non-IV route:

$$c(t) = \int_0^t r(t-\tau)[I(\tau)]\mathrm{d}\tau \tag{14}$$

If there is no first-pass effect involved, then $I(t)$ is equal to the rate of intestinal absorption upon administration of equal doses (in IV and non-IV forms). In first-pass metabolism, $I(t)$ is the systemic availability of the drug upon oral (or sublingual, rectal, buccal, etc.) dosing. The function $r(t)$ is obtained by fitting the data upon IV administration to a variety of exponential equations and selecting the best fit through residual mean error of fit. The duration of infusion can be instantaneous (a few seconds for bolus input) but, more realistically, is usually a few minutes. Although it is desirable to obtain the sample as early as possible, sampling earlier than two minutes after injection is not advised to allow time for venous mixing. Longer-term IV infusions are also used to obtain the $r(t)$ function. Mathematical solutions of the deconvolution are easily obtained by using such validated software as PKQuest requiring Maple software. Several methods are used for decovolution; γ-distribution input is a parametric fitting technique. Although polyexponential fitting techniques are widely used, better fits are obtained by using a parametric approach for simulating $I(t)$ where A is the amount of drug reaching circulation, Γ is the γ-function, a is the γ-number that ranges from 1 to 6, and b has inverse time units:

$$I(t) = \frac{(Ab)^a \times t^{a-1} \times \mathrm{e}^{-bt}}{[\Gamma(a)]} \quad \text{or} \quad I(t) = \frac{(Ab)^a \times t^{a-1} \times \mathrm{e}^{-bt}}{\Gamma(a)} \tag{15}$$

This approach offers a superior simulation, particularly in situations where there is a delay in the input function such as in intestinal absorption and gastric emptying variations. The three parameters given above are estimated by global (also called simulated annealing) and local (also called Powell) nonlinear optimization. The fitting of data using γ-deconvolution method smoothes data noise, and with no user adjustable parameters, the bias is removed. If the input is not possible to define using a single γ-distribution, then other deconvolution approaches, such as analytical, spline, or uniform approaches, which remove the "roughness" of the input rate are used; the choice of parameters is additionally improved by experimental Akaike criterion and the "generalized cross-validation."

The analytical deconvolution involves approximation of $C(t)$ by an interopolating or smoothed-spline function and the devonvolution. The analytical deconvolution method is most commonly used for the advantage of being fast and where data are exact, excellent results are obtained; however, the robustness of this approach depends on the value chosen for the smoothing parameter, which is poorly estimated even when standard deviation is available (very rare). Where there is noisy data, it adds more error in analytical deconvolution compared with spline and uniform methods. Also, analytical deconvolution does not allow use of negative values for input. In spline function input consideration, the input $I(t)$ is parameterized using a general B-spline function and then obtaining deconvolution by a constrained regression. In using uniform input, $I(t)$ is estimated on dense uniform sequence of time points and then using stochastic regularization procedure for deconvolution.

Pharmacologic Evaluation of Bioavailability

The estimations of bioavailability discussed above are based on plasma and/or urine levels of the drug and/or its biotransformation products. It is understood in these calculations that these concentrations relate in some manner to the pharmacologic or clinical response of the drug. Ideally, therefore, it is desirable to measure bioavailability as a function of pharmacologic or clinical effect. In order to do so, a specific and discriminating test is needed. Some quantitative endpoint must be available which measures the efficacy or quantitates the drug effect. For example, lowering of blood sugar by an antidiabetic agent, lowering of blood pressure by a hypotensive agent, weight loss produces by an anorexic agent, etc., would be appropriate

measures. Less reliable measures, such as psychologic rating score and a physician's opinion of efficacy, cannot be of great value in these studies.

Before any comparative bioavailability testing is performed using pharmacologic or clinical response, a satisfactory dose–response curve should be obtained on one of the formulations to be included in the study. The success of the application of the dose–response curve should be established on one of the formulations to be included in the study. The success of the application of this dose–response curve depends on two factors. First, the curve should be steep, indicating that significant changes in pharmacologic response occur with a small change in the dose, and second, the dose contained in the formulations should be such that the response lies between 20% and 80% of maximum response to assure linear measurements. Responses falling beyond these ranges are more difficult to quantify.

Few studies have reported the use of dose–response curves in bioavailability measurements, but the idea is certainly attractive and relevant to drug therapy.

Studies in healthy volunteers or patients using PD measurements may be used for establishing equivalence between two pharmaceutical products. These studies may become necessary if quantitative analysis of the drug and/or metabolite(s) in plasma or urine cannot be made with sufficient accuracy and sensitivity. Furthermore, PD studies in humans are required if measurements of drug concentrations cannot be used as surrogate endpoints for the demonstration of efficacy and safety of the particular pharmaceutical product, e.g., for topical products without an intended absorption of the drug into the systemic circulation.

If PD studies are to be used they must be performed as rigorously as bioequivalence studies, and the principles of good clinical practice (GCP) (see WHO Guidelines for GCP for Trials on Pharmaceutical Products) must be followed.

The following requirements must be recognized when planning, conducting, and assessing the results of a study intended to demonstrate equivalence by means of measuring PD drug responses:

1. The response that is being measured should be a pharmacologic or therapeutic effect that is relevant to the claims of efficacy and/or safety.
2. The methodology must be validated for precision, accuracy, reproducibility, specificity, and ruggedness.
3. Neither the test nor the reference product should produce a maximal response in the course of the study, since it may be impossible to distinguish differences between formulations given in doses that give maximum or near-maximum effects. Investigation of dose–response relationships may be a necessary part of the design.
4. The response should be measured quantitatively under double blind conditions and be recorded in an instrument-produced or instrument-recorded fashion on a repetitive basis to provide a record of the PD events that are substitutes for plasma concentrations. In those instances where such measurements are not possible, recordings on visual analogue scales may be used. In other instances where the data are limited to qualitative (categorized) measurements, appropriate special statistical analysis will be required.
5. Nonresponders should be excluded from the study through prior screening. The criteria by which responders versus nonresponders are identified must be stated in the protocol.
6. In instances where an important placebo effect can occur, comparison between pharmaceutical products can only be made by a priori consideration of the placebo effect in the study design. This may be achieved by adding a third phase with placebo treatment in the design of the study.
7. The underlying pathology and natural history of the condition must be considered in the study design. There should be knowledge of the reproducibility of baseline conditions.
8. A crossover design may be used. Where this is not appropriate, a parallel group study design should be chosen.

In studies in which continuous variables could be recorded, the time course of the intensity of the drug action can be described in the same way as in a study in which plasma

concentrations were measured, and parameters can be derived which describe the area under the effect–time curve, the maximum response and the time when maximum response occurred.

The statistical considerations for the assessment of the outcome of the study are, in principle, the same as outlined for the bioequivalence studies. However, a correction for the potential nonlinearity of the relationship between the dose and the area under the effect–time curve should be performed on the basis of the outcome of the dose-ranging study as mentioned above. However, it should be noted that the conventional acceptance range as applied for bioequivalence assessment is not appropriate (too large) in most of the cases but should be defined on a case-by-case basis and described in the protocol.

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4 Waiver of Bioavailability/Bioequivalence Studies

BACKGROUND

Bioavailability (BA) and bioequivalence (BE) studies are expensive to conduct and given the need for a multitude of these studies in the development of a new drug application (NDA) or abbreviated new drug application (ANDA), there had always existed a need to justify these needs on scientific grounds. This is particularly important for the generic drug industry since the generic competitors must keep their cost of regulatory approval to as low a level as possible. Recently, guidelines have emerged that would allow waiver of both BA and BE studies in some situations. There are also provisions available for the sponsor to challenge the requirement and if the basic criteria set are met, there is a very good possibility of receiving waivers. These waivers are intended to apply to

- subsequent in vivo BA or BE studies of formulations after the initial establishment of the in vivo BA of immediate release (IR) dosage forms during the investigational new drug application (IND) period and
- in vivo BE studies of IR dosage forms in ANDAs. Regulations at 21 CFR part 320 address the requirements for BA and BE data for the approval of drug applications and supplemental applications.

Provision for waivers of in vivo BA/BE studies (biowaivers) under certain conditions is provided at 21 CFR 320.22.

THE BIOPHARMACEUTICS CLASSIFICATION SYSTEM

The biopharmaceutics classification system (BCS) is a scientific framework for classifying drug substances based on their aqueous solubility and intestinal permeability. When combined with the dissolution of the drug product, the BCS takes into account three major factors that govern the rate and extent of drug absorption from IR solid oral dosage forms: dissolution, solubility, and intestinal permeability. According to the BCS, the drug substances are classified as follows:

Class 1: High solubility—high permeability
Class 2: Low solubility—high permeability
Class 3: High solubility—low permeability
Class 4: Low solubility—low permeability

In addition, IR solid oral dosage forms are categorized as having rapid or slow dissolution. Within this framework, when certain criteria are met, the BCS can be used as a drug development tool to help sponsors justify requests for biowaivers. There are several factors that affect the classification of drugs in different classes. Table 1 expands this classification to include a more detailed description including the effect of transporter efflux factors.

Observed in vivo differences in the rate and extent of absorption of a drug from two pharmaceutically equivalent solid oral products may be due to differences in drug dissolution in vivo. However, when the in vivo dissolution of an IR solid oral dosage form is rapid in relation to gastric emptying and the drug has high permeability, the rate and extent of drug

TABLE 1 The Biopharmaceutics Classification System as Defined by the FDA and Modified by Recent Findings

	High solubility (e.g., when the highest dose strength is soluble in 250 mL or less of aqueous media over a pH range of 1 to 7.5 at 37°C)	**Low solubility**
High permeability [e.g., absorption >90% when compared with intravenous dose (drug + metabolite)]	Class 1: (generally about 8% of new leads) High solubility High permeability Rapid dissolution for biowaiver Route of elimination: metabolism, extensive Transporter effects: minimal Examples: abacavir; acetaminophen; *acyclovir*; *amiloride*^{S,I}; amitryptyline^{S,I}; ntipyrine; *atropine*; **buspirone**; caffeine; *captopril*; chloroquine^{S,I}; **chlorpheniramine**; cyclophosphamide; desipramine; **diazepam**; **diltiazem**^{S,I}; **dihenhydramine**; disopyramide; **doxepin**; oxycycline; enalapril; ephedrine; ergonovine; ethambutol; ethinyl estradiol; fluoxetine^{I}; glucose; imipramine^{I}; ketoprofen; **ketorolac**; labetolol; levodopa^{S}; levofloxacin^{S}: **lidocaine^{I}**: lomefloxacin: **meperidine**; metoprolol; metronidazole; **midazolam**^{S,I}; **minocycline**; misoprostol; **nifedipine**^{S}; phenobarbital; phenylalanine; prednisolone; **primaquine**^{S}; promazine; propranolol^{I}; **quinidine**^{S,I}; **rosiglitazone**; salicylic acid; theophylline; valproic acid; **verapamil**^{I}; zidovudine	Class 2 Low solubility High permeability Route of elimination: metabolism, extensive Transporter: efflux transporter effects predominant Examples: **amiodarone**^{I}; **atorvastatin**^{S,I}; **azithromycin**^{S,I}; **carbamazepine**^{S,I}; **carvedilol**; chlorpromazine^{I}; *ciprofloxacin*^{S}; **cisapride**^{S}; **cyclosporine**^{S,I}; **danazol**; **dapsone**; diclofenac; diflunisal; digoxin^{S}; *erythromycin*^{S,I}; flurbiprofen; **glipizide**; glyburide^{S,I}; griseofulvin; ibuprofen; **indinavir**^{S}; **indomethacin**; **itraconazole**^{S,I}; **ketoconazole**^{I}; **lansoprazole**^{I}; **lovastatin**^{S,I}; *mebendazole*; naproxen; nelfinavir^{S,I}; ofloxacin; oxaprozin; phenazopyridine; phenytoin^{S}; piroxicam; raloxifene^{S}; **ritonavir**^{S,I}; **saquinavir**^{S,I}; saquinavir^{S,I}; **sirolimus**^{S}; sirolimus^{S}; spironolactone^{I}; spironolactone^{I}; **tacrolimus**^{S,I}; tacrolimus^{S,I}; talinolol^{S}; talinolol^{S}; **tamoxifen**^{I}; tamoxifen^{I}; **terfenadine**^{I}; terfenadine^{I}; warfarin; warfarin
Low permeability	Class 3 High solubility Low permeability Route of elimination: renal and/or biliary elimination of unchanged drug; metabolism poor Transporter: absorptive effects predominant Examples: *acyclovir*; *amiloride*^{S,I}; amoxicillin^{S,I}; atenolol; *atropine*; bidisomide; bisphosphonates; *captopril*; cefazolin; cetirizine; cimetidine^{S}; *ciprofloxacin*^{S}; cloxacillin; dicloxacillin^{S}; *erythromycin*^{S,I}; famotidine; fexofenadine^{S}; folinic acid; *furosemide*; ganciclovir; *hydrochlorothiazide*; lisinopril; metformin; *methotrexate*; nadolol; penicillins; pravastatin^{S}; ranitidine^{S}; tetracycline; trimethoprim^{S}; valsartan; zalcitabine	Class 4 Low solubility Low permeability Route of elimination: renal and/or biliary elimination of unchanged drug; metabolism poor Transporter: absorptive and efflux transporters can be predominant Examples: amphotericin B; chlorothiazide; chlorthalidone; *ciprofloxacin*^{S}; colistin; *furosemide*; *hydrochlorothiazide*; *mebendazole*; *methotrexate*; neomycin

Note: The compounds listed in italic are those falling in more than one category by different authors, which could be a result of the definition of the experimental conditions. The compounds listed in bold are primarily CYP3A substrates where metabolism accounts for more than 70% of the elimination; superscript I and/or S indicate P-gp inhibitors and/or substrate, respectively. Class 1 and Class 2 compounds are eliminated primarily via metabolism, whereas Class 3 and Class 4 compounds are primarily eliminated unchanged into the urine and bile.

Abbreviations: CYP3A, cytochrome P450 3A; FDA, Food and Drug Administration; P-gp, P-glycoprotein.

absorption is unlikely to be dependent on drug dissolution and/or gastrointestinal (GI) transit time. Under such circumstances, demonstration of in vivo BA or BE may not be necessary for drug products containing Class 1 drug substances, as long as the inactive ingredients used in the dosage form do not significantly affect absorption of the active ingredients. The BCS approach outlined in this guidance can be used to justify biowaivers for highly soluble and highly permeable drug substances (i.e., Class 1) in IR solid oral dosage forms that exhibit rapid in vitro dissolution using the recommended test methods [21 CFR 320.22(e)]. The recommended methods for determining solubility, permeability, and in vitro dissolution are discussed below.

Solubility

The solubility class boundary is based on the highest dose strength of an IR product that is the subject of a biowaiver request. A drug substance is considered highly soluble when the highest dose strength is soluble in 250 mL or less of aqueous media over the pH range of 1 to 7.5. The volume estimate of 250 mL is derived from typical BE study protocols that prescribe administration of a drug product to fasting human volunteers with a glass (about 8 oz) of water.

Permeability

The permeability class boundary is based indirectly on the extent of absorption (fraction of dose absorbed, not systemic BA) of a drug substance in humans and directly on measurements of the rate of mass transfer across human intestinal membrane. Alternatively, nonhuman systems capable of predicting the extent of drug absorption in humans can be used (e.g., in vitro epithelial cell culture methods). In the absence of evidence suggesting instability in the GI tract, a drug substance is considered to be highly permeable when the extent of absorption in humans is determined to be 90% or more of an administered dose based on a mass balance determination or in comparison to an intravenous reference dose.

Dissolution

An IR drug product is considered rapidly dissolving when no less than 85% of the labeled amount of the drug substance dissolves within 30 minutes, using Unites States Pharmacopoeia (USP) Apparatus I at 100 rpm (or Apparatus II at 50 rpm) in a volume of 900 mL or less in each of the following media: (*i*) 0.1 N HCl or simulated gastric fluid USP without enzymes, (*ii*) a pH 4.5 buffer, and (*iii*) a pH 6.8 buffer or simulated intestinal fluid USP without enzymes.

METHOD OF CLASSIFICATION

The following approaches are recommended for classifying a drug substance and determining the dissolution characteristics of an IR drug product according to the BCS.

Determining Drug Substance Solubility Class

An objective of the BCS approach is to determine the equilibrium solubility of a drug substance under physiological pH conditions. The pH-solubility profile of the test drug substance should be determined at 37 ± 1°C in aqueous media with a pH range of 1 to 7.5. A sufficient number of pH conditions should be evaluated to accurately define the pH-solubility profile. The number of pH conditions for a solubility determination can be based on the ionization characteristics of the test drug substance. For example, when the pK_a of a drug is in the range of three to five, solubility should be determined at $pH = pK_a$, $pH = pK_a + 1$, $pH = pK_a - 1$, and at $pH = 1$ and 7.5. A minimum of three replicate determinations of solubility in each pH condition is recommended. Depending on study variability, additional replication may be necessary to provide a reliable estimate of solubility. Standard buffer solutions described in the USP are considered appropriate for use in solubility studies. If these buffers are not suitable for physical or chemical reasons, other buffer solutions can be used. Solution pH should be verified after addition of the drug substance to a buffer. Methods other than the traditional shake-flask method, such as acid or base titration methods, can also be used with justification to support the ability of such methods to predict equilibrium solubility of the test drug substance. The concentration of the drug substance in selected buffers (or pH conditions) should be determined using a validated stability-indicating assay that can distinguish the drug substance from its degradation products. If the degradation of the drug substance is observed as a function of buffer composition and/or pH, it should be reported along with other stability data.

The solubility class should be determined by calculating the volume of an aqueous medium sufficient to dissolve the highest dose strength in the pH range of 1 to 7.5. A drug substance should be classified as highly soluble when the highest dose strength is soluble in ≤ 250 mL of aqueous media over the pH range of 1 to 7.5.

Determining Drug Substance Permeability Class

The permeability class of a drug substance can be determined in human subjects using mass balance, absolute BA, or intestinal perfusion approaches. Recommended methods not involving human subjects include in vivo or in situ intestinal perfusion in a suitable animal model (e.g., rats), and/or in vitro permeability methods using excised intestinal tissues, or monolayers of suitable epithelial cells. In many cases, a single method may be sufficient (e.g., when the absolute BA is 90% or more, or when 90% or more of the administered drug is recovered in urine). When a single method fails to conclusively demonstrate a permeability classification, two different methods may be advisable. Chemical structure and/or certain physicochemical attributes of a drug substance (e.g., partition coefficient in suitable systems) can provide useful information about its permeability characteristics. The sponsors may wish to consider use of such information to support further a classification.

Pharmacokinetic Studies in Humans

Mass Balance Studies

Pharmacokinetic mass balance studies using unlabeled, stable isotopes or a radio-labeled drug substance can be used to document the extent of absorption of a drug. Depending on the variability of the studies, a sufficient number of subjects should be enrolled to provide a reliable estimate of the extent of absorption. Because this method can provide highly variable estimates of drug absorption for many drugs, other methods described below may be preferable.

Absolute BA Studies

Oral BA determination using intravenous administration as a reference can be used. Depending on the variability of the studies, a sufficient number of subjects should be enrolled in a study to provide a reliable estimate of the extent of absorption. When the absolute BA of a drug is shown to be 90% or more, additional data to document drug stability in the GI fluid are not necessary.

The following methods can be used to determine the permeability of a drug substance from the GI tract: (*i*) in vivo intestinal perfusion studies in humans, (*ii*) in vivo or in situ intestinal perfusion studies using suitable animal models, (*iii*) in vitro permeation studies using excised human or animal intestinal tissues, or (*iv*) in vitro permeation studies across a monolayer of cultured epithelial cells.

In vivo or in situ animal models and in vitro methods, such as those using cultured monolayers of animal or human epithelial cells, are considered appropriate for passively transported drugs. The observed low permeability of some drug substances in humans could be caused by efflux of drugs via membrane transporters such as P-glycoprotein (P-gp). When the efflux transporters are absent in these models, or their degree of expression is low when compared with that in humans, there may be a greater likelihood of misclassification of permeability class for a drug subject to efflux when compared with that transported passively. Expression of known transporters in selected study systems should be characterized. Functional expression of efflux systems (e.g., P-gp) can be demonstrated with techniques such as bidirectional transport studies, demonstrating a higher rate of transport in the basolateral-to-apical direction when compared with apical-to-basolateral direction using selected model drugs or chemicals at concentrations that do not saturate the efflux system (e.g., cyclosporin A, vinblastine, and rhodamine 123). An acceptance criterion for intestinal efflux that should be present in a test system cannot be set at this time. Instead, this guidance recommends limiting the use of nonhuman permeability test methods for drug substances that are transported by passive mechanisms. Pharmacokinetic studies on dose linearity or proportionality may provide useful information for evaluating the relevance of observed in vitro efflux of a drug. For example, there may be fewer concerns associated with the use of in vitro methods for a drug that has a higher rate of transport in the basolateral-to-apical direction at low drug concentrations but exhibits linear pharmacokinetics in humans.

Poor absorption or permeation is more likely when there are more than 5H-bond donors, 10H-bond acceptors, the molecular weight is greater than 500, and the calculated log *P*

is greater than five. This is also often referred to as Rule 5 of Lipinski. However, Lipinski specifically states that Rule 5 holds only for compounds that are *not* substrates for active transporters. Since almost all drugs are substrates for some transporter, much remains to be studied about the Lipinski's rule. In addition, unless a drug molecule can passively gain intracellular access, it is not possible to simply investigate whether the molecule is a substrate for efflux transporters.

Several generalizations can be made about the interplay of transporters and the BCS classification.

1. *Transporter effects are minimal for Class 1 compounds*. The high permeability/high solubility of such compounds allows high concentrations in the gut to saturate any transporter, both efflux and absorptive. Class 1 compounds may be substrates for both uptake and efflux transporters in vitro in cellular systems under the right conditions (e.g., midazolam and nifedipine are substrates for P-gp), but transporter effects are not important clinically. It is therefore possible that some compounds that should be considered as Class 1 in terms of drug absorption and disposition are not actually Class 1 in the BCS due to the requirement of good solubility and rapid dissolution at low pH values. Such pH effects would not be limiting in vivo where absorption takes place in the intestine. Examples of this include the NSAIDs, such as diclofenac, diflunisal, flurbiprofen, indomethacin, naproxen, and piroxicam; warfarin is almost completely bioavailable. In contrast, ofloxacin is listed as Class 2 because of its low solubility at pH 7.5.
2. *Efflux transporter effects will predominate for Class 2 compounds*. The high permeability of these compounds will allow ready access to the gut membranes and uptake transporters will have no effect on absorption, but the low solubility will limit the concentrations coming into the enterocytes, thereby preventing saturation of the efflux transporters. Consequently, efflux transporters will affect the extent of oral BA and the rate of absorption of Class 2 compounds.
3. *Transporter–enzyme interplay in the intestine will be important primarily for Class 2 compounds that are substrates for CYP3A and Phase 2 conjugation enzymes.* For such compounds, intestinal uptake transporters will generally be unimportant due to the rapid permeation of the drug molecule into the enterocytes as a function of their high lipid solubility. That is, the absorption of Class 2 compounds is primarily passive and a function of lipophilicity. However, due to the low solubility of these compounds, there will be little opportunity to saturate apical efflux transporters and intestinal enzymes such as cytochrome P450 3A4 (CYP3A4) and uridine 5′-diphospho glucuronosyltransferase (UDP). Thus, changes in transporter expression and inhibition or induction of efflux transporters will cause changes in intestinal metabolism of drugs that are substrates for the intestinal metabolic enzymes. A large number of Class 2 compounds are shown in Table 1, which are primarily substrates of cytochrome P450 3A (CYP3A) (compounds listed in bold), as well as substrates or inhibitors of the efflux transporter P-gp (indicated by superscripts S and I, respectively). Work in our laboratory has characterized this interplay in the absorptive process for the investigational cysteine protease inhibitor K77 and sirolimus, substrates for CYP3A and P-gp, and more recently for raloxifene, a substrate for UGTs and P-gp.
4. *Absorptive transporter effects will predominate for Class 3 compounds*. For Class 3 compounds, sufficient drugs are available in the gut lumen due to good solubility, but an absorptive transporter is necessary to overcome the poor permeability characteristics of these compounds. However, intestinal apical efflux transporters may also be important for the absorption of such compounds when sufficient enterocyte penetration is achieved via an uptake transporter.

Table 2 lists model drugs suggested for use in establishing suitability of a permeability method. The permeability of these compounds was determined based on data available to the Food and Drug Administration (FDA). Potential internal standards (IS) and efflux pump substrates (ES) are also identified.

TABLE 2 Model Drugs to Establish Permeability of Drugs

Drug	Permeability class
Antipyrine	High (potential IS candidate)
Caffeine	High
Carbamazepine	High
Fluvastatin	High
Ketoprofen	High
Metoprolol	High (potential IS candidate)
Naproxen	High
Propranolol	High
Theophylline	High
Verapamil	High (potential ES candidate)
Amoxicillin	Low
Atenolol	Low
Furosemide	Low
Hydrochlorthiazide	Low
Mannitol	Low (potential IS candidate)
Methyldopa	Low
Polyethylene glycol (400)	Low
Polyethylene glycol (1000)	Low
Polyethylene glycol (4000)	Low (zero permeability marker)
Ranitidine	Low

Abbreviations: ES, efflux pump substrates; IS, internal standards.

For application of the BCS, an apparent passive transport mechanism can be assumed when one of the following conditions is satisfied:

- A linear (pharmacokinetic) relationship between the dose (e.g., relevant clinical dose range) and measures of BA (area under the concentration–time curve) of a drug is demonstrated in humans.
- Lack of dependence of the measured in vivo or in situ permeability is demonstrated in an animal model on initial drug concentration (e.g., 0.01, 0.1, and 1 time the highest dose strength dissolved in 250 mL) in the perfusion fluid.
- Lack of dependence of the measured in vitro permeability on initial drug concentration (e.g., 0.01, 0.1, and 1 time the highest dose strength dissolved in 250 mL) is demonstrated in donor fluid and transport direction (e.g., no statistically significant difference in the rate of transport between the apical-to-basolateral and basolateral-to-apical directions for the drug concentrations selected) using a suitable in vitro cell culture method that has been shown to express known efflux transporters (e.g., P-gp).

To demonstrate the suitability of a permeability method intended for application of the BCS, a rank order relationship between test permeability values and the extent of drug absorption data in human subjects should be established using a sufficient number of model drugs. For in vivo intestinal perfusion studies in humans, six model drugs are recommended. For in vivo or in situ intestinal perfusion studies in animals and in vitro cell culture methods, 20 model drugs are recommended. Depending on study variability, a sufficient number of subjects, animals, excised tissue samples, or cell monolayers should be used in a study to provide a reliable estimate of drug permeability. This relationship should allow precise differentiation between drug substances of low and high intestinal permeability attributes.

For demonstration of the suitability of a method, model drugs should represent a range of low (e.g., $<50\%$), moderate (e.g., 50–89%), and high ($\geq 90\%$) absorption. The sponsors may select compounds from the list of drugs and/or chemicals provided in Table 2, or they may choose to select other drugs for which there is information available on mechanism of absorption and reliable estimates of the extent of drug absorption in humans.

After demonstrating the suitability of a method and maintaining the same study protocol, it is not necessary to retest all selected model drugs for subsequent studies intended to classify

a drug substance. Instead, a low and a high permeability model drug should be used as IS (i.e., included in the perfusion fluid or donor fluid along with the test drug substance). These two IS are in addition to the fluid volume marker (or a zero permeability compound such as PEG 4000) that is included in certain types of perfusion techniques (e.g., closed loop techniques). The choice of IS should be based on compatibility with the test drug substance (i.e., they should not exhibit any significant physical, chemical, or permeation interactions). When it is not feasible to follow this protocol, the permeability of IS should be determined in the same subjects, animals, tissues, or monolayers, following evaluation of the test drug substance. The permeability values of the two IS should not differ significantly between different tests, including those conducted to demonstrate the suitability of the method. At the end of an in situ or in vitro test, the amount of drug in the membrane should be determined.

For a given test method with set conditions, selection of a high permeability IS with permeability in close proximity to the low/high permeability class boundary may facilitate the classification of a test drug substance. For instance, a test drug substance may be determined to be highly permeable when its permeability value is equal to or greater than that of the selected IS with high permeability.

Instability in the GI Tract

Determining the extent of absorption in humans based on mass balance studies using total radioactivity in urine does not take into consideration the extent of degradation of a drug in the GI fluid prior to intestinal membrane permeation. In addition, some methods for determining permeability could be based on loss or clearance of a drug from fluids perfused into the human and/or animal GI tracts either in vivo or in situ. Documenting the fact that drug loss from the GI tract arises from intestinal membrane permeation, rather than a degradation process, will help establish permeability. Stability in the GI tract may be documented using gastric and intestinal fluids obtained from human subjects. Drug solutions in these fluids should be incubated at 37°C for a period that is representative of in vivo drug contact with these fluids; for example, one hour in gastric fluid and three hours in intestinal fluid. Drug concentrations should then be determined using a validated stability-indicating assay method. Significant degradation (>5%) of a drug in this protocol could suggest potential instability. Obtaining GI fluids from human subjects requires intubation and may be difficult in some cases. Use of GI fluids from suitable animal models and/or simulated fluids such as gastric and intestinal fluids USP can be substituted when properly justified.

Determining Drug Product Dissolution Characteristics and Dissolution Profile Similarity

Dissolution testing should be carried out in USP Apparatus I at 100 rpm or Apparatus II at 50 rpm using 900 mL of the following dissolution media: (*i*) 0.1 N HCl or simulated gastric fluid USP without enzymes, (*ii*) a pH 4.5 buffer, and (*iii*) a pH 6.8 buffer or simulated intestinal fluid USP without enzymes. For capsules and tablets with gelatin coating, simulated gastric and intestinal fluids USP (with enzymes) can be used.

Dissolution testing apparatus used in this evaluation should conform to the requirements in USP (<711> dissolution). Selection of the dissolution testing apparatus (USP Apparatus I or II) during drug development should be based on a comparison of in vitro dissolution and in vivo pharmacokinetic data available for the product. The USP Apparatus I (basket method) is generally preferred for capsules and products that tend to float, and USP Apparatus II (paddle method) is generally preferred for tablets. For some tablet dosage forms, in vitro (but not in vivo) dissolution may be slow due to the manner in which the disintegrated product settles at the bottom of a dissolution vessel. In such situations, USP Apparatus I may be preferred over Apparatus II. If the testing conditions need to be modified to better reflect rapid in vivo dissolution (e.g., use of a different rotating speed), such modifications can be justified by comparing in vitro dissolution with in vivo absorption data (e.g., a relative BA study using a simple aqueous solution as the reference product).

A minimum of 12 dosage units of a drug product should be evaluated to support a biowaiver request. Samples should be collected at a sufficient number of intervals to characterize the dissolution profile of the drug product (e.g., 10, 15, 20, and 30 minutes).

When comparing the test and reference products, dissolution profiles should be compared using a similarity factor (f_2). The similarity factor is a logarithmic reciprocal square root transformation of the sum of squared error and is a measurement of the similarity in the percentage of dissolution between the two curves.

$$f_2 = 50 \cdot \log\left\{\left[1 + \frac{1}{n}\sum_{t=1}^{n}(R_t - T_t)^2\right]^{-0.5} \cdot 100\right\}$$

Two dissolution profiles are considered similar when the f_2 value is ≥ 50. To allow the use of mean data, the coefficient of variation should not be more than 20% at the earlier time points (e.g., 10 minutes), and should not be more than 10% at other time points. Note that when both the test and reference products dissolve 85% or more of the labeled amount of the drug in ≤ 15 minutes using all three dissolution media recommended above, the profile comparison with an f_2 test is unnecessary.

ADDITIONAL CONSIDERATIONS

When requesting a BCS-based waiver for in vivo BA/BE studies for IR solid oral dosage forms, applicants should note that the following factors can affect their request or the documentation of their request.

Excipients

Excipients can sometimes affect the rate and extent of drug absorption. In general, using excipients that are currently in FDA-approved IR solid oral dosage forms will not affect the rate or extent of absorption of a highly soluble and highly permeable drug substance that is formulated in a rapidly dissolving IR product. To support a biowaiver request, the quantity of excipients in the IR drug product should be consistent with the intended function (e.g., lubricant). When new excipients or atypically large amounts of commonly used excipients are included in an IR solid dosage form, additional information documenting the absence of an impact on BA of the drug may be requested by the agency. Such information can be provided with a relative BA study using a simple aqueous solution as the reference product. Large quantities of certain excipients, such as surfactants (e.g., polysorbate 80) and sweeteners (e.g., mannitol or sorbitol) may be problematic, and the sponsors are encouraged to contact the review division when this is a factor.

Prodrugs

Permeability of prodrugs will depend on the mechanism and (anatomical) site of conversion to the drug substance. When the prodrug-to-drug conversion is shown to occur predominantly after intestinal membrane permeation, the permeability of the prodrug should be measured. When this conversion occurs prior to intestinal permeation, the permeability of the drug should be determined. Dissolution and pH-solubility data on both the prodrug and drug can be relevant. The sponsors may wish to consult with appropriate review staff before applying the BCS approach to IR products containing prodrugs.

Exceptions

The BCS-based biowaivers are not applicable for the following.

Narrow Therapeutic Range Drugs

The narrow therapeutic range drug products are defined as those containing certain drug substances that are subject to therapeutic drug concentration or pharmacodynamic monitoring, and/or where product labeling indicates a narrow therapeutic range designation. Examples

include digoxin, lithium, phenytoin, theophylline, and warfarin. Because not all drugs subject to therapeutic drug concentration or pharmacodynamic monitoring are narrow therapeutic range drugs, the sponsors should contact the appropriate review division to determine whether a drug should be considered to have a narrow therapeutic range.

Products Designed to Be Absorbed in the Oral Cavity

A request for a waiver of in vivo BA/BE studies based on the BCS is not appropriate for dosage forms intended for absorption in the oral cavity (e.g., sublingual or buccal tablets).

REGULATORY ASPECTS

INDs/NDAs

Evidence demonstrating in vivo BA or information to permit the FDA to waive this evidence must be included in the NDAs [21 CFR 320.21(a)]. A specific objective is to establish in vivo performance of the dosage form used in the clinical studies that provided primary evidence of efficacy and safety. The sponsor may wish to determine the relative BA of an IR solid oral dosage form by comparison with an oral solution, suspension, or intravenous injection [21 CFR 320.25 (d)(2) and 320.25 (d)(3)]. The BA of the clinical trial dosage form should be optimized during the IND period.

Once the in vivo BA of a formulation is established during the IND period, waivers of subsequent in vivo BE studies, following major changes in components, composition, and/or method of manufacture (e.g., similar to SUPAC-IR Level 3 changes) may be possible using the BCS. The BCS-based biowaivers are applicable to the to-be-marketed formulation when changes in components, composition, and/or method of manufacture occur to the clinical trial formulation, as long as the dosage forms have rapid and similar in vitro dissolution profiles. This approach is useful only when the drug substance is highly soluble and highly permeable (BCS Class 1), and the formulations pre- and post-change are pharmaceutical equivalents [under the definition at 21 CFR 320.1 (c)]. The BCS-based biowaivers are intended only for BE studies. They do not apply to food effect BA studies or other pharmacokinetic studies.

ANDAs

The BCS-based biowaivers can be requested for rapidly dissolving IR test products containing highly soluble and highly permeable drug substances, provided that the reference listed drug product is also rapidly dissolving and the test product exhibits similar dissolution profiles to the reference listed drug product (see "The Biopharmaceutics Classification System" and "Method of Classification"). This approach is useful when the test and reference dosage forms are pharmaceutical equivalents. The choice of dissolution apparatus (USP Apparatus I or II) should be the same as that established for the reference listed drug product.

Post-Approval Changes

The BCS-based biowaivers can be requested for significant post-approval changes (e.g., Level 3 changes in components and composition) to a rapidly dissolving IR product containing a highly soluble, highly permeable drug substance, provided that dissolution remains rapid for the post-change product and both the pre- and post-change products exhibit similar dissolution profiles (see "The Biopharmaceutics Classification System" and "Method of Classification"). This approach is useful only when the drug products pre- and post-change are pharmaceutical equivalents.

DATA TO SUPPORT BIOWAIVER

The drug substance for which a waiver is being requested should be highly soluble and highly permeable. Sponsors requesting biowaivers based on the BCS should submit the following

information to the agency for review by the Office of Clinical Pharmacology and Biopharmaceutics (for NDAs) or Office of Generic Drugs, Division of Bioequivalence (for ANDAs).

Data Supporting High Solubility

Data supporting high solubility of the test drug substance should be developed (see "Determining Drug Substance Solubility Class"). The following information should be included in the application:

- A description of test methods, including information on analytical method and composition of the buffer solutions
- Information on chemical structure, molecular weight, nature of the drug substance (acid, base, amphoteric, or neutral), and dissociation constants (pK_as)
- Test results (mean, standard deviation, and coefficient of variation) summarized in a table under solution pH, drug solubility (e.g., mg/mL), and volume of media required to dissolve the highest dose strength
- A graphic representation of mean pH-solubility profile

Data Supporting High Permeability

Data supporting high permeability of the test drug substance should be developed (see "Determining Drug Substance Solubility Class"). The following information should be included in the application:

- For human pharmacokinetic studies, information on study design and methods used along with the pharmacokinetic data
- For direct permeability methods, information supporting the suitability of a selected method that encompasses a description of the study method; criteria for selection of human subjects, animals, or epithelial cell line; drug concentrations in the donor fluid; description of the analytical method; method used to calculate extent of absorption or permeability; and where appropriate, information on efflux potential (e.g., bidirectional transport data)

A list of selected model drugs along with data on the extent of absorption in humans (mean, standard deviation, and coefficient of variation) used to establish the suitability of a method, permeability values for each model drug (mean, standard deviation, and coefficient of variation), permeability class of each model drug, and a plot of the extent of absorption as a function of permeability (mean $\pm$ standard deviation or 95% confidence interval) with identification of the low/high permeability class boundary and selected IS. Information to support high permeability of a test drug substance should include permeability data on the test drug substance, the IS (mean, standard deviation, and coefficient of variation), stability information, data supporting passive transport mechanism where appropriate, and methods used to establish high permeability of the test drug substance.

Data Supporting Rapid and Similar Dissolution

For submission of a biowaiver request, an IR product should be rapidly dissolving. Data supporting rapid dissolution attributes of the test and reference products should be developed. The following information should be included in the application:

- A brief description of the IR products used for dissolution testing, including information on batch or lot number, expiry date, dimensions, strength, and weight.
- Dissolution data obtained with 12 individual units of the test and reference products using recommended test methods as in "Determining Drug Product Dissolution Characteristics and Dissolution Profile Similarity." The percentage of labeled claim dissolved at each specified testing interval should be reported for each individual dosage unit. The mean percent dissolved, range (highest and lowest) of dissolution, and coefficient of variation (relative standard deviation) should be tabulated. A graphic representation of the mean

dissolution profiles for the test and reference products in the three media should also be included.
- Data supporting similarity in dissolution profiles between the test and reference products in each of the three media, using the f_2 metric.

Additional Information

The manufacturing process used to make the test product should be described briefly to provide information on the method of manufacture (e.g., wet granulation vs. direct compression). A list of excipients used, the amount used, and their intended functions should be provided. Excipients used in the test product should have been used previously in FDA-approved IR solid oral dosage forms.

SURROGATE METHODS

As the U.S. FDA has begun accepting recommendations for the waiver of BE requirement, protocols that prove extremely expensive in the drug development cycle, there is a greater need to develop surrogate models that one day may prove useful in securing waivers for all classes of drugs. Generally, the methods available currently show that the complexity of assay is directly proportional to its correlation with absorption of drugs in humans (Fig. 1). In this chapter, we examine more complex assay systems.

Data from both complex biological and artificial permeation assays can provide valuable information regarding the absorption of a drug (Courtesy of Millipore Corporation, Billerica, Massachusetts, U.S.A.).

Drug transport across epithelial cell barriers, especially the human small intestine, is difficult to predict. The intestinal epithelial cell barrier is a sophisticated organ that has evolved over hundreds of millions of years to become a smart, effective, and selective xenobiotic screen. Nevertheless, there is large interindividual variability in the intestinal transport of drugs. Genetic variability in key proteins is believed to be causal. There is a pressing need to better understand the key processes and how the system components interact at the molecular, cellular, and tissue level to control drug transport and determine drug absorption in the small intestine.

Is it feasible to construct an in silico framework to represent the drug absorption in the small intestine at the cellular level with internal dynamic property and concert with the update molecular biochemical mechanism? This new generation of models and computational tools might integrate the available and emerging information at different levels to better account for and predict observed experimental results. Predicting aqueous solubility with in silico tools solubility is a key drug property. It is however difficult to measure accurately, especially for poorly soluble compounds, and thus numerous in silico models have been developed for its prediction. Some in silico models can predict aqueous solubility of simple, uncharged organic chemicals reasonably well; however, solubility prediction for charged species and drug-like chemicals is not very accurate. However, extrapolating solubility data to intestinal absorption

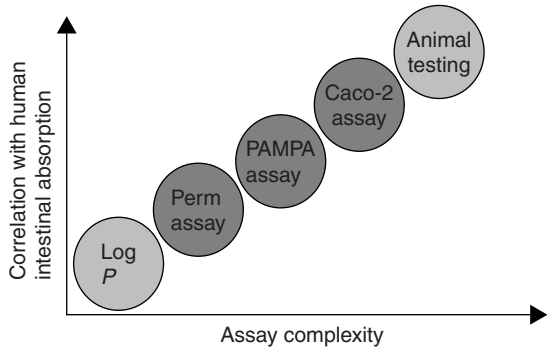

FIGURE 1 Assay complexity versus correlation with human absorption. *Abbreviation*: PAMPA, parallel artificial membrane permeability analysis.

from pharmacokinetic and physicochemical data and elucidating crucial parameters for absorption and the potential for improvement of BA are important at the preformulation stages.

The poor oral BA of drugs is generally assumed to be due to physiochemical problems, which result in poor solubility in GI tract or difficulty in diffusion through the small intestine epithelial membrane. Furthermore, the biochemical process also contributes to oral BA. The in vitro cell culture models of the intestinal epithelial cell barrier have evolved to become widely used experimental devices.

In the previous chapter, the log P factor was discussed in detail; in this chapter, we examine other methods of testing transport across membranes.

Permeability Assays

The permeability assay uses an artificial membrane composed of hexadecane. The automated systems comprise multiwell systems as shown in Figure 2.

The support membrane is 3 μm track-etched polycarbonate, 10 μm thick; the artificial membrane is hexadecane and the recommended incubation time is four to six hours (Courtesy of Millipore Corporation).

Permeability Assay Protocol

1. Into each well, add 15 μL of a 5% solution of hexadecane in hexane.
2. Dry for 45 minutes to 1 hour to ensure complete evaporation of hexane.
3. Add 300 μL of buffer with 5% DMSO at a desired pH to acceptor plate.
4. Place donor plate into the acceptor plate making sure underside of membrane is in contact with buffer.
5. Dissolve drugs of interest to the desired concentration. Add 150 μL of the drug at the desired concentration in 5% DMSO/PBS at the desired pH to each well in the donor plate.
6. Cover and incubate at room temperature for four to six hours.
7. Transfer 100 μL/well from the donor plate and 250 μL/well from the acceptor plate to separate UV–Vis compatible plates and measure the UV–Vis absorption from 250 to 500 nm (SpectraMax® plate reader, Molecular Devices, Sunnyvale, California, U.S.A.) for both plates.
8. Prepare drug solutions at the theoretical equilibrium (i.e., the resulting concentration if the donor and acceptor solutions were simply combined) and measure UV–Vis absorption from 250 to 500 nm for 250 μL/well of each.
9. Calculate log P_e and membrane retention using the equation.

$$\log P_e = \log\left\{C\cdot -\ln\left\{1-\frac{[\text{drug}]_{\text{Acceptor}}}{[\text{drug}]_{\text{equilibrium}}}\right\}\right\} \quad \text{where } C = \left\{\frac{V_D\cdot V_A}{(V_D+V_A)\text{Area}\cdot\text{time}}\right\} \tag{1}$$

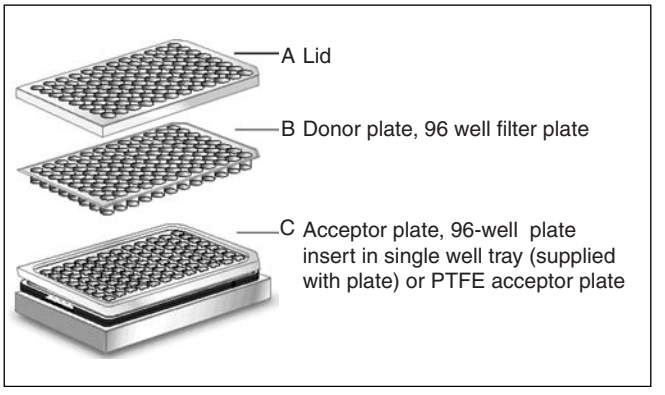

FIGURE 2 The 96-well permeability testing method. *Abbreviation*: PTFE, polytetrafluoroethylene.

PAMPA

Early drug discovery absorption, distribution, metabolism, excretion (ADME) assays, such as fast Caco-2 screens (see below), can help in rejecting test compounds that lack good pharmaceutical profiles. A cost-effective, high-throughput method—parallel artificial membrane permeability analysis (PAMPA)—that uses a phospholipid artificial membrane that models passive transport of epithelial cells is becoming increasingly popular. The PAMPA assay utilizes a range of lipid components that model a variety of different plasma membranes. The support membrane is 0.45 μm hydrophobic polyvinylidene fluoride, 130 μm thick, and artificial membrane is lecithin in dodecane; recommended incubation time is 16 to 24 hours. The protocol involves the following:

1. Dissolve drugs of interest to the desired concentration.
2. Add 300 μL of buffer with 5% DMSO at a desired pH to acceptor plate.
3. Into each well add 5 μL of lipids in organic solvent (e.g., 2% lecithin in dodecane).
4. Add 150 μL of the drug at the desired pH and concentration in 5% DMSO/PBS to each well in the donor plate.
5. Place donor plate into the acceptor plate making sure underside of membrane is in contact with buffer. Steps 3 to 5 should be completed quickly, within 10 minutes.
6. Cover and incubate at room temperature for 16 to 24 hours.
7. Transfer 100 μL/well from the donor plate and 250 μL/well from the acceptor plate to separate UV–Vis compatible plates and measure the UV–Vis absorption from 250 to 500 nm (SpectraMax plate reader) for both plates.
8. Prepare drug solutions at the theoretical equilibrium (i.e., the resulting concentration if the donor and acceptor solutions were simply combined) and measure UV–Vis absorption from 250 to 500 nm for 250 μL/well of each.
9. Calculate log P_e and membrane retention using the equation (1) above.

The permeability and PAMPA assays as described above are robust and reproducible assays for determining passive, transcellular compound permeability. Permeability and PAMPA are automation compatible, relatively fast (4–16 hours), inexpensive, straightforward, and their results correlate with human drug absorption values from the published methods. The PAMPA assay provides the benefits of a more biologically relevant system. It is also possible to tailor the lipophilic constituents so that they mimic specific membranes such as the blood–brain barrier. Optimization of incubation time, lipid mixture, and lipid concentration will also enhance the assay's ability to predict compound permeability.

Modifications of permeability and PAMPA systems have been reported, e.g., using the pION PAMPA Evolution 96 System with double sink and Gut-Box (http://www.pion-inc.com/products.htm) as a new surrogate assay that predicts the GI tract absorption of candidate drug molecules at different pH conditions. Using Beckman Coulter's Biomek FX Single Bridge Laboratory Automation Workstation PAMPA Assay System that features a 30-minute incubation time and an on-deck integrated Gut-Box and a SpectraMax microplate spectrophotometer, the permeability coefficients of drug standards with diverse physiochemical properties can be compared from both the PAMPA and Caco-2 assays automated using the Biomek FX Workstation.

These automated assays can be used for high-throughput ADME screening in early drug discovery. The double-sink PAMPA permeability assay mimics in vivo conditions by the use of a chemical sink in the acceptor wells and pH gradient in the donor wells. The use of the pION Gut-Box integrated on the deck has shortened the PAMPA assay incubation time to 30 minutes. The permeability coefficient and rank order correlates well with data obtained using the in vitro Caco-2 assay and in vivo permeability properties measured in rat intestinal perfusions.

Caco-2 Drug Transport Assays

Drug absorption generally occurs either through passive transcellular or paracellular diffusion, active carrier transport, or active efflux mechanisms. Several methods have been developed

to aid in the understanding of the absorption of new lead compounds. The most common ones use an immortalized cell line (e.g., Caco-2, Madin-Darby carine kidney (MDCK)) to mimic the intestinal epithelium. These in vitro models provide more predictive permeability information than artificial membrane systems (i.e., PAMPA and permeability assays described above) based on the cells' ability to promote (active transport) or resist (efflux) transport. Various in vitro methods listed in the U.S. FDA guidelines, acceptable to evaluate the permeability of a drug substance, include monolayers of suitable epithelial cells, and one such epithelial cell line that has been widely used as a model system of intestinal permeability is the Caco-2 cell line.

The kinetics of intestinal drug absorption, permeation enhancement, chemical moiety structure–permeability relationships, dissolution testing, in vitro–in vivo correlation (IVIVC), BE, and the development of novel polymeric materials are closely associated with the concept of Caco-2. Since most drugs are known to absorb via the intestine without using cellular pumps, passive permeability models came in the limelight. In a typical Caco-2 experiment, a monolayer of cells is grown on a filter separating two stacked micro-well plates. The permeability of drugs through the cells is determined after the introduction of a drug on one side of the filter. The entire process is automated, and when used in conjunction with chromatography and/or mass spectroscopy detection, it enables any drug's permeability to be determined. The method requires careful sample analysis to calculate permeability correctly. Limitations of the Caco-2 experiments are 21 days for preparing a stable monolayer, stringent storage conditions; however, tight junction formation prior to use is the better choice. The villus in the small intestine contains more than one cell type, the Caco-2 cell line does not produce the mucus as observed in the small intestine, and no P450 metabolizing enzyme activity has been found in the Caco-2 cell line. Test compound solubility may pose a problem in the Caco-2 assays because of the assay conditions. Finally, Caco-2 cells also contain endogenous transporter and efflux systems, the later of which works against the permeability process and can complicate data interpretation for some drugs.

The Caco-2 cell line is heterogeneous and was derived from a human colorectal adenocarcinoma. Caco-2 cells are used as in vitro permeability models to predict human intestinal absorption because they exhibit many features of absorptive intestinal cells. This includes their ability to spontaneously differentiate into polarized enterocytes that express high levels of brush border hydrolases and form well-developed junctional complexes. Consequently, it becomes possible to determine whether passage is transcellular or paracellular based on a compound's transport rate. Caco-2 cells also express a variety of transport systems including dipeptide transporters and P-gps. Due to these features, drug permeability in Caco-2 cells correlates well with human oral absorption, making Caco-2 an ideal in vitro permeability model. Additional information can be gained on metabolism and potential drug–drug interactions as the drug undergoes transcellular diffusion through the Caco-2 transport model.

Although accurate and well researched, the Caco-2 cell model requires a high investment of time and resources. Depending on the number of factors, including initial seeding density, culturing conditions, and passage number, Caco-2 cells can take as much as 20 days to reach confluence and achieve full differentiation. During this 20-day period, they require manual or automated exchange of media as frequently as every other day. The transport assays consume valuable drug compounds and normally require expensive, posttransport sample analyses (e.g., LC–MS). Therefore, the use of the Caco-2 transport model in a high-throughput laboratory setting is only possible if the platform is robust, automation compatible, reproducible, and provides high-quality data that correlate well with established methodologies.

The Millipore MultiScreen Caco-2 assay system is a reliable 96-well platform for predicting human oral absorption of drug compounds (using Caco-2 cells or other cell lines whose drug transport properties have been well characterized). The MultiScreen system format is automation compatible and is designed to offer more cost-effective, higher throughput screening of drugs than a 24-well system. The MultiScreen Caco-2 assay system exhibits good uniformity of cell growth and drug permeability across all 96 wells and low variability between production lots. The plate design supports the use of lower volumes of expensive media and reduced amounts of the test compounds. Using the MultiScreen Caco-2 assay system, standard drug compounds are successfully categorized as either "high" or "low" permeable, as defined

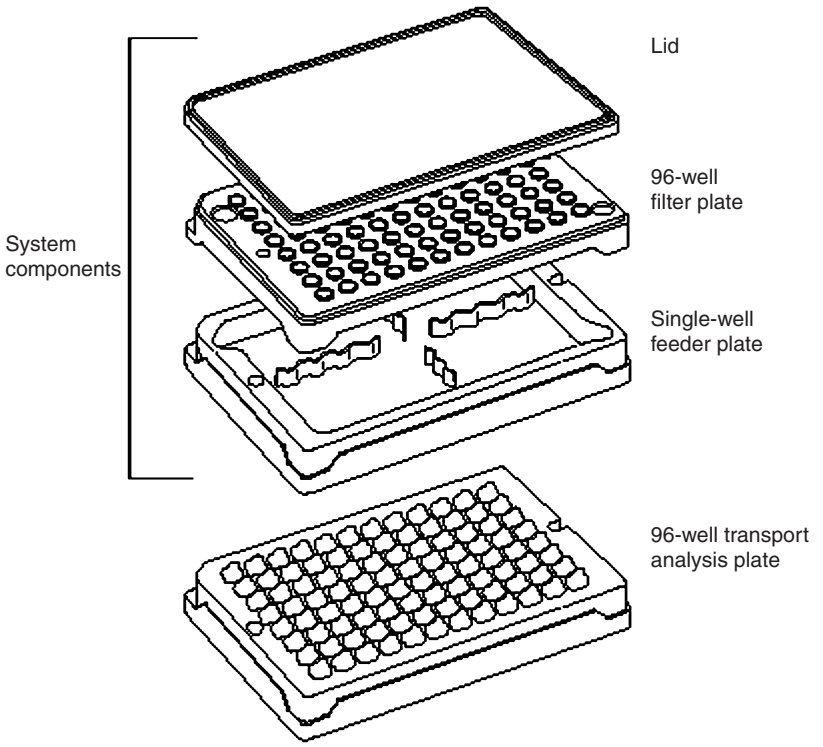

FIGURE 3 The MultiScreen Caco-2 assay system.

by the FDA, and the permeability data correlate well with established human absorption values. The components of Caco-2 Assay system are shown in Figure 3.

Components with single-well feeder plate and 96-well transport analysis plate (Courtesy of Millipore Corporation).

The apparent permeability (P_{app}) in cm/sec can be calculated for Caco-2 drug transport assays using the following equation:

$$P_{app} = \left(\frac{V_A}{\text{Area time}}\right)\left(\frac{[\text{drug}]_{\text{acceptor}}}{[\text{drug}]_{\text{initial,donor}}}\right) \quad (2)$$

where V_A is the volume (mL) in the acceptor well, "Area" is the surface area of the membrane (0.11 cm^2 for MultiScreen Caco-2 plate and 0.3 cm^2 for the 24-well plate), and "time" is the total transport time in seconds. For radio-labeled drug transport experiments, the count per minute (CPM) units obtained from the Trilux Multiwell Plate Scintillation Counter are used directly for the drug acceptor and initial concentrations such that the formula becomes

$$P_{app} = \left(\frac{V_A}{\text{Area time}}\right)\left(\frac{\text{CPM}_{\text{acceptor}}}{\text{CPM}_{\text{initial,donor}}}\right) \quad (3)$$

Historically, it has been shown that a sigmoidal relationship exists between drug absorption rates as measured with the in vitro Caco-2 model and human absorption.

$$\%\ \text{human absorption} = 100 \times \exp(a + b \times P_{app})/[1 + \exp(a + b \times P_{app})] \quad (4)$$

The Caco-2 cells are heterogeneous and their properties in final culture may differ based on the selection pressures of a particular laboratory. Direct comparison of compound permeability rates between laboratories is not possible unless the same Caco-2 cells and conditions are used. Therefore, transport rates and permeability classification ranges of specific drugs are expected to vary between reported studies. Most important is the ability

to successfully classify compounds as low-, medium-, or high-permeable drugs and produce transport results that correlate to established human absorption values.

Several modifications of the Caco-2 cell model have been tested; for example, CYP3A4-transfected Caco-2 cells are also used to define the biochemical absorption barriers. Oral BA and intestinal drug absorption can be significantly limited by metabolizing enzymes and efflux transporters in the gut. The most prevalent oxidative drug-metabolizing enzyme present in the intestine is CYP3A4. Currently, more than 50% of the drugs on the market metabolized by P450 enzymes are metabolized by CYP3A4. Oral absorption of CYP3A4 substrates can also be limited by the multidrug resistance transporter P-gp, because there is extensive substrate overlap between these two proteins. P-gp is an ATP-dependent transporter on the apical plasma membrane of enterocytes, which functions to limit the entry of the drugs into the cell. There is significant interaction between CYP3A4 and P-gp in the intestine can serve. Although Caco-2 cells express a variety of uptake and efflux transporters found in the human intestine, a major drawback to the use of Caco-2 cells is that they lack CYP3A4. As such, no data regarding the importance of intestinal metabolism on limiting drug absorption can be obtained from normal Caco-2 cells. Caco-2 cells pretreated with 1,25-dihydroxyvitamin-D_3 (vitamin D_3) express higher levels of CYP3A4 when compared with Caco-2 but still underestimate the amount of CYP3A4 in the human intestine.

Animal Model Testing

Although the quantity of substance available at the preformulation stages is generally small, in some instances, early animal testing for absorption potential is needed, particularly if the solid form of the new drug offers many options such as amorphous forms, solvates, etc. The absorption models used in animals are well described and would not be discussed here. Establishing good IVIVC at this stage proves useful because of limited access to sufficient compound to run the entire absorption profiles. The IVIVC analysis can be made extensive or general conclusions can be drawn from limited studies, and the choice depends on the amount of compound available and the nature or robustness of correlation observed.

IVIVC

The selection of a drug candidate marks the most crucial stage in the life cycle of drug development. Such selection is primarily based on the drug "developability" criteria, which include physicochemical properties of the drug and the results obtained from preliminary studies involving several in vitro systems and in vivo animal models, which address efficacy and toxicity issues. During this stage, exploring the relationship between in vitro and in vivo properties of the drug in animal models provide an idea about the feasibility of the drug delivery system for a given drug. In such correlations, study designs including study of more than one formulation of the modified release dosage forms and a rank order of release (fast/slow) of the formulations should be incorporated. Even though the formulations and methods used at this stage are not optimal, they prompt better design and development efforts in the future.

The four levels of IVIVC, which have been described in the FDA guidance, include A, B, C, and multiple C.

- *Level A correlation*: This correlation represents a point-to-point relationship between in vitro dissolution and in vivo dissolution (input/absorption rate). Level A IVIVC is also viewed as a predictive model for the relationship between the entire in vitro release time course and entire in vivo response time course. In general, correlations are linear at this level. Although a concern of acceptable nonlinear correlation has been addressed, no formal guidance on the nonlinear IVIVC has been established. Level A correlation is the most informative and very useful from a regulatory perspective.
- *Level B correlation*: In Level B correlation, the mean in vivo dissolution or mean residence time is compared with the mean in vitro dissolution time using statistical moment analytical methods. This type of correlation uses all of the in vitro and in vivo data; thus, it is not considered as a point-to-point correlation. This is of limited interest and use because more than one kind of plasma curve produces similar mean residence time.

- *Level C correlation*: This correlation describes a relationship between the amount of drug dissolved (e.g., percent dissolved at one hour) at one time point and one pharmacokinetic parameter (e.g., either AUC or C_{max}). Level C correlation is considered the lowest correlation level as it does not reflect the complete shape of the plasma concentration–time curve. Similarly, a multiple Level C correlation relates one or more pharmacokinetic parameters to the percent drug dissolved at several time points of the dissolution profile and thus may be more useful. Level B and C correlations can be useful in early formulation development, including selecting the appropriate excipients, to optimize manufacturing processes, for quality control purposes, and to characterize the release patterns of newly formulated IR and modified release products relative to the reference.

The most basic IVIVC models are expressed as a simple linear equation between the in vivo drug absorption and in vitro drug dissolved (released).

$$\text{Y (in vivo absorbed)} = mX \text{ (in vitro drug dissolved)} + C \tag{5}$$

In this equation, m is the slope of the relationship and C the intercept. Ideally, $m=1$ and $C=0$, indicating a linear relationship. However, depending on the nature of the modified release system, some data are better fitted using nonlinear models, such as Sigmoid, Weibull, Higuchi, or Hixson–Crowell.

In vivo release rate (X'_{vivo}) can also be expressed as a function of in vitro release rate ($X'_{rel,vitro}$) with parameters a and b, which may be empirically selected and refined using appropriate mathematical processes.

$$X'_{vivo}(t) = X'_{rel,vitro}(a + bt) \tag{6}$$

An iterative process may be used to compute the time-scaling and time-shifting parameters. Integral to the model development exercise is model validation, which can be accomplished using data from the formulations used to build the model (internal validation) or using data obtained from a different (new) formulation (external validation). While internal validation serves the purpose of providing basis for the acceptability of the model, external validation is superior and affords greater "confidence" in the model.

Generally, a plot of the fraction of drug absorbed (F_a) against the fraction of drug dissolved (F_d) is made, wherein the fraction absorption absorbed is obtained by deconvoluting the plasma profile. Often the goal is to develop a profile that need not a priori be a linear or even a predefined function. For example,

$$F_a = \frac{1}{f_a}\left[1 - \frac{\alpha}{\alpha - 1}(1 - F_d) + \frac{1}{\alpha - 1}(1 - F_d)^{\alpha}\right] \tag{7}$$

where F_a is the fraction of the total amount of drug absorbed at time t, f_a is the fraction of the dose absorbed at t, α is the ratio of the apparent first-order permeation rate constant (k_{paap}) to the first-order dissolution rate constant (k_d), and F_d the fraction of drug dose dissolved at time t.

Internal Validation

Using the IVIVC model, for each formulation, the relevant exposure parameters (C_{max} and AUC) are predicted and compared with the actual (observed) values. The prediction errors are calculated using

$$\text{Prediction error (\%PE)} = [(C_{max,observed} - C_{max,predicted})/C_{max,observed}] \times 100 \tag{8}$$

C_{max} can be replaced with the corresponding AUC. The criteria set in the FDA guidance on IVIVC are as follows: For C_{max} and AUC, the mean absolute percent prediction error should not exceed 10%, and the prediction error for individual formulations should not exceed 15%.

For establishing external predictability, the exposure parameters for a new formulation are predicted using its in vitro dissolution profile and the IVIVC model, and the predicted parameters are compared with the observed parameters. The prediction errors are computed as for the internal validation. For C_{max} and AUC, the prediction error for the external validation

formulation should not exceed 10%. A prediction error of 10% to 20% indicates inconclusive predictability and illustrates the need for further study using additional data sets. For drugs with narrow therapeutic index, external validation is required despite acceptable internal validation, whereas internal validation is usually sufficient with non-narrow therapeutic index drugs.

Several commercial software programs are available to study IVIVC; for example, PDx-IVIVC (http://www.globomaxservice.com/pdxivivc.htm) which is a comprehensive IVIVC software program that performs deconvolution, calculating the fraction or percentage of drug absorbed and correlating it with in vitro fraction or percentage dissolved data. It also allows Level C correlations (single or multiple), wherein a single point relationship between a dissolution parameter, for example, percent dissolved in four hours and a pharmacokinetic parameter (e.g., AUC, C_{max}, T_{max}) is determined. A successful IVIVC model can be developed if in vitro dissolution is the rate-limiting step in the sequence of events leading to appearance of the drug in the systemic circulation following oral or other routes of administration. Thus, the dissolution test can be utilized as a surrogate for BE studies (involving human subjects) if the developed IVIVC is predictive of in vivo performance of the product.

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5 Regulatory Review Process

BACKGROUND

The bioequivalence (BE) review process establishes that the proposed generic drug is bioequivalent to the reference listed drug, based upon a demonstration that both the rate and the extent of absorption of the active ingredient of the generic drug fall within established parameters when compared to that of the reference listed drug.

The Food and Drug Administration (FDA) requires an applicant to provide detailed information to establish bioequivalency. Applicants may request a waiver from performing in vivo (testing done in humans) BE studies for certain drug products where bioavailability (the rate and extent to which the active ingredient or active moiety is absorbed from the drug product and becomes available at the site of action) may be demonstrated by submitting data such as (*i*) a formulation comparison for products whose bioavailability is self evident, for example, oral solutions, injectables, or ophthalmic solutions where the formulations are identical, or (*ii*) comparative dissolution.

Alternatively, in vivo BE testing comparing the rate and extent of absorption of the generic versus the reference product is required for most tablet and capsule dosage forms. For certain products, a head-to-head evaluation of comparative efficacy based upon clinical endpoints may be required.

The Manual of Policies and Procedures of the Center for Drug Evaluation and Research (CDER) (Generic Drugs) (MAPP 5210.6) describes the following procedures for review of BE study protocols.

PROTOCOLS

Background

BE studies are frequently needed to support the filing and approval of abbreviated new drug applications (ANDAs). To conduct an adequate study and avoid unnecessary human research, any sponsor planning to conduct a bioavailability or BE study should submit the proposed study protocol to the office of generic drugs (OGD) for review prior to the initiation of the study. OGD reviews the protocol and provides advice on appropriate study design, reference material, and the proposed analytical and statistical methods to be used. Sponsors or contract research organizations (CROs) can submit protocols.

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21 CFR 320.30, inquiries regarding bioavailability and bioequivalence requirements and review of protocols by the FDA; 21 CFR 10.90, FDA regulations, recommendations, and agreements.

Policy

The division of bioequivalence (DBE) will review submitted BE protocols. The protocols will be randomly assigned to BE reviewers, unless a protocol requires the expertise of a particular reviewer. The reviewers will perform a search of the literature and the agency's databases

and prepare the review. After the protocol review, comments will be provided in a letter to the generic firm.

Procedures

When a protocol is received in the DBE, the Project Manager (PM) assigns it randomly to the next available reviewer. All protocols received are entered in the protocol tracking system and assigned a control number. The protocol receipt date, firm name, drug name, reviewer assigned, and date of assignment are recorded. The reviewer searches the literature and the agency's databases [e.g., Excalibur, WinBio, drug files (hard copy and electronic)]. If a protocol has been previously submitted and found acceptable by the division, this should be used as a model in the preparation of responses to subsequent protocols for the same drug. The reviewer should state in the review whether other protocols for the same drug have been previously reviewed. If no other protocols have been reviewed for the product, a statement to that effect should be included in the review. The reviewer prepares a review with recommendations to the requestor. The review must have the concurrence of the team leader and division director. If the reviewer discovers discrepancies in BE criteria or appropriate study design in recommendations provided to industry in previous protocols or correspondence for the same drug product, the reviewer prepares a memorandum to the team leaders and division director. The memo should specify the name of the sponsor or CROs that received conflicting information/guidance in protocol responses. ANDAs affected by this information should also be noted. Once the review is finalized and has the concurrence of the division director, it is forwarded to the PM. The PM or Transportation Investment Area (TIA) drafts a letter based on the reviewer's recommendation. The PM ensures that all recommendations are provided to the firm. The letter will be routed through the team leader for corrections and endorsement, and to the division director for signature. Once the letter is signed by the division director, the PM or TIA enters into the protocol tracking system the date the review was finalized and the date the letter was issued. The protocol is then forwarded to the Document Room. Document Room personnel mail the letter and store the protocol in the designated area. The PM drafts letters to sponsors or CROs that have received outdated information to ensure that consistent information is provided to industry.

PRODUCTIVITY DOCUMENTATION

Background

The Company Officer Management Information System (COMIS) database was created, in part, to keep track of the workload of all divisions. Information on all submissions received in OGD on ANDAs is entered into this system, including the applicant's name, ANDA number, drug name, dosage form, strengths, letter date, and receipt date. The BE section of an ANDA contains data on the demonstration of BE, such as BE studies, studies with clinical endpoints, dissolution data, and waiver requests. The BE data entry screen in COMIS keeps a record of (*i*) the reviewer assigned to the submission, (*ii*) the type of studies submitted in the BE section, and (*iii*) the dates when the review was initiated and satisfactorily completed by the reviewer. Other work, such as controlled correspondence and protocols, is tracked in separate databases. The overall productivity of the division and the reviewers is monitored using the information in COMIS and the other databases.

Policy

Information entered into the COMIS database on the study types in the BE section of an ANDA documents the overall productivity of the reviewers and the division. Consistent and fair classification of these study types ensures objective evaluation of reviewers. Non-ANDA-related work is tracked in separate databases. That information includes a control number, name of sponsor, drug name, name of assigned reviewer, date of assignment, date of completion of the review, and dates when letters are issued.

Procedures

When the Document Room assigns an ANDA to the DBE, a description of the BE section is entered into the BE data entry screen in COMIS, using the study types below.

BE Studies

1. *Fasting Study* (*STF*). This includes replicate study designs and combined studies (e.g., combined fasting and multiple-dose studies where the same subjects are used).
2. *Food Study* (*STP*).
3. *Multiple-Dose Study* (*STM*).
4. *Study* (*STU*). This category is generally used for a BE study with clinical endpoints, in vitro studies for metered-dose inhalers and nasal sprays, pilot and pivotal studies for vasoconstrictors, or any pharmacokinetic/pharmacodynamic study other than a standard BE study (such as 1–3 above).

Dissolution Data (DIS)

This code is usually used when dissolution data are the only basis for approval. Examples are AA drugs and supplements for which changes in formulation or manufacturing require dissolution data only. In vitro release data for topical products may also be coded under DIS.

Note: Dissolution data submitted for the same strength drug that was the subject of a BE study are not separately coded. The dissolution information is considered part of the study.

Other (OTH)

1. *Study amendment* (*STA*). This category is for responses to deficiency comments. Whether the amendment contains dissolution data or addresses a deficiency such as incomplete information on analytical methods or a study, the submission should be coded as STA unless a new study is submitted for review. In that case, the appropriate code under BE studies should be selected. If an amendment to a previously submitted BE study is included with a new, not previously submitted BE study required to establish BE, then STA should be coded for the amendment, and the new study should be coded separately. Retesting of subjects classified as outliers in the original submission should not be classified as a separate study, but as part of the original study. Frequently, the division telephones sponsors to request information needed to finalize the review. These requests should be made for information the sponsor can respond to within 10 working days, and should be coded as STA. If the sponsor submits incorrect information or partial data, the submission should be coded as new correspondence (NC). Once the correct information is received, the submission should be coded as STA.
2. *Waiver* (*WAI*). This category is used for injectable, ophthalmic, otic, oral, and topical solutions. A formulation in the same concentration packaged in different sizes is not coded separately, but different concentrations of the same product are coded separately.
3. *Dissolution Waiver* (*DIW*). This code is used for lower strengths that can be approved based on proportionality of the formulation and an acceptable study on the highest strength or the strength of the reference listed drug. A dissolution waiver should be coded for each strength for which dissolution data are submitted, except the strength for which BE studies have been conducted.
4. *Other* (*OTH*). This category is used for correspondence or addenda revising the original review. The Division of Scientific Investigations (DSI) inspection reports may generate an addendum to the review. If a significant statistical analysis is needed based on the recommendation of the DSI, or if the issuance of a Form 483 (Inspectional Observations) indicates serious violations by the laboratory, then the review of the DSI report may be coded as OTH. If the DSI report is acceptable, the DSI report should be filed in the ANDA, and no addendum to the review is necessary. Addenda to the reviews are entered as U.S.

documents (FDA generated), because these reviews are not prompted by industry submissions, but are due to internal policy changes or inspection reports. Diskettes containing the data already coded in a previous submission will not be coded separately.

Protocols

1. *Protocol* (*PRO*). This is used for protocols submitted as part of an investigational new drug application (IND) or an ANDA. An example of a protocol submitted as part of an ANDA would be a skin irritation study protocol.
2. *Protocol Amendment* (*PRA*). Amendment to a Protocol.
3. *Other Protocols*. There are also protocols sent to the DBE for review to obtain comments on the proposed study design prior to the submission of ANDAs. Pilot studies submitted with a protocol to justify a particular study design are not coded separately. A review is generated and comments are provided to the firm by letter. This is not recorded in COMIS. It is tracked in a separate database and is counted as part of the overall productivity of individual reviewers. Occasionally, sponsors submit protocols for studies that are not necessary (i.e., a waiver request for in vivo testing). In this case, the additional protocol does not have to be reviewed and credit will not be given.

Controlled Correspondence

BE information requests sent as correspondence are also randomly assigned to DBE reviewers for evaluation and generation of a review. These reviews are not recorded in COMIS, but are tracked in a separate database and counted as part of the overall productivity of individual reviewers. A citizen petition is counted as controlled correspondence. If additional information is submitted for pending correspondence and/or citizen petitions prior to the completion of the response to the original piece, the issues raised by the additional supplement to the submission should be addressed in the review underway. If a review has been finalized and an additional supplement is submitted raising new issues, another review can be generated.

PROCESSING OF WORK

The reviewers sign their names in the assignment logbook. When an assignment is available, the BE PM assigns it to the next reviewer and enters the reviewer's name and date of assignment in the appropriate database (COMIS, protocols, controlled correspondence) and the assignment logbook. The PM also verifies study codes at this time. The reviewer obtains the submission from the document room. When the review is completed, the reviewer states on the last page of the review the study types reviewed in the submission and comments on the acceptability of the data provided by the firm. The following decision codes should be used when determining the acceptability of each study type.

1. AC (acceptable). The submission was complete and all data were found acceptable.
2. UN (unacceptable). A study failed to meet standard criteria for BE (e.g., 90% CI for fasting study, incorrect dissolution methods).
3. IC (incomplete). Information was missing from the submission.
4. NC (no action). No action or review was necessary.

The team leaders verify that study codes and decision codes are accurate. Once the review is finalized and has the division director's concurrence, it is forwarded to the BE PM, who forwards acceptable comments to the chemistry PM or prepares fax cover sheets for deficiencies to be transmitted to the firm. The BE PMs then deliver acceptable completed reviews to the Document Room. Reviews containing deficiencies to be transmitted to the firm are delivered to the Review Support Branch Chief, who gathers any comments from other

disciplines (chemistry, labeling, and microbiology), and faxes all deficiencies and comments together. The Document Room staff enters data into the BE data entry screen in COMIS, including the completion date (the date when the Director of BE signed the review). The Document Room staff also verifies study codes and enters decision codes. This closes the submission, indicating that the review has been completed. Once the submission is closed, reviewers are credited for their work.

INSPECTIONS

Background

This MAPP outlines policies and procedures to use in (*i*) identifying when to request inspections of clinical facilities or analytical laboratories associated with BE studies and (*ii*) applying inspection information to the review of ANDAs.

In vivo BE studies are used to support the approval of many ANDAs. To help ensure that these studies are reliable, the OGD needs information on the inspection status of clinical facilities and analytical laboratories where the studies are conducted.

OGD requests information on the compliance status of relevant clinical facilities and laboratories from the Good Laboratory Practice (GLP)/bioequivalence investigations branch (GBIB), DSI, Office of Medical Policy.

OGD requests that GBIB initiate a *routine inspection* of clinical facilities or analytical laboratories conducting BE studies included in an unapproved ANDA if

1. A clinical facility or analytical testing site is identified in the ANDA that has no inspection history, was classified official action indicated OAI on its last inspection, or has not been inspected within the past three years.
2. A clinical facility and/or analytical laboratory is performing a nonconventional BE study for which it has never been inspected by DSI (e.g., a study using pharmacodynamic endpoints to assess BE).
3. OGD requests a *directed inspection* of a facility if there is a question about the quality or integrity of the data submitted in an ANDA. Instances of suspect data may include missing data points, errors in calculation, or inadequate documentation.
4. Any material information derived from inspection and during the review of applications and documents.

METHODS VALIDATION FOR ANDAS

Background

Since 1981, methods validation has not been an approval criterion for new drug applications (NDAs). Until 1997, however, OGD's policy was to require satisfactory methods validation before approval of ANDAs for noncompendial drug products. In some cases, ANDA approvals were delayed pending completion of methods validation. Validation of the analytical methods and testing procedures was considered an important component when ensuring application approvability. However, there were circumstances when a delay in completion of the methods validation process was beyond the control of the applicant. In those instances, OGD wanted to ensure that an application that was otherwise eligible for approval was approved without undue delay. Therefore, in November 1998, OGD revised its policy regarding methods validation for applications that have been recommended for approval to allow approval of an ANDA if (*i*) there was no undue delay in sample submission by the applicant, (*ii*) there is no apparent problem with the validation in progress or the validation has not been initiated by the servicing laboratory, and (*iii*) there is a commitment from the applicant to resolve any problems with methods validation. Now, to better use the limited resources of the program to ensure adequacy of critical and/or complex methods, OGD has determined that there are other situations in which methods validation is not needed to support approval of ANDAs. Consequently, OGD is revising its policy regarding methods validation consistent with this determination.

References

Compliance Program on Preapproval Inspections CP7346.832
21 CFR 314.50(e), samples and labeling
21 CFR 314.70, supplements and other changes to an approved application

Policy

Methods validation requests will be limited to noncompendial drug products and, with team leader and division director (or deputy) concurrence, will be further subject to reviewer discretion because of specific concerns (i.e., for cause) relating to a drug product or an analytical method. Representative for cause examples include (but are not limited to):

- New emerging analytical technologies
- Analytical methods for novel/complex drug delivery systems [e.g., transdermal delivery system (TDS), metered-dose inhaler (MDI), nasal spray]
- Chromatographic methods for quantitation of low dose drugs
- Chromatographic methods for resolving multiple drug components with concomitant impurities/degradants.

OGD does not require or request methods verification by an FDA laboratory of a product for which a United States Pharmacopoeia (USP) monograph exists. However, FDA laboratories may conduct methods verification analyses of compendial products at their option. Application approval is not dependent on receipt of these test results. Proposals for alternative analytical methods for products that are the subject of a USP monograph will be evaluated during the review process. There is no need for FDA laboratories to validate the alternative methods since the official methods for regulatory purposes are those of the USP and, therefore, OGD does not request methods validation for alternative methods for compendial products.

If there is no USP monograph for a drug substance or drug product, the applicant's proposed regulatory analytical methods may be validated by an FDA laboratory.

Under certain other circumstances, methods validation for an ANDA for a noncompendial drug product may clearly be waived. The final decision should be documented in the application. Circumstances that support a waiver include, but are not limited to:

- The proposed analytical methods have been validated previously in an FDA laboratory under another of the same applicant's ANDAs for a similar drug product (e.g., different strength, different packaging configuration).
- There exists in the compendium a monograph for a similar dosage form (e.g., for injection vs. injection) containing the applicant's proposed regulatory methods, and the reviewer has verified that the change in dosage form will cause no analytical interferences in the compendial procedures. That is, the reviewer has verified the suitability of the compendial methods under actual use conditions.
- The division director will sign off on an approval package if all aspects of the ANDA are complete and satisfactory, excluding methods validation and establishment evaluation request (EER) results.
- OGD will not wait for completion of methods validation to begin the administrative review process.

Upon completion of the administrative review process, the application will be approved if all other aspects of the ANDA, including the EER and office-level BE review, are satisfactory and the following criteria are met:

- There is no undue delay in sample submission by the applicant.
- There is no apparent problem encountered with the validation in progress, or the validation has not been initiated by the servicing laboratory.

- There is a commitment from the applicant in the ANDA to resolve any problems with methods validation.

OGD expects the applicant to provide samples to the servicing laboratory within 10 working days of the request and will consider longer time frames to be *undue delay*. If it is determined that there were delays in the provision of samples to the laboratory, or if significant problems are identified in the course of methods validation, OGD will not approve the application before the *completion* of the methods validation and the resolution of the deficiencies. Whether pre- or post-approval, the chemistry review branch will evaluate negative laboratory findings and determine their impact on the applicable submission.

Procedures

A request for validation of the applicant's proposed regulatory analytical methods is sent by the review chemist to the Office of Regulatory Affairs (ORA) coordinator in the division of field science (DFS) using form FDA 2871a. This action should be taken as soon as the need is identified and the test methods are determined to be adequate by the review chemist.

- A copy of the methods, testing specifications, and composition statement is to be included with the request. The package is sent to DFS by current procedures.
- Requests are processed and carried out as detailed in the supplement to the Compliance Program on Preapproval Inspections CP7346.832.

The chemistry/microbiology review is included in the approval package, along with the BE and labeling reviews. Upon concurrence by the chemistry team leader, the package proceeds through the final administrative review channels. If, after administrative review, the application remains approvable (including an acceptable EER and office-level BE endorsement), the PM determines the status of the methods validation process. The application can be approved with or without results of the methods validation, except under the circumstances noted below.

- There was an undue delay in sample submission by the applicant.
- There are problems identified in the course of methods validation by the servicing laboratory.
- There is no commitment from the applicant to resolve any problems subsequently found by the FDA laboratory.

Any problem identified with the method or the product is evaluated by the review chemist for its significance. Any problem that potentially affects the quality of the drug product must be resolved before application approval. When approval is granted in the absence of a completed methods validation, the approval letter is revised to include the following statement as the last paragraph. *Validation of the regulatory methods has not been completed. It is the general policy of the OGD not to withhold approval until the validation is complete.*

The approval letter is endorsed by the chemistry reviewer and team leader as well as the division director. If the laboratory results are received during the administrative review process for approval and they reveal problems with the methods or the product, the approval of the application is delayed and the results transmitted to the applicant. The applicant is asked to address these issues as soon as possible in an amendment to the application. This amendment is given priority review in consultation, if necessary, with the servicing laboratory. If the amended methods are satisfactory to OGD and they address the concerns of the laboratory, the application can then be approved, provided all other aspects of the application are acceptable. Out-of-specification results on products already expired at the time of testing are evaluated for their significance and relevance. Any product failures must be satisfactorily resolved before application approval. Routine revalidation can be done after approval of the application. The review chemist can request testing at a second FDA laboratory to resolve conflicting results obtained by an applicant and by the FDA servicing laboratory. The team leader and the division director must concur with the request. For methods validation completed after an application

is approved, any deficiencies identified are communicated promptly to the applicant. Generally, the response addressing the deficiencies can be submitted as a changes-being-effected supplement. If the methods validation is waived, this fact must be documented and filed in the ANDA.

Regulatory Audit of BE Studies Submitted

When BE studies are submitted as part of an ANDA, the U.S. FDA inspections include an audit of the studies submitted under the Compliance Program 7348.001. It is important to review these directives since it allows firms to prepare studies and have them ready for presentation in a format that is readily accessible and comprehensible. This applies to both domestic and international inspections. When the clinical and analytical portions of a study have been performed at separate locations, separate reports should be prepared and submitted for each site.

PART I—BACKGROUND

The BE Regulations (21 CFR 320) of January 7, 1977, and its amendments stated the requirements for submission of in vivo bioavailability and BE data as a condition of marketing a new (i.e., new chemical compound; new formulation, new dosage form, or new route of administration of a marketed drug) or generic drug. 21 CFR 320 also provided general guidance concerning the design and conduct of bioavailability/BE studies. However, it should be noted that BE studies conducted to support ANDAs involve testing of already approved drug entities and therefore, generally do not require an IND. However, sponsors of generic drugs need to file INDs when studies involve a route of administration or dosage level or use in a patient population or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug product [21 CFR 312.2(b)(iii)].

The FDA does not require BE studies on pre-1938 drug products. It is however the responsibility of the firm to assure that the studies are submitted in accordance with the most current guidelines as amended, BE studies involve both a clinical component and an analytical component. The objective of a typical BE study is to demonstrate that the test and reference products achieve a similar pharmacokinetic profile in plasma, serum, and/or urine. BE studies usually involve administration of test and reference drug formulations to 18 to 36 normal healthy subjects, but patients with a target disease may also be used.

Formulations to be tested are administered either as a single dose or as multiple doses. Sometimes formulations can be labeled with a radioactive component to facilitate subsequent analysis. In a BE study, serial samples of biologic fluid (plasma, serum, or urine) are collected just before and at various times after dose administration. These samples are later analyzed for drug and/or metabolite concentrations. The study data are used in subsequent pharmacokinetic analyses to establish BE.

In some situations, the clinical and analytical facilities for a study may be part of the same organization and therefore may be covered by one district. In other situations, the two facilities may be located in different districts. For the purpose of this program, the district where the clinical facility is located will be referred to as the clinical component district, and the district where the analytical facility is located will be referred to as the analytical component district.

PART II—IMPLEMENTATION

Objectives

1. To verify the quality and integrity of scientific data from BE studies submitted to the CDER;
2. To ensure that the rights and welfare of human subjects participating in drug testing are protected; and

3. To ensure compliance with the regulations (21 CFR 312, 320, 50, and 56) and promptly follow-up on significant problems, such as research misconduct or fraud.

Program Management Instructions

Coverage

It is important to draw distinctions between a clinical laboratory, a clinical facility, and an analytical facility. A clinical laboratory generally uses blood and/or urine to conduct medical screening or diagnostic tests such as complete blood counts (CBC), liver function tests [alanine aminotransferase (ALT), aspartate aminotransferase (AST)] or kidney function (blood urea nitrogen (BUN), creatinine clearance, etc.) tests. Clinical laboratories are usually certified under programs based on the Clinical Laboratories Improvement Act (42 USC 263a), and are not routinely inspected by the FDA. A clinical laboratory may be visited during a BE study audit to confirm that reported screening or diagnostic laboratory work was indeed performed. The clinical facility and the analytical facility as described above are the laboratories that will be routinely inspected under this program.

1. *Clinical Facilities*. Clinical facilities conduct BE studies (including screening, dosing, monitoring of subjects' safety, etc.) in order to obtain biological specimens (e.g., plasma, serum, urine) for analysis of drug and/or drug metabolite concentrations. Facilities that conduct BE studies in human research subjects for pharmacodynamic measurements (i.e., clinical or pharmacological effects) are also included.
2. *Analytical Facilities*. Analytical facilities analyze biological specimens collected in BE studies and other human clinical studies for drug and/or metabolite concentrations to measure the absorption and disposition of the drug.
3. *Clinical and Analytical Investigators*. The clinical investigator in a BE study is involved in the screening and dosing of human subjects, and will ordinarily be a physician. Ph.D. clinical pharmacologists and Pharm.D.'s are acceptable if a physician is available to cover medical emergencies. The clinical investigator may also perform pharmacodynamic measurement(s) and evaluation activities of clinical or pharmacological endpoints. The analytical investigator in a BE study is the scientist in the analytical facility responsible for assay development and validation, and analyses of biological specimens, e.g., Scientific Director or Laboratory Director.

Process

Facilities where BE studies are conducted are to be to include a review of the clinical and analytical testing procedures plus an audit of source data from one or more specified studies. Assignments under this program are of two basic categories:

1. *Directed Data Audit*: Covers studies and/or facilities in which gross problems/inadequacies are suspected (including, but not limited to research misconduct, or fraud). Such assignments require rapid evaluation and resolution.
2. *Routine Data Audit*: Covers (*i*) pivotal studies under current review in the divisions of Pharmaceutical Evaluation I (HFD-860), II (HFD-870), or III (HFD-880) in the Office of Clinical Pharmacology and Biopharmaceutics (HFD-850); and (*ii*) BE studies supporting the approval of a generic product.

Assignments will be issued by the GLP and the (HFD-48) to the field. For each assignment, a scientific reviewer in GBIB with expertise in chemical assays, bioavailability/BE, biopharmaceutics, pharmacokinetics or pharmacodynamics will (*i*) assist the field in coordinating and as necessary conducting the inspection; (*ii*) provide technical guidance and on-site support to the field as necessary; and (*iii*) serve as the liaison between the field investigator(s) and the Review divisions in CDER.

GBIB will generate assignments under this program based on information provided by the Review divisions in CDER. GBIB will send assignment memos to the director of the Investigations Branch in the appropriate district office(s) (for domestic inspections),

or the division of Emergency and Investigational Operations, International Operations Group (for foreign inspections). The assignment memo will include the following information:

1. NDA/ANDA number
2. Name of the drug
3. Name of the sponsor
4. Study/protocol number(s)
5. Title of each study identified for inspection
6. Address(es) of the clinical and analytical facilities
7. Instructions on inspectional areas
8. Deadline(s) such as preferred date for completion of inspection, Review division action goal date, or the user fee goal date, etc.
9. The name of the GBIB contact

After a field investigator has been assigned, background material [including source data from the specific study(ies)] will be forwarded to the field investigator. In the event that a clinical or analytical facility designated for inspection is found to be located elsewhere, the district should contact GBIB immediately in order to redirect the assignment.

For all inspections in which a Form FDA-483 is issued, a copy of the Form FDA-483 should be forwarded by facsimile to the GBIB contact or the Branch Chief of GBIB.

PART III—INSPECTIONAL

Operations

Inspectional

A complete inspection report under this compliance program consists of inspectional findings covering:

1. *Clinical testing*, which includes the adequacy of facilities and procedures utilized by the clinical investigator along with a data audit of the specific study(ies) identified by GBIB; and
2. *Analytical testing*, which includes the adequacy of the facilities, equipment, personnel, and methods and procedures utilized at the analytical facility including an audit of the method validation and analytical data for the study(ies) identified by GBIB.

A full narrative report of any deviations from existing regulations is required. Deviation(s) must be documented sufficiently to support legal or administrative action. For example, any records containing data that are inconsistent with data submitted to FDA should be copied and the investigator should identify the discrepancy. Generally, serious violations will require more extensive documentation a discussion between the inspector and his supervisor and the appropriate Center contact prior to embarking on this type of coverage.

Investigational

If inspections of institutional review boards and/or clinical laboratories are indicated, the inspector is required to contact his supervisor and GBIB for guidance prior to initiating the inspection.

Refusals

1. If access to, or copying of records is refused for any reason, the inspector promptly contacts his supervisor so that the GBIB contact can be advised of the refusal. Send follow-up information via Smart Messaging Service (SMS) to GBIB, and Office of Regulatory Affairs (ORO) contacts. The same procedure is followed when it becomes evident that delays by the firm constitute a de facto refusal.

2. If actions by the firm take the form of a partial refusal for inspection of documents or areas to which FDA is entitled under the law, inspector calls attention to 301(e) and (f) and 505(k)(2) of the FD&C Act; if the refusal persists, he telephones his supervisor and the GBIB contact for instructions.
3. If the proper course of action to deal with a refusal cannot be resolved expeditiously by GBIB or ORO, GBIB will notifies the Bioresearch Program Coordinator (HFC-230).

Findings

1. If the inspector encounters serious problems with the data, methodology, quality control (QC) practices, etc., he will continue with the originally assigned inspection, but contact GBIB for advice on possibly expanding the inspection. GBIB will determine if an in depth inspection, involving additional BE studies, should be initiated.
2. If the inspector encounters questionable or suspicious records and is unable to review or copy them immediately and have reason to preserve their integrity by officially sealing them, the inspector contacts his supervisor immediately for instructions. Procedures exist for the inspector district to clear this type of action by telephone with the ORA/Bioresearch Program Coordinator (HFC-230). See *Inspection Operations Manual*, Section 453.5.
3. Issuance of a Form FDA-483, Inspectional Observations, is appropriate when (*i*) practice at the clinical site deviates from the standards for conduct of a clinical study as set forth in 21 CFR 312 and 320 and 361, (*ii*) practice at the analytical site deviates from the standards of laboratory practices as set forth in 21 CFR 320, and (*iii*) discrepancies have occurred between source data and reported data in the case report forms. Examples of noncompliance to study standards at the clinical and analytical sites are listed in Part V of this guidance. Observed deficient practices should be discussed with the responsible officials.

PART IV—REGULATORY/ADMINISTRATIVE STRATEGY

Clinical Testing

Examples of Noncompliance

1. Subjects not receiving the test or reference drug formulation according to the study randomization codes.
2. Biological samples compromised by improper identification, handling or storage.
3. Failure to report adverse experiences, such as vomiting, and diarrhea, which may affect absorption and elimination of drugs.
4. Inadequate drug accountability records.
5. Inadequate medical supervision and coverage.
6. Significant problems/protocol deviations/adverse events not reported to the sponsor.
7. Failure to adhere to the inclusion/exclusion criteria of the approved protocol.
8. Inadequate or missing informed consent for participating subjects.
9. Any other situation in which the health and welfare of the subjects are compromised.

Analytical Testing

Examples of Noncompliance

1. Inconsistencies between data reported to the FDA and at the site.
2. Inadequate or missing validation of assay methodology with respect to specificity (related chemicals, degradation products, metabolites), linearity, sensitivity, precision, and reproducibility.
3. Failure to employ standard, scientifically sound QC techniques, such as use of appropriate standard curves and/or analyte controls that span the range of subjects' analyte levels.

4. Failure to include all data points, not otherwise documented as rejected for a scientifically sound reason, in determination of assay method precision, sensitivity, accuracy, etc.
5. Samples are allowed to remain for prolonged periods of time without proper storage.
6. Failure to maintain source data, e.g., source data written on scrap paper and/or discarded in trash after transferring to analytical documents.
7. Lack of objective standard for data acceptance of calibration standards, QCs, etc.
8. Unskilled personnel conducting analytical procedures.
9. No documentation of analytical findings.
10. Inadequate or no written procedures for drug sample receipt and handling.
11. Inadequate or missing standard operating procedures.

Note: The above are not all-inclusive lists of examples of clinical and analytical noncompliance.

BE INSPECTION REPORT

PART I—FACILITIES AND PROCEDURES (CLINICAL AND ANALYTICAL)

Facilities (Clinical and/or Analytical)

1. Evaluate the general facilities for adequate space, work flow patterns, separation of operations, etc.
2. Comment on potential or actual problems, such as:
   a. adjacent clinic rooms housing concurrent studies;
   b. open windows allowing ingress of unauthorized food, drugs, etc., into clinic rooms;
   c. are dropped ceilings sealed or monitored to prevent storage of nonpermitted materials;
   d. other conditions that may compromise study security, contribute to the potential for sample mix-up, sample contamination/degradation, etc.
3. Comment if the facilities do not appear adequate to support their normal workload.
4. Are there written, dated, and approved standard operating procedures, readily available to all personnel in their work areas? Are working copies kept current?
5. Are outdated procedures archived for future reference?
6. Are visitors to the clinical facility permitted? How are visitors monitored to prevent passage of nonpermitted materials to the study subjects?
7. Are off site trips for smoking or other reasons monitored to prevent consumption of nonpermitted materials or passage of such materials to or from unauthorized persons?

Personnel

1. Check the relevant qualifications, training, and experience of personnel. Assess staff's ability to perform assigned functions. Document any deficiencies that relate to the audited study(ies).

Specimen Handling and Integrity

In the Clinic

Check and describe:

1. Procedures for positive subject and sample identification so that study, drug, subject, sampling time, etc., are linked.
2. Procedures for adherence to processing time, temperature, and light conditions as specified by analytical method.
3. Storage conditions before and after processing, as well as during transit to the laboratory.
4. Precautions against sample loss and mix-up during storage, processing, and transit to the laboratory.

In the Analytical Laboratory

1. Determine if the analytical facility receives BE samples from other locations. If yes:
   a. Are there freight receipts for sending/receiving samples?
   b. Is a documented history of sample integrity available (e.g., the sample storage time and conditions prior to shipment)?
   c. Is the length of time in shipment recorded?
   d. Evaluate the type of transportation employed, and type of protection provided (e.g., shipped by air in insulated containers of dry ice). Report any questionable practices.
   e. What arrangement(s) can be made for receiving shipments outside of normal working hours?
   f. Are the conditions of the samples noted upon arrival at the analytical laboratory, along with the identity of the person(s) receiving the samples?
   g. Are there procedures and documentation to assure that the samples remained at the proper temperature during shipment and holding?
2. Describe the storage equipment for BE samples until analysis (e.g., GE Freezer, chest type, Model #417, etc.)
3. Evaluate the equipment and procedures (e.g., ultraviolet light protection) for storing and maintaining BE samples, prior to and during analysis.
   a. Compare storage capacity versus number of samples in storage.
   b. Examine set points for alarms and temperature controlling/recording devices.
   c. Review procedures for calibration and maintenance of alarms and controllers/recorders.
   d. Determine practices for monitoring, review, and storage of temperature records.
   e. Report any evidence of sample thawing.
   f. Check integrity of study samples.
   g. Determine if action plans are in place in case of power loss leading to abnormal storage conditions, i.e., emergency procedures.
4. Determine if samples are labeled and separated in storage and during analysis to prevent sample loss or mix-up between studies, subjects, and test/reference drug?
5. Examine how sample identification is maintained through transfer steps during analysis.
6. Is there accurate documentation to show how many freeze and thaw cycles the samples have been subjected to, including accidental thawing due to equipment failure(s)?

ELECTRONIC RECORDS AND SIGNATURES

The FDA published the Electronic Records; Electronic Signatures; Final Rule (21 CFR 11) on March 20, 1997. The rule became effective on August 20, 1997. Records in electronic form that are created, modified, maintained, archived, retrieved, or transmitted under any records requirement set forth in agency regulations must comply with 21 CFR 11. The following questions are provided to aid evaluation of electronic records and electronic signatures:

1. Are electronic data systems used to gather clinical (e.g., adverse experiences, concomitant medications) and analytical data (e.g., peak heights, peak areas of chromatograms)? Are such systems used to store, analyze, and/or calculate pharmacokinetic/pharmacodynamic modeling, or to transmit clinical and analytical data to the sponsor? If so, identify the system(s), and summarize the system(s)' capabilities. If electronic data systems are not used, omit coverage of the remainder of this section.
2. Determine the source(s) of data entered into the computer for accuracy, security, and traceability.
   a. Direct electronic transfer of on-line instrument data.
   b. Case report forms, analytical worksheets or similar records requiring manual data entry.
   c. Chromatograms requiring evaluation prior to manual extraction of data.
   d. Other.

3. Determine the following:
   a. Who enters data and when?
   b. Who verifies data entry and when?
   c. Who has access to computer and security codes?
   d. How are data in computers changed? By whom? Audit trail?
4. Determine if the sponsor gets source data or tabulated, evaluated data.
5. Determine how data are transmitted to sponsor (hard copy, computer disk, fax, modem, etc.).
6. If the *sponsor* discovers errors, omissions, etc., in the final report, what contacts are made with the investigator; how are corrections effected, and how are they documented?
7. Determine how data are retained by the investigator (hard copy, electronic, etc.).
8. Determine if the firm has standard operating procedures (SOPs) for validation of computer systems involved in storing, analyzing, calculating, modeling, and/or transmitting clinical and analytical data. Have the computer systems been validated according to the SOPs? Are results of the validations documented and available for audit? Summarize the validated capabilities of the computer systems with respect to their effect on the validity of the study data.

CLINICAL DATA AND OPERATIONS

General

Inspections of clinical facilities should include a comparison of the practices and procedures of the clinical investigator with the requirements of 21 CFR 312, 320.

Inspections should also include a comparison of the source data in the clinical investigator's files with the data submitted to the FDA. Original records should be reviewed, including medical records, dosing records, clinical laboratory test reports, adverse reaction reports, concomitant medications records, nurses' notes, etc.

Inspection Procedures

This part identifies the minimum information that must be obtained during an inspection to determine if the clinical investigator is complying with the regulations. Each FDA investigator should expand the inspection as facts emerge. The inspections should be sufficient in scope to determine the clinical investigator's general practices for each point identified, as well as the particular practices employed for the study(ies) under audit.

Study Responsibility and Administration

1. Determine if the clinical investigator was aware of the status of the test article(s), nature of the protocol, and the obligations of the clinical investigator.
2. Determine whether authority for the conduct of various aspects of the study was delegated properly so that the investigator retained control and knowledge of the study.
3. Determine if the investigator discontinued the study before completion. If so, provide reason.
4. Determine the name and address of any clinical laboratory performing clinical laboratory tests for qualifying and/or safety monitoring of study subjects.
   a. If any clinical laboratory testing was performed in the investigator's own facility, determine whether that facility is equipped to perform each test specified.
   b. Determine if individuals performing the clinical tests are adequately qualified.

Protocol

Obtain a copy of the written protocol. Unavailability should be reported and documented. If a copy of the protocol is sent with the assignment background material, it should be compared to the protocol on site. If the protocols are identical, a duplicate copy does not need to be obtained. The narrative should note that the protocols were identical. If the protocol has been accepted

by a Review division in CDER, a copy of the acceptance letter should be attached to the establishment inspection report EIR. If the Agency has recommended the incorporation of additional material, method, or information into the protocol, verify that appropriate modifications were made.

1. Compare the written protocol and all Institutional Review Board (IRB) approved modifications against the protocol provided with the assignment package. Report and document any differences.
2. Determine if the approved protocol was followed with respect to:
   a. Subject selection (inclusion/exclusion criteria)
   b. Number of subjects.
   c. Drug dose form, strength, and route of administration.
   d. Frequency of subject dosing, monitoring, and sampling.
   e. Washout period between study arms (test vs. reference drug)
   f. Other (specify)?
3. Determine whether all significant changes to the protocol were:
   a. Documented by an approved amendment that is maintained with the protocol;
   b. Dated by the investigator.
   c. Approved by the IRB and reported to the sponsor before implementation except where necessary to eliminate apparent immediate hazard to human subjects.
   d. Implemented after IRB approval.

Note: Changes in protocol are not violations of protocol.

Subjects' Records

1. Describe the investigator's source data files in terms of their organization, condition, accessibility, completeness, and legibility.
2. Determine whether there is adequate documentation to assure that all audited subjects did exist and were alive and available for the duration of their stated participation in the study.
3. Compare the source data in the clinical investigator's records with the case reports completed for the sponsor. Determine whether clinical laboratory testing (including blood work, EKGs, X-rays, eye exams, etc.), as noted in the case report forms, was documented by the presence of completed laboratory records among the source data.
4. Determine whether all adverse experiences were reported in the case report forms. Determine whether they were regarded as caused by or associated with the test article and if they were previously anticipated (specificity, severity) in any written information regarding the test article.
5. Concomitant therapy and/or intercurrent illnesses might interfere with the evaluation of the effect of the test article. Check whether concomitant therapy or illness occurred. If so, was such information included in the case report forms?
6. Determine whether the number and type of subjects entered into the study were confined to the protocol limitations and whether each record contains:
   a. Observations, information, and data on the condition of each subject at the time the subject entered into the clinical study;
   b. Records of exposure of each subject to the test article;
   c. Observations and data on the condition of each subject throughout participation in the investigation including time(s) of drug administration; dosing according to pre-established, randomization schedules; results of lab tests; development of unrelated illness; bleeding times and any other specimen collections; washout periods for subjects; and other factors which might alter the effects of the test article; and
   d. The identity of all persons and locations obtaining source data or involved in the collection or analysis of such data.

Other Study Records

Review information in the clinical investigator's records that would be helpful in assessing any under reporting of adverse experiences by the sponsor to the agency. The following information will ordinarily be obtained from the sponsor and sent with the assignment:

1. The total number of subjects entered into the study;
2. The total number of dropouts from the study (identified by subject number);
3. The number of evaluable subjects and the number of nonevaluable subjects (the latter identified by subject number); and
4. The adverse experiences identified by subject number and a description of the adverse experience.

Compare the information submitted to the sponsor according to the clinical investigator's files with the information obtained from the sponsor, and document any discrepancies found.

Consent of Human Subjects

1. Obtain a copy of the consent form actually used.
2. Determine whether proper informed consent was obtained from *all* subjects *prior* to their entry into the study. Identify the staff who obtain and witness the signing of informed consent for study subjects.

IRB

1. Identify the name, address, and chairperson of the Institutional Review Board for this study.
2. Determine whether the investigator maintains copies of all reports submitted to the IRB and reports of all actions by the IRB. Determine the nature and frequency of periodic reports submitted to the IRB.
3. Determine whether the investigator submitted reports to the IRB of all deaths and serious adverse experiences and unanticipated problems involving risk to human subjects (21 CFR 312.66).
4. Determine if the investigator submitted to and obtained IRB approval of the following *before* subjects were allowed to participate in the investigation:
   a. Protocol.
   b. Modifications to the protocol.
   c. Materials to obtain human subject consent.
   d. Media advertisements for subject recruitment.
5. Determine if the investigator disseminated any promotional material or otherwise represented that the test article was safe and effective for the purpose for which it was under investigation. Were the promotional material(s) submitted to the IRB for review and approval before use?

Sponsor

1. Did the investigator provide a copy of the IRB approved consent form to the sponsor?
2. Determine whether the investigator maintains copies of all reports submitted to the sponsor.
3. Determine if and how the investigator submitted any report(s) of deaths and adverse experiences to the sponsor.
4. Determine whether all intercurrent illnesses and/or concomitant therapy(ies) were reported to the sponsor.
5. Determine whether all case report forms on subjects were submitted to the sponsor shortly (within six months) after completion.

6. Determine whether all dropouts, and the reasons therefore were reported to the sponsor.
7. Did the sponsor monitor the progress of the study to assure that investigator obligations were fulfilled? Briefly describe the method (on-site visit, telephone, contract reserach organization, etc.) and *frequency* of monitoring. Do the study records include a log of on-site monitoring visits and telephone contacts?

Test Article Accountability

1. Determine whether unqualified or unauthorized persons administered or dispensed the test article(s).
2. What names are listed on the FDA-1571 (for Sponsor-Investigator) and FDA-1572 (for studies conducted under an IND)? Obtain a copy of all FDA-1572s.
3. Determine accounting procedures for test articles:
   a. Receipt date(s) and quantities.
   b. Dates and quantities dispensed.
   c. Quantities of BE testing samples retained (see sample collection Section under Part III).
4. Inspect storage area.
   a. Reconcile amounts of test article used with amounts received, returned, and retained. Report any discrepancy.
   b. If not previously sampled under CP 7346.832, collect samples of both the test and reference products for FDA analysis.
5. If test articles are controlled substances, determine if proper security is provided.

Records Retention

1. Determine who maintains custody of the required records and the means by which prompt access can be assured.
2. Determine whether the investigator notified the sponsor in writing regarding alternate custody of required records, if the investigator does not maintain them.
3. Be aware that records should be retained at the study site for the specified time as follows:
   a. two years following the date on which the test article is approved by FDA for marketing for the purposes which were the subject of the clinical investigation; or
   b. two years following the date on which the entire clinical investigation (not just the investigator's part in it) is terminated or discontinued *by the sponsor*. If the investigator was terminated or discontinued, was the FDA notified?

ABBREVIATED REPORT FORMAT

For inspection of a clinical facility, abbreviated report is allowed if (*i*) there are no significant violations and no FDA Form 483 is issued, and (*ii*) in cases where there are objectionable findings but the findings are not serious and clearly do not have any impact on data integrity and study outcomes. The following is a guideline for preparation of the abbreviated report:

1. Reason for inspection
   a. Identify the headquarters unit that initiated and/or issued the assignment.
   b. State the purpose of the inspection.
2. What was covered
   a. Identify the clinical study, protocol number, sponsor, NDA, ANDA, etc.
   b. Location of study.
3. Administrative procedures
   a. Report the name, title, and authority of the person to whom credentials were shown and FDA-482 Notice of Inspection was issued.
   b. Persons interviewed.
   c. Who accompanied the inspector during establishment inspection.

   d. Who provided relevant information.
   e. Identify the IRB.
   f. Prior inspectional history.
4. Individual responsibilities
   a. Identify study personnel and summarize their responsibilities relative to the clinical study (e.g., who screened the subjects, who administered the drugs, who supervised collection, identification, and processing of samples, etc.)
   b. A statement about: (*i*) who obtained informed consent, (*ii*) how it was obtained, and (*iii*) was informed consent signed by each subject.
   c. Identify by whom the clinical study was monitored, and when, etc.
5. Inspectional findings
   a. A statement regarding the comparison of data on the case report forms to the source data at the investigator's site. Indicate the number of records compared and what was compared (patient charts, hospital records, lab slips, etc.), and specific information about any discrepancies.
   b. A statement indicating if the drug accountability records were sufficient to reconcile the amount of drug received, dispensed, returned, and retained.
   c. A statement about protocol adherence. Describe in detail any nonadherence.
   d. A statement concerning doses in accordance with preestablished, randomization schedules.
   e. The EIR should identify the IRB and state if it approved the study and was kept informed of the progress of the study.
   f. A statement on: (*i*) follow-up activities in response to reports of adverse experiences (including death) if any occurred; (*ii*) whether there was evidence of under reporting of adverse experiences/events.
   g. Discussion of 483 observations, reference the exhibits/documentation collected.
6. Discussion with management
   a. Discussion of 483 observations and non-483 observations.
   b. Clinical investigator's response to observations.

Remember that the above deals with abbreviated reports, not abbreviated inspections. All assignments issued for cause must have full reporting. The assignment EMS or memo will indicate the need for full reporting for any special inspection.

ANALYTICAL DATA AND OPERATIONS

Information required by this section must be obtained with the assistance of a qualified analyst from the field and/or a reviewer in GBIB with expertise in the type of analysis used in the BE study under review.

At random, compare the analytical source data with data provided in the inspection assignment for accuracy of transference and for scientific soundness/bearing on the validity of the study. Analytical source data are: codes used to blind samples; data establishing the sensitivity, linearity, specificity, and precision of the analytical assay; data determining the stability of the drug in the biological specimen; all standard curves; blinded and unblinded spiked control samples; blanks; data on reagent preparation; instrumental readings; calculations; etc. The data comparison and the testing procedural review should include an evaluation of any discrepancies found.

Prestudy Analysis

If the analytical laboratory is involved in analysis of drug standards and products employed in the BE studies, determine if:

1. Appropriate samples were analyzed by the laboratory to determine potency and content uniformity for tablets and capsules. Include a description of procedures used to prepare the sample(s) used in the study.

2. If testing of the samples described above was not performed by the analytical laboratory, did the sponsor provide test results to the laboratory?
3. For both the test and reference drug products studied, were the products' appearance, potency, dosage form (capsule, tablet, suspension, controlled release, etc.), lot numbers and expiration dates the same as that reported to FDA?

Protocol Acceptance

If the Review Division reviewed the protocol and recommended protocol modifications, verify that the modifications were incorporated into the protocol.

Equipment

Check on the following with respect to both current equipment and practices and those in place at the time of the study:

1. Does the laboratory have the same type, brand, and model (not serial) numbers of all major pieces of analytical equipment and instrumentation used in their testing procedures, as reported in the ANDA or NDA? (For example, gas chromatographs, high performance liquid chromatographs, ultraviolet spectrophotometers, colorimeter, fluorescence or atomic absorption spectrophotometer, pH meter, etc.). If not, describe the discrepancy and include its effect on the validity of the study data.
2. Assess the general condition of the major pieces of equipment (e.g., gross mistreatment) which may render them inaccurate or unreliable. Examples: damaged gas chromatograph inlet port, dry pH meter electrodes, etc. Review maintenance and repair logs for indications of past problems.
3. Are there written operating instructions for these major pieces of equipment, and are they available to the laboratory personnel?
4. Are there written and scheduled calibration/standardization procedures, and preventative maintenance procedure for all analytical instruments employed in the study? Determine whether these calibration/standardization procedures are actually employed and documented? If not, describe the deficiencies and determine whether the instruments have been calibrated during the time of the study.
5. Were specific instrument operating parameters documented during the study? If so, where?

Analytical Methods Validation

Determine through data and procedural review if:

1. The analytical laboratory has scientifically sound data to support claims for the specificity of the assay employed in this study. Ascertain the laboratory's justification for noninterferences, both endogenous and exogenous (e.g., metabolites, solvent contamination, etc.) in measuring the analytes (drug, metabolites, etc.) studied.
2. The analytical laboratory has data to support the claims for the linearity of the assay employed in this study.
3. The laboratory analyst who analyzed the biological samples has generated data demonstrating the sensitivity of the assay using the same instrumentation as that employed in the BE study. The sensitivity of the assay (or limit of detection) may be defined as the lowest quantifiable limit that can be *reproducibly determined* for the measured analyte(s) being carried through the method.
4. The laboratory analyst who analyzed the biological specimen has generated data demonstrating the precision of the assay using the instrumentation employed in the BE study. The data should be available for both standard and QC samples and should include the consistency of precision of the standard and control samples carried through the assay

procedure. Ascertain the laboratory's justification for the precision based on the separation procedure, instrumentation, and analyte concentration levels in the biological fluids.
5. The laboratory has data to demonstrate drug recoveries (percent recovery) for the measured analyte(s). This should include both analyte extraction efficiency from the biological fluid *and* recovery of the analyte(s) carried through the analytical testing procedure.
6. The analytical laboratory determined the stability of the drug both in the biological specimen and in the sample preparation medium under the same condition as in actual analysis of subject samples.
7. The analytical laboratory showed that the storage procedures (e.g., freezing and number of freeze/thaw cycles) have no adverse effect on drug stability for the period of time the samples were stored, from subject dosing until last sample analysis.
8. The water quality specified for sample and reagent preparation is consistently and readily available in the lab.

Sample Analyses

Determine if:

1. The analytical assay employed was the same as that specified in the ANDA or NDA.
2. The assay parameters observed for the study's sample analysis are similar to those (e.g., specificity, precision, etc.) obtained during method validation. Review study subjects' source analytical data to check this; pay particular attention to analytical runs determined toward the end of analytical testing.
3. Coding techniques were used to blind the analytical laboratory to the sample. Was the code available to the analytical chemist?
4. The samples were analyzed in a randomized fashion or in some specific order. Were samples of test and reference products for the same subject analyzed at the same time under identical conditions with the same standard curve, same control, and same instrument?
5. Standard curves are prepared each time a batch of unknown samples is assayed. If not, how often are standards run? Have all the standard curves run during the study been reported? How many standards are used to define each standard curve (should be 5–8, excluding blank). Does the laboratory have scientifically sound procedures for acceptance or rejection of a standard point and/or a standard curve?
6. The standard curve encompasses the concentration values reported. Were any values reported which were derived from points extrapolated on the standard curve?
7. The laboratory has a scientifically sound SOP in place to guide the acceptance/rejection of data. Did the laboratory adhere to the SOPs in the reporting of repeated determinations, or was supervisory discretion used to accept/reject data points?
8. Blinded or nonblinded spiked control samples have been included and reported with each run. Who prepared these samples? Were the controls made from a standard weight different from the standard weight used to prepare standards for the standard curve (i.e., two separate independent weighings for calibration standards and QC stock solutions)? Do the controls span the expected analyte concentration range (low, midrange, and high) found in the subjects' samples? Have all control values been reported individually, as opposed to averages?
9. The control samples were processed and analyzed exactly the same as the unknown samples. Were the controls interspersed throughout the entire analytical run?
10. The source of blank biological fluids. (Was each subject's zero hour serum used as the blank, pooled plasma, etc.?) Were interferences noted in the analytical source data for these samples? Specifications should be established to assure that blank biological fluids are as similar as possible to the biological matrix for the subject samples.
11. The source of the drug standards used for the in vivo sample analysis. If not compendial standards, how was the quality and purity of the standard assured?
12. All sample values were recorded and reported. If not, were reasons for rejection documented and justified? Were any samples rerun? When repeated determinations were made, were new standard curves and control samples run concurrently?

13. The procedure employed for determining which value of a rerun sample is reported. Was this procedure scientifically sound and consistently followed? Was an established written procedure followed?
14. The submitted chromatograms are representative of the quality of the chromatograms generated throughout the study.
15. There are written procedures for preparing reagents used in these assays. Are reagents properly labeled with date of preparation, storage requirements, as well as chemist who prepared them? Were the original weighings for calibration standard and QC stock solutions checked and countersigned by a second party?
16. Copies of the following chromatograms are available: (If not submitted by the applicant, the Field investigator or chemist should obtain copies.)
    a. Reagent blank
    b. Sample blank
    c. Internal standard
    d. A standard run
    e. A QC run
    f. A set of chromatograms for one subject over the entire span of the study.

For Antibiotic Analyses

Determine:

1. Are incubators available? Specify dimensions and type.
2. Whether:
    a. The bench tops are level.
    b. The room temperature is controlled and, if so, what are the temperature tolerances.
    c. Agar, propagation cultures and other necessary resources are available and properly monitored.
    d. Zone readers are available, if so, specify type.
    e. Autoclaves are available and, if so, specify type and determine if the autoclave sterilization process has been validated.
3. The room where these studies are conducted is "environmentally sterile" and what monitoring is done to determine the degree of "environmental sterility."
4. Whether the samples were run properly through the incubator, i.e., times and temperatures are controlled to desired specifications and properly documented.
5. Whether the standards, controls, and samples are incubated at the same time, in the same incubator.
6. Whether the microorganisms used in the media are the same as described in the AADA.
7. Whether a burner is used to heat the wire for transfer purposes.
8. Whether calibrated zone readers were used for zone size determinations.
9. Whether turbidimetric methodology was employed. Also, determine the type of spectrophotometry used.
10. Whether the turbidimetric standardization procedure was the same as that specified in the AADA. If not, describe differences.
11. Whether all samples were read in duplicate. Were all samples read by the same person? Did zone diameters or turbidimetric readings correlate with drug concentration levels?
12. Are standard operating procedures in place to calibrate the incubator, autoclave, etc., used in antibiotic analysis? Are the SOPs readily available to laboratory personnel?

For Radiometric Analyses—In Addition to the General Guidance Above

Determine:

1. How the specific activity of the radiochemical standards employed was determined.
2. Whether all counts specified in records submitted to the Agency were actually counted for the time interval specified.

3. Whether an inventory of all radiolabeled compounds is maintained by the laboratory.
4. If the background level has been determined? If yes, by what method?
5. For RIA methodology, determine if a commercial kit was used in the analysis. If so, report the type of kit, the expiration date and whether the laboratory validated the accuracy, specificity, precision, sensitivity, and linearity of the kit assay in relation to the reported study assay procedure.

Data Handling and Storage

Determine:

1. Whether bound notebooks and/or source data worksheets are used by the laboratory.
2. If bound notebooks are used, are the pages filled in sequentially on a chronological basis? Does the analyst sign the notebook/worksheet daily? Does a supervisor initial the notebook/worksheet after checking it for accuracy?
3. Whether the laboratory retains all source data, such as notebooks, worksheets, chromatograms, standard curves, etc. Is there justification for source data excluded from the study report, such as rejected runs, missing samples, etc.?
4. Whether the analyst(s) sign and date all source data records.
5. How long the source data is retained.
6. Describe the maintenance and accessibility of laboratory source data (e.g., repeated determinations, rejected analytical runs, etc.). Document problems with data recording and verification, such as lack of dates and signatures, erasures, white-out, etc.

BE TESTING REPORT SUMMARY

1. District:
2. Date(s) of Inspection:
3. Application No. (if applicable):
4. Application Sponsor (if any):
   a. Name:
   b. Address:
   c. City: State: Zip:
5. Location where testing performed:
   a. Clinical Facility Name: Address:
   b. City: State: Zip:
   c. Central File No.:
   d. Analytical Facility Name:
   e. Address:
   f. City: State: Zip:
   g. Central File No.:
6. Responsible Official (Recipient of Notice of Inspection):
   a. Name and Title:
7. Person receiving FDA-483 (if issued):
   a. Name and Title:
8. Drug under study:
   a. Generic Name:
   b. Trade Name:
   c. Dosage Form:
   d. Strength(s):
9. Number of subjects in clinical test:
10. Status of clinical testing:
    a. Date Started:
    b. Completion Date:
11. Sample Collection Sample Lot #_____
12. FDA Investigator(s):
13. Remarks:

GLPs

In the 1970s, FDA inspections of nonclinical laboratories revealed that some studies submitted in support of the safety of regulated products had not been conducted in accord with acceptable practice, and that accordingly data from such studies was not always of the quality and integrity to assure product safety. As a result of these findings, FDA promulgated the GLP Regulations, 21 CFR Part 58, on December 22, 1978 (43 FR 59986). The regulations became effective June 1979. The regulations establish standards for the conduct and reporting of nonclinical laboratory studies and are intended to assure the quality and integrity of safety data submitted to the FDA.

The FDA relies on documented adherence to GLP requirements by nonclinical laboratories in judging the acceptability of safety data submitted in support of research and/or marketing permits. The FDA has implemented this program of regular inspections and data audits to monitor laboratory compliance with the GLP requirements.

The objective of this program is

1. To verify the quality and integrity of data submitted in a research or marketing application.
2. To inspect (approximately every two years) nonclinical laboratories conducting safety studies that are intended to support applications for research or marketing of regulated products.
3. To audit safety studies and determine the degree of compliance with GLP regulations.
   a. Types of Inspections
      i. *Surveillance Inspections*. Surveillance inspections are periodic, routine determinations of a laboratory's compliance with GLP regulations. These inspections include a facility inspection and audits of ongoing and/or recently completed studies.
      ii. *Directed Inspections*
         – Directed inspections are assigned to achieve a specific purpose, such as:
         – Verifying the reliability, integrity, and compliance of critical safety studies being reviewed in support of pending applications.
         – Investigating issues involving potentially unreliable safety data and/or violative conditions brought to the FDA's attention.
         – Reinspecting laboratories previously classified OAI (usually within six months after the firm responds to a Warning Letter).
         – Verifying the results from third party audits or sponsor audits submitted to the FDA for consideration in determining whether to accept or reject questionable or suspect studies.

Inspections

1. The investigator will determine the current state of GLP compliance by evaluating the laboratory facilities, operations, and study performance.
2. Organization chart—If the facility maintains an organization chart, obtain a current version of the chart for use during the inspection and submit it in the EIR.
3. Facility floor-plan diagram—Obtain a diagram of the facility. The diagram may identify areas that are not used for GLP activities. If it does not, request that appropriate facility personnel identify any areas that are not used for GLP activities. Use during the inspection and submit it in the EIR.
4. Master schedule sheet—Obtain a copy of the firm's master schedule sheet for all studies listed since the last GLP inspection or last two years and select studies as defined in 21 CFR 58.3(d). If the inspection is the first inspection of the facility, review the entire master schedule. If studies are identified as non-GLP, determine the nature of several studies to verify the accuracy of this designation. See 21 CFR 58.1 and 58.3(d). In contract laboratories determine who decides if a study is a GLP study.
5. Identification of studies
   a. *Directed Inspections*—Inspection assignments will identify studies to be audited.
   b. *Surveillance Inspections*—Inspection assignments may identify one or more studies to be

audited. If the assignment does not identify a study for coverage, or if the referenced study is not suitable to assess all portions of current GLP compliance, the investigator will select studies as necessary to evaluate all areas of laboratory operations. When additional studies are selected, first priority should be given to FDA studies for submission to the assigning Center.

6. Ongoing studies—Obtain a copy of the study protocol and determine the schedule of activities that will be underway during the inspection. This information should be used to schedule inspections of ongoing laboratory operations, as well as equipment and facilities associated with the study. If there are no activities underway in a given area for the study selected, evaluate the area based on ongoing activities.
7. Completed studies—The data audit should be carried out as outlined in Part III. If possible, accompany laboratory personnel when they retrieve the study data to assess the adequacy of data retention, storage, and retrieval as described in Part III.

The facility inspection should be guided by the GLP regulations. The following areas should be evaluated and described as appropriate.

1. Organization and personnel (21 CFR 58.29, 58.31, 58.33)
   a. Purpose—To determine whether the organizational structure is appropriate to ensure that studies are conducted in compliance with GLP regulations, and to determine whether management, study directors, and laboratory personnel are fulfilling their responsibilities under the GLPs.
   b. Management responsibilities (21 CFR 58.31)—Identify the various organizational units, their role in carrying out GLP study activities, and the management responsible for these organizational units. This includes identifying personnel who are performing duties at locations other than the test facility and identifying their line of authority. If the facility has an organization chart, much of this information can be determined from the chart.
2. Determine if management has procedures for assuring that the responsibilities in 58.31 can be carried out. Look for evidence of management involvement, or lack thereof, in the following areas:
   a. Assigning and replacing study directors.
   b. Control of study director workload (use the Master Schedule to assess workload).
   c. Establishment and support of the quality assurance unit (QAU), including assuring that deficiencies reported by the QAU are communicated to the study directors and acted upon.
   d. Assuring that test and control articles or mixtures are appropriately tested for identity, strength, purity, stability, and uniformity.
   e. Assuring that all study personnel are informed of and follow any special test and control article handling and storage procedures.
   f. Providing required study personnel, resources, facilities, equipment, and materials.
   g. Reviewing and approving protocols and SOPs.
   h. Providing GLP or appropriate technical training.
3. Personnel (21 CFR 58.29)—Identify key laboratory and management personnel, including any consultants or contractors used, and review personnel records, policies, and operations to determine if:
   a. Summaries of training and position descriptions are maintained and are current for selected employees.
   b. Personnel have been adequately trained to carry out the study functions that they perform.
   c. Personnel have been trained in GLPs.
   d. Practices are in place to ensure that employees take necessary health precautions, wear appropriate clothing, and report illnesses to avoid contamination of the test and control articles and test systems.
4. If the firm has computerized operations, determine the following:
   a. Who was involved in the design, development, and validation of the computer system?

   b. Who is responsible for the operation of the computer system, including inputs, processing, and output of data?
   c. Whether computer system personnel have training commensurate with their responsibilities, including professional training and training in GLPs?
   d. Whether some computer system personnel are contractors who are present on-site full-time, or nearly full-time. The investigation should include these contractors as though they were employees of the firm. Specific inquiry may be needed to identify these contractors, as they may not appear on organization charts.
   e. Interview and observe personnel using the computerized systems to assess their training and performance of assigned duties.
5. Study director (21 CFR 58.33)
   a. Assess the extent of the study director's actual involvement and participation in the study. In those instances when the study director is located off-site, review any correspondence/records between the testing facility management and QAU and the off-site study director. Determine that the study director is being kept immediately apprised of any problems that may affect the quality and integrity of the study.
   b. Assess the procedures by which the study director:
   c. Assures the protocol and any amendments have been properly approved and are followed.
   d. Assures that all data are accurately recorded and verified.
   e. Assures that data are collected according to the protocol and SOPs.
   f. Documents unforeseen circumstances that may affect the quality and integrity of the study and implements corrective action.
   g. Assures that study personnel are familiar with and adhere to the study protocol and SOPs.
   h. Assures that study data are transferred to the archives at the close of the study.
6. EIR Documentation and Reporting—Collect exhibits to document deficiencies. This may include SOPs, organizational charts, position descriptions, and CVs, as well as study-related memos, records, and reports for the studies selected for review. The use of outside or contract facilities must be noted in the EIR. The assigning Center should be contacted for guidance on inspection of these facilities.
7. QAU (QAU; 21 CFR 58.35)
   a. Purpose: To determine if the test facility has an effective, independent QAU that monitors significant study events and facility operations, reviews records and reports, and assures management of GLP compliance.
8. QAU Operations—[21 CFR 58.35(b–d)]—Review QAU SOPs to assure that they cover all methods and procedures for carrying out the required QAU functions, and confirm that they are being followed. Verify that SOPs exist and are being followed for QAU activities including, but not limited to, the following:
   a. Maintenance of a master schedule sheet.
   b. Maintenance of copies of all protocols and amendments.
   c. Scheduling of its in-process inspections and audits.
   d. Inspection of each nonclinical laboratory study at intervals adequate to assure the integrity of the study, and maintenance of records of each inspection.
   e. Immediately notify the study director and management of any problems that are likely to affect the integrity of the study.
   f. Submission of periodic status reports on each study to the study director and management.
   g. Review of the final study report.
   h. Preparation of a statement to be included in the final report that specifies the dates inspections were made and findings reported to management and to the study director.
9. Inspection of computer operations.
   a. Verify that, for any given study, the QAU is entirely separate from and independent of the personnel engaged in the conduct and direction of that study. Evaluate the time QAU personnel spend in performing in-process inspection and final report audits.

Determine if the time spent is sufficient to detect problems in critical study phases and if there are adequate personnel to perform the required functions.

b. *Note*: The investigator may request the firm's management to certify in writing that inspections are being implemented, performed, documented, and followed-up in accordance with this section [See 58.35(d)].

10. EIR Documentation and reporting—Obtain a copy of the master schedule sheet dating from the last routine GLP inspection or covering the past two years. If the master schedule is too voluminous, obtain representative pages to permit headquarters review. When master schedule entries are coded, obtain the code key. Deficiencies should be fully reported and documented in the EIR. Documentation to support deviations may include copies of QAU SOPs, list of QAU personnel, their curriculum vitae (CVs) or position descriptions, study-related records, protocols, and final reports.
11. Facilities (21 CFR 58.41-51)
    a. Purpose: Assess whether the facilities are of adequate size and design.
    b. Facility inspection
        i. Review environmental controls and monitoring procedures for critical areas (i.e., animal rooms, test article storage areas, laboratory areas, handling of bio-hazardous material, etc.) and determine if they appear adequate and are being followed.
        ii. Review the SOPs that identify materials used for cleaning critical areas and equipment, and assess the facility's current cleanliness.
        iii. Determine whether there are appropriate areas for the receipt, storage, mixing, and handling of the test and control articles.
        iv. Determine whether separation is maintained in rooms where two or more functions requiring separation are performed.
        v. Determine that computerized operations and archived computer data are housed under appropriate environmental conditions (e.g., protected from heat, water, electromagnetic forces).
12. EIR documentation and reporting—Identify which facilities, operations, SOPs, etc., were inspected. Only significant changes in the facility from previous inspections need be described. Facility floor plans may be collected to illustrate problems or changes. Document any conditions that would lead to contamination of test articles or to unusual stress of test systems.
13. Equipment (21 CFR 58.61-63)
    a. Purpose: To assess whether equipment is appropriately designed and of adequate capacity and is maintained and operated in a manner that ensures valid results.
    b. Equipment Inspection—Assess the following:
        i. The general condition, cleanliness, and ease of maintenance of equipment in various parts of the facility.
        ii. The heating, ventilation, and air conditioning system design and maintenance, including documentation of filter changes and temperature/humidity monitoring in critical areas.
        iii. Whether equipment is located where it is used and that it is located in a controlled environment, when required.
        iv. Nondedicated equipment for preparation of test and control article carrier mixtures is cleaned and decontaminated to prevent cross contamination.
        v. For representative pieces of equipment check the availability of the following:
            – SOPS and/or operating manuals.
            – Maintenance schedule and log.
            – Standardization/calibration procedure, schedule, and log.
            – Standards used for calibration and standardization.
        vi. For computer systems, assess that the following procedures exist and are documented:
            – Validation study, including validation plan and documentation of the plan's completion.
            – Maintenance of equipment, including storage capacity and backup procedures.
            – Control measures over changes made to the computer system, which include

the evaluation of the change, necessary test design, test data, and final acceptance of the change.
- Evaluation of test data to assure that data are accurately transmitted and handled properly when analytical equipment is directly interfaced to the computer.
- Procedures for emergency backup of the computer system (e.g., backup battery system and data forms for recording data in the event of a computer failure or power outage).

14. EIR documentation and reporting—The EIR should list which equipment, records, and procedures were inspected and the studies to which they are related. Detail any deficiencies that might result in contamination of test articles, uncontrolled stress to test systems, and/or erroneous test results.
15. Testing facility operations (21 CFR 58.81)
    a. Purpose: To determine if the facility has established and follows written SOPs necessary to carry out study operations in a manner designed to ensure the quality and integrity of the data.
    b. SOP Evaluation
        i. Review the SOP index and representative samples of SOPs to ensure that written procedures exist to cover at least all of the areas identified in 58.81(b).
        ii. Verify that only current SOPs are available at the personnel workstations.
        iii. Review key SOPs in detail and check for proper authorization signatures and dates, and general adequacy with respect to the content (i.e., SOPs are clear, complete, and can be followed by a trained individual).
        iv. Verify that changes to SOPs are properly authorized and dated and that a historical file of SOPs is maintained.
        v. Ensure that there are procedures for familiarizing employees with SOPs.
        vi. Determine that there are SOPs to ensure the quality and integrity of data, including input (data checking and verification), output (data control), and an audit trail covering all data changes.
        vii. Verify that a historical file of outdated or modified computer programs is maintained. If the firm does not maintain old programs in digital form, ensure that a hard copy of all programs has been made and stored.
        viii. Verify that SOPs are periodically reviewed for current applicability and that they are representative of the actual procedures in use.
        ix. Review selected SOPs and observe employees performing the operation to evaluate SOP adherence and familiarity. EIR Documentation and Reporting-Submit SOPs, data collection forms, and raw data records as exhibits that are necessary to support and illustrate deficiencies.
16. Reagents and solutions (21 CFR 58.83)
    a. Purpose: To determine that the facility ensures the quality of reagents at the time of receipt and subsequent use.
        i. Review the procedures used to purchase, receive, label, and determine the acceptability of reagents and solutions for use in the studies.
        ii. Verify that reagents and solutions are labeled to indicate identity, titer or concentration, storage requirements, and expiration date.
        iii. Verify that for automated analytical equipment, the profile data accompanying each batch of control reagents are used.
        iv. Check that storage requirements are being followed.
17. Test and control articles (21 CFR 58.105-113)
    a. Purpose: To determine that procedures exist to assure that test and control articles and mixtures of articles with carriers meet protocol specifications throughout the course of the study, and that accountability is maintained.
    b. Characterization and Stability of Test Articles (21 CFR 58.105)—The responsibility for carrying out appropriate characterization and stability testing may be assumed by the facility performing the study or by the study sponsor. *When test article characterization and stability testing is performed by the sponsor, verify that the test facility has received documentation that this testing has been conducted.*

c. Verify that procedures are in place to ensure that
   i. The acquisition, receipt and storage of test articles, and means used to prevent deterioration and contamination are as specified.
   ii. The identity, strength, purity, and composition (i.e., characterization) to define the test and control articles are determined for each batch and are documented.
   iii. The stability of test and control articles is documented.
   iv. The transfer of samples from the point of collection to the analytical laboratory is documented.
   v. Storage containers are appropriately labeled and assigned for the duration of the study.
   vi. Reserve samples of test and control articles for each batch are retained for studies lasting more than four weeks.

d. Test and control article handling (21 CFR 58.107). Determine that there are adequate procedures for:
   i. Documentation for receipt and distribution.
   ii. Proper identification and storage.
   iii. Precluding contamination, deterioration, or damage during distribution.
   iv. Inspect test and control article storage areas to verify that environmental controls, container labeling, and storage are adequate.
   v. Observe test and control article handling and identification during the distribution and administration to the test system.
   vi. Review a representative sample of accountability records and, if possible, verify their accuracy by comparing actual amounts in the inventory. For completed studies verify documentation of final test and control article reconciliation.

18. Protocol and conduct of nonclinical laboratory study (21 CFR 58.120-130)
    a. Purpose: To determine if study protocols are properly written and authorized, and that studies are conducted in accordance with the protocol and SOPs.
    b. Study protocol (21 CFR 58.120)
       i. Review SOPs for protocol preparation and approval and verify they are followed.
       ii. Review the protocol to determine if it contains required elements.
       iii. Review all changes, revisions, or amendments to the protocol to ensure that they are authorized, signed, and dated by the study director.
       iv. Verify that all copies of the approved protocol contain all changes, revisions, or amendments.
19. Conduct of the nonclinical laboratory study (21 CFR 58.130). Evaluate the following laboratory operations, facilities, and equipment to verify conformity with protocol and SOP requirements for:
    a. Test system monitoring.
    b. Recording of raw data (manual and automated).
    c. Corrections to raw data (corrections must not obscure the original entry and must be dated, initialed, and explained).
    d. Randomization of test systems.
    e. Collection and identification of specimens.
    f. Authorized access to data and computerized systems.
20. Records and reports (21 CFR 58.185-195)
    a. Purpose: To assess how the test facility stores and retrieves raw data, documentation, protocols, final reports, and specimens.
    b. Reporting of Study Results (21 CFR 58.185)—Determine if the facility prepares a final report for each study conducted.
21. Storage and retrieval of records and data (21 CFR 58.190)
    a. Verify that raw data, documentation, protocols, final reports, and specimens have been retained.
    b. Identify the individual responsible for the archives. Determine if delegation of duties to other individuals in maintaining the archives has occurred.

c. Verify that archived material retained or referred to in the archives is indexed to permit expedient retrieval. It is not necessary that all data and specimens be in the same archive location. For raw data and specimens retained elsewhere, the archives index must make specific reference to those other locations.
d. Verify that access to the archives is controlled and determine that environmental controls minimize deterioration.
e. Ensure that there are controlled procedures for adding or removing material. Review archive records for the removal and return of data and specimens. Check for unexplained or prolonged removals.
f. Determine how and where computer data and backup copies are stored, that records are indexed in a way to allow access to data stored on electronic media, and that environmental conditions minimize deterioration.
g. Determine to what electronic media such as tape cassettes or ultra high capacity portable discs the test facility has the capacity of copying records in electronic form. Report names and identifying numbers of both copying equipment type and electronic medium type to enable agency personnel to bring electronic media to future inspections for collecting exhibits.

22. *Data audit*. In addition to the procedures outlined above for evaluating the overall GLP compliance of a firm, the inspection should include the audit of at least one completed study. Studies for audit may be assigned by the Center or selected by the investigator as described in Part III. The audit will include a comparison of the protocol (including amendments to the protocol), raw data, records, and specimens against the final report to substantiate that protocol requirements were met and that findings were fully and accurately reported. For each study audited, the study records should be reviewed for quality to ensure that data are
    a. Attributable—the raw data can be traced, by signature or initials and date to the individual observing and recording the data. Should more than one individual observe or record the data, that fact should be reflected in the data.
    b. Legible—the raw data are readable and recorded in a permanent medium. If changes are made to original entries, the changes:
        i. Must not obscure the original entry.
        ii. Indicate the reason for change.
        iii. Must be signed or initialed and dated by the person making the change.
    c. Contemporaneous-the raw data are recorded at the time of the observation.
    d. Original-the first recording of the data.
    e. Accurate—the raw data are true and complete observations. For data entry forms that require the same data to be entered repeatedly, all fields should be completed or a written explanation for any empty fields should be retained with the study records.
23. General
    a. Determine if there were any significant changes in the facilities, operations, and QAU functions other than those previously reported.
    b. Determine whether the equipment used was inspected, standardized, and calibrated prior to, during, and after use in the study. If equipment malfunctioned, review the remedial action, and ensure that the final report addresses whether the malfunction affected the study.
    c. Determine if approved SOPs existed during the conduct of the study. Compare the content of the protocol with the requirements in 21 CFR. Review the final report for the study director's dated signature and the QAU statement as required in 21 CFR 58.35(b)(7).
24. Protocol versus final report—Study methods described in the final report should be compared against the protocol and the SOPs to confirm those requirements were met.
25. Final report versus raw data. The audit should include a detailed review of records, memorandum, and other raw data to confirm that the findings in the final report completely and accurately reflect the raw data. Representative samples of raw data should be audited against the final report.

26. Samples. Collection of samples should be considered when the situation under audit or surveillance suggests that the facility had, or is having, problems in the area of characterization, stability, storage, contamination, or dosage preparation.
27. Inspectional observations. An FDA 483 listing inspectional observations will be issued under this program. Findings should not be listed on the FDA 483 if in the opinion of the field investigator:
    a. The findings are problems that have been observed and corrected by the firm through its internal procedures.
    b. The findings are minor and are one-time occurrences that have no impact on the firm's operations, study conduct, or data integrity.
    c. Findings that are not considered significant enough to be listed on the FDA 483 may be discussed with the firm's management. Such discussions must be reported in the EIR. Analyzing Laboratories.

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6 Statistical Evaluation of Bioequivalence Data

BACKGROUND

Defined as relative bioavailability (BA), bioequivalence (BE) involves comparison between a test (*T*) and reference (*R*) drug product, where *T* and *R* can vary, depending on the comparison to be performed (e.g., to-be-marketed dosage form vs. clinical trial material, generic drug vs. reference listed drug, drug product changed after approval vs. drug product before the change). Although BA and BE are closely related, BE comparisons normally rely on (*i*) a criterion, (*ii*) a confidence interval for the criterion, and (*iii*) a predetermined BE limit. BE comparisons could also be used in certain pharmaceutical product line extensions, such as additional strengths, new dosage forms (e.g., changes from immediate release to extended release), and new routes of administration. In these settings, the approaches described in this guidance can be used to determine BE. The general approaches discussed in this guidance may also be useful when assessing pharmaceutical equivalence or performing equivalence comparisons in clinical pharmacology studies and other areas.

In the July 1992 guidance on *Statistical Procedures for BE Studies Using a Standard Two-Treatment Crossover Design* (the 1992 guidance), Center for Drug Evaluation and Research (CDER) recommended that a standard in vivo BE study design be based on the administration of either single or multiple doses of the *T* and *R* products to healthy subjects on separate occasions, with random assignment to the two possible sequences of drug product administration. The 1992 guidance further recommended that statistical analysis for pharmacokinetic (PK) measures, such as area under the curve (AUC) and peak concentration (C_{max}), be based on the two one-sided tests (TOST) procedure to determine whether the average values for the PK measures determined after administration of the *T* and *R* products were comparable. This approach is termed average bioequivalence (ABE) and involves the calculation of a 90% confidence interval for the ratio of the averages (population geometric means) of the measures for the *T* and *R* products. To establish BE, the calculated confidence interval should fall within a BE limit, usually 80% to 125% for the ratio of the product averages. (For a broad range of drugs, a BE limit of 80–125% for the ratio of the product averages has been adopted for use of an ABE criterion. Generally, the BE limit of 80–125% is based on a clinical judgment that a test product with BA measures outside this range should be denied market access.) In addition to this general approach, the 1992 guidance provided specific recommendations for (*i*) logarithmic transformation of PK data, (*ii*) methods to evaluate sequence effects, and (*iii*) methods to evaluate outlier data.

Although ABE is recommended for a comparison of BA measures in most BE studies, the current method of testing requires evaluating both the *population* and *individual BE*. This is useful, in some instances, for analyzing in vitro and in vivo BE studies. The ABE approach focuses only on the comparison of population averages of a BE measure of interest and not on the variances of the measure for the *T* and *R* products. The ABE method does not assess a subject-by-formulation interaction (SFI) variance, that is, the variation in the average *T* and *R* difference among individuals. In contrast, population and individual BE approaches include comparisons of both averages and variances of the measure. The population BE approach assesses total variability of the measure in the population. The individual BE approach assesses within-subject variability for the *T* and *R* products, as well as the SFI.

EXPERIMENTAL DESIGNS

A good knowledge of statistics is essential in designing meaningful BA studies. There are too many factors that, if not properly considered, can render BA data less meaningful such as healthy subjects versus patients; effects of disease, age, diet, environmental conditions; the number of subjects, their sex and age; choice of blood levels versus urinary excretion; monitoring metabolites versus parent drug; frequency and number of blood/urine samples obtained; relative importance of the various phases of plasma concentration to therapeutic or toxic response; single-dose studies versus multiple-dose steady state estimations; sensitivity of analytic methodology.

In comparing formulations, the basic statistical designs depend on whether it is a comparison between two formulations or more than two formulations.

Comparisons of Two Formulations

When two formulations are compared with each other, one of the formulations serves as the standard. The standard is generally the innovator's product whose BA has been accepted as a standard by the Food and Drug Administration (FDA). Two basic designs are possible for this type of comparison, i.e., parallel groups or crossover. The parallel group design takes two groups of subjects in equal number and matched as much as possible for age, sex, height, weight, etc. An assumption is made that the overall biologic variability is equal in both groups. One group is given the standard formulation and the other group receives the formulation being evaluated. However, it can be understood why there may be significant variability between groups, a factor that can be significantly reduced by giving both formulations to all subjects in the study and thus each subject serving its own control. There must, however, be sufficient time between trials to wash out the entire drug from the body, which requires at least 10 half-lives (to reduce levels to below 0.1% level) and it is generally about one week for most drugs. However, for drugs like digoxin, phenobarbital, or reserpine, which have long half-lives, more than one week may be needed to wash out the entire drug. Further care must be exercised to take into account disposition of metabolites. Since there may be an interaction in disposition between the metabolite and the drug, it is necessary to rid the body of all. The crossover designs are called randomized balanced crossover designs.

Randomized Balanced Crossover Design

Subject no.	Week 1	Week 2
1	A	B
2	B	A
3	B	A
4	B	A
5	A	B
6	B	A
7	A	B
8	A	B
9	B	A
10	A	B

Analysis of Variance for Crossover Design (in 10 Subjects)

Source of variation	Degrees of freedom
Days	1
Subjects	9
Formulations	1
Error	8

Comparisons of More than Two Formulations

If a crossover design is used, the study becomes too large to handle. Also, the order in which the formulations are given becomes important. To overcome these disadvantages, for a small number of formulations, a Latin square design can be used.

Subject no.	Week 1	Week 2	Week 3
1	A	B	C
2	C	A	B
3	B	C	A

For large number of formulations, a balanced incomplete block design is used where each formulation occurs in the same number of times and every pair of formulation occurs together in the same number of subjects.

Subject no.	Week 1	Week 2
1	A	B
2	B	C
3	D	B
4	B	A
5	A	C
6	D	C
7	C	A
8	C	D
9	A	D
10	B	D
11	C	B
12	D	A

Number of Subjects in Study

The number of subjects used in the study depends on two factors: the level of statistical significance (generally taken as 95%) and the degree of difference within formulations, which make them different (generally taken as 20%). The number of subjects is calculated by

$$N = 20 \times \frac{E}{M_d} \tag{1}$$

This equation assures that there be at least 10 subjects in the study, E is the error of variance per observation and M_d is the minimum difference between formulations. Thus, depending on the degree of variability of the AUC or other parameters monitored, the number of subjects can increase, but not less than 10.

STATISTICAL MODEL

The suggested regulatory model for testing BE is expressed as follows:

$$\frac{(\mu_T - \mu_R)^2 + (\sigma^2_{WT} - \sigma^2_{WR}) + \sigma^2_D}{\sigma^2_W} \leq \theta_I \tag{2}$$

The model has three components in its numerator. The first term is the squared difference between the means $[(\mu_T - \mu_R)^2]$ of the test (T) and reference (R) formulations and a measure of ABE. The second component compares the within-subject variances of the two drug products (σ^2_{WT} and σ^2_{WR}). The third term is the variance component for the SFI (σ^2_D). The model requires that the sum of the three terms, normalized by a variance (σ^2_W) and with an associated confidence interval, should not exceed a preset regulatory limit (θ_I). The "trade-off" of the first and second terms supposedly provides a reward for a better formulation. SFI is thought to

have potential clinical importance. Also, the model claims to evaluate the "switchability" rather than the "prescribability" of drug formulations.

The normalizing variance term is constant ($\sigma_W^2 = \sigma_0^2$) if the intrasubject variance of the reference formulation does not exceed a preset value ($\sigma_{WR}^2 \leq \sigma_0^2$), but takes the value of $\sigma_W^2 = \sigma_{WR}^2$ at larger variances ($\sigma_{WR}^2 > \sigma_0^2$). $\sigma_0^2 = 0.04$ has been suggested to separate the "constant-scaled" and "reference-scaled" calculations.

Several concerns arise in the use of this model as follows:

- There is no clinical evidence of the inadequacy of the ABE approach.
- There are only tenuous indications of the prevalence of SFIs.
- The original assumptions for the properties of the interaction term had to be modified in this model.
- The mean–variability trade-off is asymmetric, and can result not only in rewards but also in penalties which, in the presence of random variations, can be large.
- The usefulness of the proposed aggregate criterion is questioned since it intends to accomplish various goals simultaneously, whereas stepwise procedures could be less problematic and more effective.

Statistical analyses of BE data are typically based on a statistical model for the logarithm of the BA measures (e.g., AUC and C_{max}). The model is a mixed effects or two-stage linear model. Each subject, j, theoretically provides a mean for the log-transformed BA measure for each formulation, μ_{Tj} and μ_{Rj} for the T and R formulations, respectively. The model assumes that these subject-specific means come from a distribution with population means μ_T and μ_R, and between-subject variances σ_{BT}^2 and σ_{BR}^2, respectively. The model allows for a correlation between μ_{Tj} and μ_{Rj}. The SFI variance component, σ_{D^2}, is related to these parameters as follows:

$$\sigma_D^2 = \text{variance of } (\mu_{Tj} - \mu_{Rj}) = (\sigma_{BT} - \sigma_{BR})^2 + 2(1-\rho)\sigma_{BT}\sigma_{BR} \quad (3)$$

For a given subject, the observed data for the log-transformed BA measure are assumed to be independent observations from distributions with means μ_{Tj} and μ_{Rj}, and within-subject variances σ_{WT}^2 and σ_{WR}^2. The total variances for each formulation are defined as the sum of the within- and between-subject components (i.e., $\mu_{TT}^2 = \mu_{WT}^2 + \mu_{BT}^2$ and $\mu_{TR}^2 = \mu_{WR}^2 + \mu_{BR}^2$). For analysis of crossover studies, the means are given additional structure by the inclusion of period and sequence effect terms.

STATISTICAL APPROACHES FOR BE

The general structure of a BE criterion is that a function (Θ) of population measures should be demonstrated to be no greater than a specified value (θ). Using the terminology of statistical hypothesis testing, this is accomplished by testing the hypothesis H_0: $\Theta > \theta$ versus H_A: $\Theta \cdot \theta$ at a desired level of significance, often 5%. Rejection of the null hypothesis H_0 (i.e., demonstrating that the estimate of Θ is statistically significantly less than θ results in a conclusion of BE). The choice of Θ and θ differs in average, population, and individual BE approaches.

A general objective in assessing BE is to compare the log-transformed BA measure after administration of the T and R products. The population and individual approaches are based on the comparison of an expected squared distance between the T and R formulations to the expected squared distance between two administrations of the R formulation. An acceptable T formulation is one where the T–R distance is not substantially greater than the R–R' distance. In both population and individual BE approaches, this appears as a comparison to the reference variance, which is referred to as *scaling to the reference variability.*

Population and individual BE approaches, but not the ABE approach, allow two types of scaling: reference scaling and constant scaling. Reference scaling means that the criterion used is scaled to the variability of the R product, which effectively widens the BE limit for more variable reference products. Although generally sufficient, use of reference scaling alone could unnecessarily narrow form should bethe BE limit for drugs and/or drug products that have low variability but a wide therapeutic range. This guidance, therefore, recommends

mixed scaling for the population and individual BE approaches. With mixed scaling, the reference-scaled form of the criterion should be used if the reference product is highly variable; otherwise, the constant-scaled used.

ABE

The following criterion is recommended for ABE:

$$(\mu_T - \mu_R)^2 \theta_A^2 \tag{4}$$

where μ_T is the population average response of the log-transformed measure for the *T* formulation and μ_R is the population average response of the log-transformed measure for the *R* formulation.

This criterion is equivalent to

$$\theta_A(\mu_T - \mu_R)\theta_A \tag{5}$$

and, usually, $\theta_A = \ln(1.25)$.

Population BE

The following mixed-scaling approach is recommended for population BE (i.e., use the reference-scaled method if the estimate of $\sigma_{TR} > \sigma_{T_0}$ and the constant-scaled method if the estimate of $\sigma_{TR} \cdot \sigma_{T_0}$).

The recommended criteria are

Reference scaled:

$$\left\{\left[(\mu_T - \mu_R)^2 + \left(\mu_{TT}^2 - \mu_{TR}^2\right)/\sigma_{TR}^2\right]\right\}\theta_P \tag{6}$$

or

Constant scaled:

$$\left\{\left[(\mu_T - \mu_R)^2 + (\mu_{TT}^2 - \mu_{TR}^2)/\sigma_{T_0}^2\right]\right\}\theta_P \tag{7}$$

where μ_T is the population average response of the log-transformed measure for the *T* formulation; μ_R is the population average response of the log-transformed measure for the *R* formulation; μ_{TT}^2 is the total variance (i.e., sum of within- and between-subject variances) of the *T* formulation; μ_{TR}^2 is the total variance (i.e., sum of within- and between-subject variances) of the *R* formulation; $\sigma_{T_0}^2$ is the specified constant total variance; and θ_P is the BE limit.

Equations (4) and (5) represent an aggregate approach where a single criterion on the left-hand side of the equation encompasses two major components: (1) the difference between the *T* and *R* population averages ($\mu_T - \mu_R$) and (2) the difference between the *T* and *R* total variances ($\sigma_{TT}^2 - \sigma_{TR}^2$). This aggregate measure is scaled to the total variance of the *R* product or to a constant value ($\sigma_{T_0}^2$, a standard that relates to a limit for the total variance), whichever is greater.

The specification of both σ_{T_0} and σ_P relies on the establishment of standards. When the population BE approach is used, in addition to meeting the BE limit based on confidence bounds, the point estimate of the geometric test/reference mean should fall within 80% to 125%.

Individual BE

The following mixed-scaling approach is one approach for individual BE (i.e., use the reference-scaled method if the estimate of $\sigma_{WR} > \sigma_{W_0}$, and the constant-scaled method if the estimate of $\sigma_{WR} \cdot \sigma_{W_0}$).

The recommended criteria are:

Reference scaled:

$$\left\{\left[(\mu_T - \mu_R)^2 + \sigma_D^2 + \left(\sigma_{WT}^2 - \sigma_{WR}^2\right)/\sigma_{WR}^2\right]\right\}\theta_I \tag{8}$$

or

Constant scaled:

$$\left\{\left[(\mu_T - \mu_R)^2 + \sigma_D^2 + \left(\sigma_{WT}^2 - \sigma_{WR}^2\right)/\sigma_{W_0}^2\right]\right\}\theta_I \tag{9}$$

where μ_T is the population average response of the log-transformed measure for the T formulation; μ_R is the population average response of the log-transformed measure for the R formulation; σ_D^2 is the SFI variance component; σ_{WT}^2 is the within-subject variance of the T formulation; σ_{WR}^2 is the within-subject variance of the R formulation; $\sigma_{W_0}^2$ is the specified constant within-subject variance; and θ_I is the BE limit.

Equations (6) and (7) represent an aggregate approach where a single criterion on the left-hand side of the equation encompasses three major components: (*i*) the difference between the T and R population averages ($\mu_T - \mu_R$), (*ii*) SFI (σ_D^2), and (*iii*) the difference between the T and R within-subject variances ($\sigma_{WT}^2 - \sigma_{WR}^2$). This aggregate measure is scaled to the within-subject variance of the R product or to a constant value ($\sigma_{W_0}^2$, a standard that relates to a limit for the within-subject variance), whichever is greater.

The specification of both σ_{W_0} and θ_I relies on the establishment of standards. The generation of these standards is described below. When the individual BE approach is used, in addition to meeting the BE limit based on confidence bounds, the point estimate of the geometric test/reference mean ratio should fall within 80% to 125%.

Standards

The equations for standards to be established (i.e., σ_{T_0} and θ_P for assessment of population BE, σ_{W_0} and θ_I for individual BE). The recommended approach to establishing these standards is described below.

σ_{T_0} and σ_{W_0}

A general objective in assessing BE should be to compare the difference in the BA log-measure of interest after the administration of the T and R formulations, $T - R$, with the difference in the same log metric after two administrations of the R' formulation, $R - R'$.

Population BE

For population BE, the comparisons of interest should be expressed in terms of the ratio of the expected squared difference between T and R (administered to different individuals) and the expected squared difference between R and R' (administered to different individuals), as shown below.

$$E(T-R)^2 = (\mu_T - \mu_R)^2 + \sigma_{TT}^2 + \sigma_{TR}^2 \tag{10}$$

$$E(R-R')^2 = 2\sigma_R^2 \tag{11}$$

$$\frac{E(T-R)^2}{E(R-R')^2} = \left\{(\mu_T - \mu_R)^2 + \sigma_{TT}^2 + \sigma_{TR}^2\right\} - 2\sigma_{TR}^2 - \tag{12}$$

The population BE criterion (PBC) in equation (4) (Section IV.B.) is derived from equation (10), such that the criterion equals zero for two identical formulations. The square root of equation (10) yields the "population difference ratio" (PDR).

$$\text{PDR} = \frac{(\mu_T - \mu_R)^2 + \sigma_{TT}^2 + \sigma_{TR}^2}{2\sigma_{TR}^2} \tag{13}$$

The PDR is the square root of the ratio of the expected squared $T-R$ difference compared to the expected squared $R-R'$ difference in the population. It should be noted that the PDR is monotonically related to the PBC described in equation (4) as follows:

$$\text{PDR} = (\text{PBC}/2 + 1) \tag{14}$$

Individual BE

For individual BE, the comparisons of interest should be expressed in terms of the ratio of the expected squared difference between T and R (administered to the same individual) and the expected squared difference between R and R' (two administrations of R to the same individual), as shown below.

$$E(T-R)^2 = (\mu_T - \mu_R)^2 + \sigma_D^2 + \sigma_{WT}^2 + \sigma_{WR}^2 \tag{15}$$

$$E(R-R')^2 = 2\sigma_{WR}^2 \tag{16}$$

$$\frac{E(T-R)^2}{E(R-R')^2} = \frac{(\mu_T - \mu_R)^2 + \sigma_D^2 + \sigma_{WT}^2 + \sigma_{WR}^2}{2\sigma_{WR}^2} \tag{17}$$

The individual BE criterion (IBC) in equation (6) is derived from equation (15), such that the criterion equals zero for two identical formulations. The square root of equation (15) is the individual difference ratio (IDR).

$$\text{IDR} = \frac{(\mu_T - \mu_R)^2 + \sigma_D^2 + \sigma_{WT}^2 + \sigma_{WR}^2}{2\sigma_{WR}^2} \tag{18}$$

The IDR is the square root of the ratio of the expected squared $T-R$ difference compared to the expected squared $R-R'$ difference within an individual. The IDR is monotonically related to the IBC described in equation (6) as follows:

$$\text{IDR} = (\text{IBC}/2 + 1) \tag{19}$$

This guidance recommends that $\sigma_{W_0} = 0.2$, based on the consideration of the maximum allowable IDR of 1.25. (The IDR upper bound of 1.25 is drawn from the currently used BE limit of 1.25 for the ABE criterion.)

θ_P and θ_I

The determination of θ_P and θ_I should be based on the consideration of ABE criterion and the addition of variance terms to the population and IBC, as expressed by the formula below.

$$\theta = \frac{\text{ABE limit} + \text{variance factor}}{\text{variance}}$$

Population BE

$$\theta_P = \frac{-(\ln 1.25)^2 + \varepsilon_P}{\sigma^2_{T_0}} \tag{20}$$

The value of ε_P for population BE is guided by the consideration of the variance term $(\sigma^2_{TT} - \sigma^2_{TR})$ added to the ABE criterion.

Individual BE

$$\theta_P = \frac{-(\ln 1.25)^2 + \varepsilon_I}{\sigma^2_{W_0}} \tag{21}$$

The value of ε_I for individual BE is guided by the consideration of the estimate of SFI (σ_D) as well as the difference in within-subject variability $(\sigma^2_{WT} - \sigma^2_{WR})$ added to the ABE criterion. The recommended allowance for the variance term $(\sigma^2_{WT} - \sigma^2_{WR})$ is 0.02. In addition, this guidance recommends a σ^2_D allowance of 0.03. The σ magnitude of σ_D is associated with the percentage of individuals whose average T to R ratios lie outside 0.8 to 1.25. It is estimated that if $\sigma_D = 0.1356$, ~10% of the individuals would have their average ratios outside 0.8 to 1.25, even if $\mu_T - \mu_R = 0$. When $\sigma_D = 0.1741$, the probability is ~20%.

Accordingly, on the basis of consideration for both σ_D and variability $(\sigma^2_{WT} - \sigma^2_{WR})$ in the criterion, this guidance recommends that $\varepsilon_I = 0.05$.

STUDY DESIGN

Experimental Design

Nonreplicated Designs

A conventional nonreplicated design, such as the standard two-formulation, two-period, two-sequence crossover design, can be used to generate data where an average or population approach is chosen for BE comparisons. Under certain circumstances, parallel designs can also be used.

Replicated Crossover Designs

Replicated crossover designs can be used irrespective of which approach is selected to establish BE, although they are not necessary when an average or population approach is used. Replicated crossover designs are critical when an individual BE approach is used to allow estimation of within-subject variances for the T and R measures and the SFI variance component. The following four-period, two-sequence, two-formulation design is recommended for replicated BE studies.

	Period			
Sequence	**1**	**2**	**3**	**4**
1	T	R	T	R
2	R	T	R	T

For this design, the same lots of the T and R formulations should be used for the replicated administration. Each period should be separated by an adequate washout period.

Other replicated crossover designs are possible. For example, a three-period design, as shown below, could be used.

	Period		
Sequence	**1**	**2**	**3**
1	T	R	T
2	R	T	R

A greater number of subjects would be encouraged for the three-period design compared to the recommended four-period design to achieve the same statistical power to conclude BE.

Sample Size Determination

Sample sizes for ABE should be obtained using published formulas. Sample sizes for population and individual BE should be based on simulated data. The simulations should be conducted using a default situation allowing the two formulations to vary as much as 5% in average BA with equal variances and certain magnitude of SFI. The study should have 80% or 90% power to conclude BE between these two formulations. Sample size also depends on the magnitude of variability and the design of the study. Variance estimates to determine the number of subjects for a specific drug can be obtained from the biomedical literature and/or pilot studies.

Tables 1–4 give sample sizes for 80% and 90% power using the specified study design, given a selection of within-subject standard deviations (natural log scale), between-subject standard deviations (natural log scale), and SFI, as appropriate.

While the above sample sizes assume equal within-subject standard deviations, simulation studies for three-period and four-period designs reveal that if $\Delta = 0$ and $\sigma^2_{WT} - \sigma^2_{WR} = 0.05$, the sample sizes given will provide either 80% or 90% power for these studies. A minimum of 12 subjects in all BE studies, one case where $n = 10$ provides 80% power should be increased to $n = 12$.

TABLE 1 Average Bioequivalence: Estimated Numbers of Samples $\Delta = 0.05$

		80% Power		90% Power	
$\sigma_{WT} = \sigma_{WR}$	σ_D	2P	4P	2P	4P
0.15	0.01	12	6	16	8
	0.10	14	10	18	12
	0.15	16	12	22	16
0.23	0.01	24	12	32	16
	0.10	26	16	36	20
	0.15	30	18	38	24
0.30	0.01	40	20	54	28
	0.10	42	24	56	30
	0.15	44	26	60	34
0.50	0.01	108	54	144	72
	0.10	110	58	148	76
	0.15	112	60	150	80

Note: 1. Results for two-period designs use method of Diletti et al. (1991). 2. Results for four-period designs use relative efficiency data of Liu (1995).

TABLE 2 Population Bioequivalence: Four-Period Design (RTRT/TRTR), Estimated Numbers of Subjects $\varepsilon = 0.02$, $\Delta = 0.05$

$\sigma_{WR} = \sigma_{WT}$	$\sigma_{BR} = \sigma_{BT}$	80% Power	90% Power
0.15	0.15	18	22
	0.30	24	32
0.23	0.23	22	28
	0.46	24	32
0.30	0.30	22	28
	0.60	26	34
0.50	0.50	22	28
	1.00	26	34

Note: Results for population BE are approximate from simulation studies (1540 simulations for each parameter combination), assuming two-sequence, four-period trials with a balanced design across sequences.

TABLE 3 Individual Bioequivalence: Estimated Numbers of Subjects $\varepsilon_I = 0.05$, $\Delta = 0.05$

		80% Power		90% Power	
$\sigma_{WT} = \sigma_{WR}$		3P 3P2S	4P	3P 3P2S	4P
0.15	0.01	14	10	18	12
	0.10	18	14	24	16
	0.15	28	22	36	26
0.23	0.01	42	22	54	30
	0.10	56	30	74	40
	0.15	76	42	100	56
0.30	0.01	52	28	70	36
	0.10	60	32	82	42
	0.15	76	42	100	56
0.50	0.01	52	28	70	36
	0.10	60	32	82	42
	0.15	76	42	100	56

Note: Results for individual BE are approximate using simulations (5000 simulations for each parameter combination). The designs used in simulations are RTR/TRT (3P) and RTRT/TRTR (4P) assuming two-sequence trials with a balanced design across sequences.

TABLE 4 Individual Bioequivalence: Estimated Numbers of Subjects $\varepsilon_I = 0.05$, $\Delta = 0.10$ with Constraint on Δ $[0.8 \leq \exp(\Delta) \leq 1.25]$

		80% Power	90% Power
$\sigma_{WT} = \sigma_{WR}$	σ_D	4P	4P
0.30	0.01	30	40
	0.10	36	48
	0.15	42	56
0.50	0.01	34	46
	0.10	36	48
	0.15	42	

Note: Results for individual BE are approximate using simulations (5000 simulations for each parameter combination). The designs used in simulations are RTRT/TRTR (4P), assuming two-sequence trials with a balanced design across sequences. When $\varepsilon_I = 0.05$, sample sizes remain the same as given in Table 3. This is because the studies are already powered for variance estimation and inference, and therefore, a constraint on the point estimate of has little influence on the sample size for small values of Δ.

Computer Software for Power Analysis of Sample Size in BE Trial with Interim Analysis

In a test of equivalence, a treatment mean and a reference mean are compared to each other. Equivalence is taken to be the alternative hypothesis, and the null hypothesis is nonequivalence. The model assumed may be additive or multiplicative. In the additive model, the focus is on the difference between the treatment mean and the reference mean, while in the multiplicative model, the focus is on the ratio of the treatment mean to the reference mean. In the additive model, the null hypothesis is that the difference between the treatment mean and the reference mean is not near zero. That is, the difference is less than the lower equivalence bound or greater than the upper equivalence bound and thus nonequivalent. The alternative is that the difference is between the equivalence bounds; therefore, the two means are considered to be equivalent.

In the multiplicative model, the null hypothesis is that the ratio of the treatment mean to the reference mean is not near one. That is, the ratio is below the lower equivalence bound or above the upper equivalence bound, and thus the two means are not equivalent. The alternative is that the ratio is between the bounds; thus, the two means are considered to be equivalent.

The power of a test is the probability of rejecting the null hypothesis when the alternative is true. In this case, the power is the probability of accepting equivalence when the treatments are in fact equivalent, that is, the treatment difference or ratio is within the prespecified boundaries.

Often, the null difference is specified to be zero; the null hypothesis is that the treatment difference is less than the lower bound or greater than the upper bound, and the alternative is that the difference is not outside the bounds specified. However, in a case where you suspect that the treatments differ slightly, for example, $\mu_1 = 6,\ \mu_2 = 5,\ \mu_1 - \mu_2 = 1$.

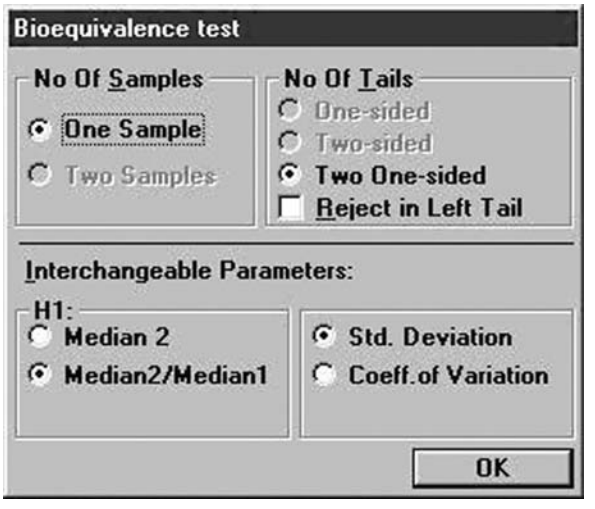
But if we want to rule out a larger difference (e.g., $|\mu_1 - \mu_2| > 2$) with the probability equal to the power you select, we would specify the null difference to be one and the lower and upper bounds to be -2 and 2, respectively. Note that the null difference must lie within the bounds specified.

Whereas a large volume of literature data report methods to calculate an appropriate sample size, a handy statistical analysis software, StudySize 2.0 (http://www.studysize.com/) works well for most types of analyses encountered. Given below is an exercise showing how to perform power analysis in multiplicative sample size analysis (courtesy of Studysize.com). The SAS software also offers a simple method of calculation of sample size.

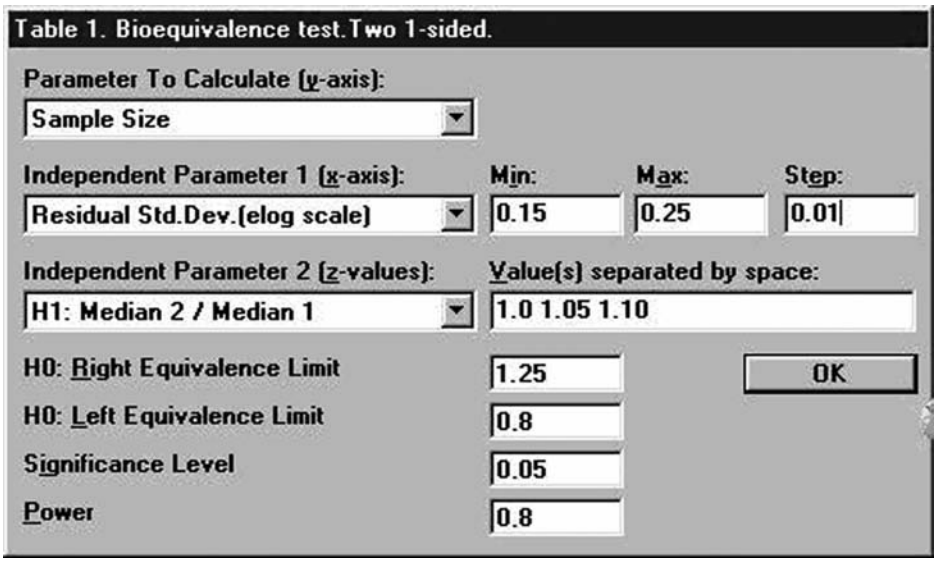
A new formulation of a drug has been developed. A two-way crossover study is planned to compare this new formulation with the existing formulation. The concentration of the active substance is measured over a 24-hour time interval and the area under the concentration curve (AUC) is calculated. The new formulation is considered bioequivalent to the old one if the ratio of the true mean AUC can be concluded to be within the interval 0.80 to 1.25. The null hypothesis is that the true ratio is outside the interval. BE is concluded if the null hypothesis is rejected. The null hypothesis is rejected at an upper significance level of 0.05 if the TOST for testing the ratio is less than 0.8 and greater than 1.25, respectively, both are rejected at the significance level of 0.05 (TOST situation). It can be shown that this is equivalent to a confidence interval for the true ratio, with confidence level 0.90 being entirely within the interval 0.8 to 1.25.

The analysis for the two-way crossover design is performed using an ANOVA on the log-transformed AUC values. The study is planned to have a power of 0.80 to conclude BE if the true ratio is approximately 1.05 at the significance level of 0.05. The expected residual standard deviation in the ANOVA (the within-subject standard deviation) for the log-transformed AUC values is assumed to be in the range 0.15 to 0.25.

1. Open the File menu and choose New Table.
2. Open the Test Procedure menu and choose BE test.
3. Set the following options and press OK.



4. Set the following values and press OK.



This will create a table where the sample size is calculated for a range of values for the residual standard deviation and for three distinct values of the true AUC ratio: 1.0, 1.05, and 1.10. Note that the sample size is treated as a continuous parameter.

Table 1

Bioequivalence test.Two 1-sided.
Sample Size as a function of Residual Std.Dev.(elog scale) and H1: Median 2 / Median 1.
H0: Right Equivalence Limit=1.25 H0: Left Equivalence Limit=0.8 Significance Level=0.05
Power=0.8

Residual Std.Dev.(elog scale)	H1: Median 2 / Median 1		
	1.00	1.05	1.10
0.15	9.5	11.1	18.6
0.16	10.5	12.4	20.9
0.17	11.6	13.7	23.4
0.18	12.8	15.2	26.0
0.19	14.0	16.7	28.8
0.20	15.3	18.3	31.7
0.21	16.7	20.0	34.8
0.22	18.2	21.8	38.1
0.23	19.7	23.7	41.5
0.24	21.3	25.7	45.0
0.25	23.0	27.7	48.7

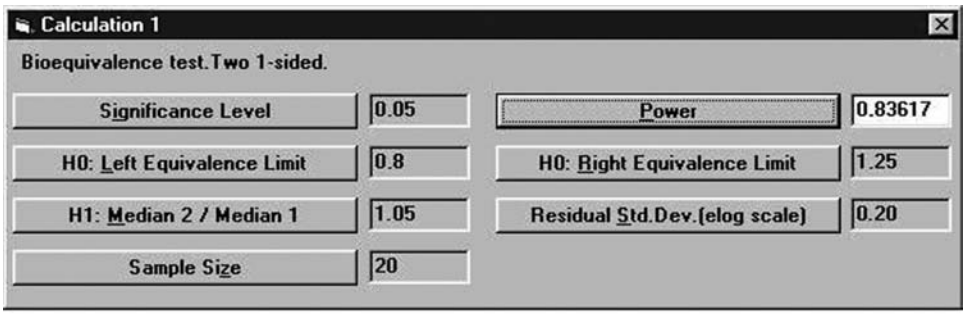
Edit Table

The table shows the sample sizes needed if the true AUC ratio is 1.05 and the residual standard deviation is in the interval 0.15 to 0.25. It also shows how much smaller and larger the sample size has to be if the assumption about the true AUC ratio instead is set to 1.0 and 1.10.

Since the study is planned to be a two-period crossover study and we usually want the same number of patients in the two possible formulation sequences, the sample sizes should be

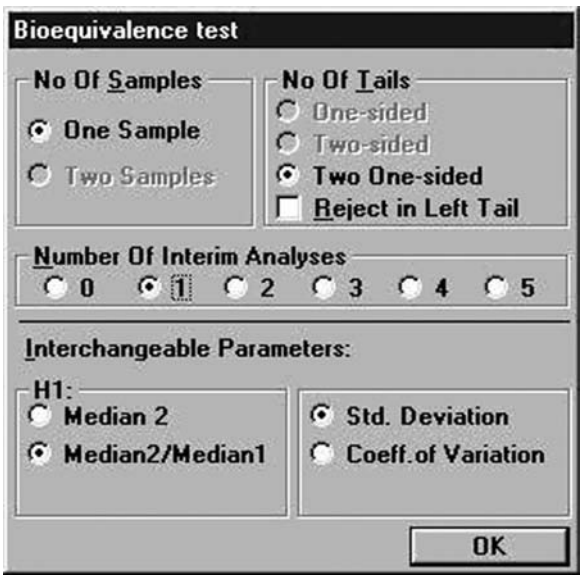
rounded upward to the nearest even integer. For example, with a ratio of 1.05 and a residual standard deviation of 0.20, the sample size 18.3 should be rounded upward to 20.

1. To calculate the power for 20 subjects.
2. Open the File menu and choose New Calculation.
3. Open the Test Procedure menu and choose BE test.
4. The dialog box with the retained values will show up. Press the OK button.
5. Set the following values except for power and then press the Power button.



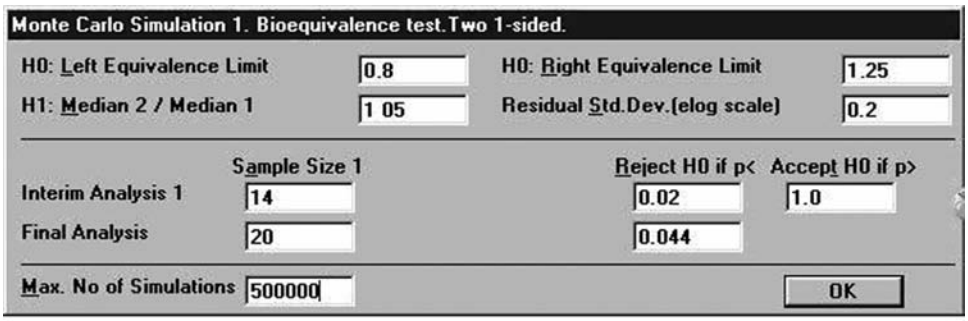
There is now a suggestion to investigate whether BE can be concluded before all the 20 subjects have entered the study.

1. Open the File Menu and choose New Monte Carlo Simulation.
2. Open the Test Procedure menu and choose BE test.
3. Set the number of interim analyses to one in the dialog box. The old parameter settings are retained. Press the OK button.



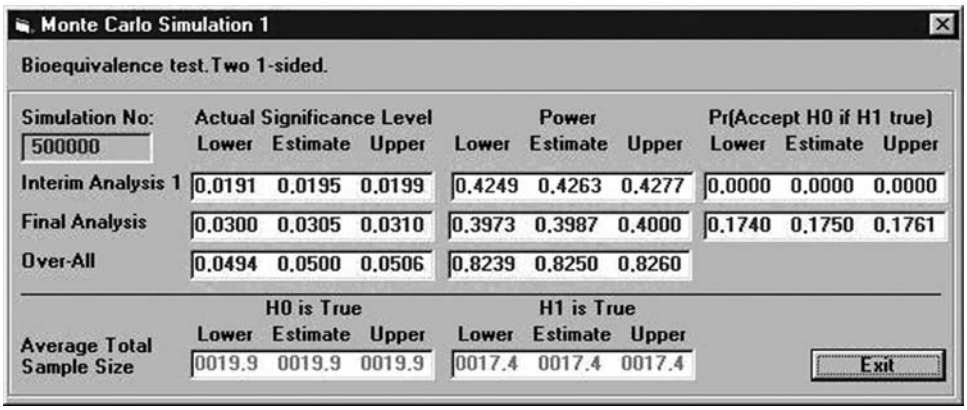
4. A new dialog box will be shown.

To compensate for the two analyses, one has to choose the significance level at the interim analysis and the final analysis in such a way that the overall significance level will be 0.05. There are many possibilities for such a design. For example, set the values as shown below and press OK.



If one prefers to use confidence intervals instead of the p values, the corresponding confidence levels are 96% at the interim analysis and 91.2% at the final analysis. BE is concluded if the respective confidence interval is within the interval 0.80 to 1.25.

The results presented below are after 500,000 Monte Carlo simulations.



The significance level is controlled and approximately 0.05 and the power is 0.825. The average number of subjects when H_1 is true is $0.4263 \times 14 + (1 - 0.4263) \times 20 = 17.4$, a gain of two subjects when compared with 20 but with somewhat lower power. However, there is a large chance (0.4263) that 14 subjects will suffice to show BE.

Sample Size and Dropouts

A minimum number of 12 evaluable subjects should be included in any BE study. When an ABE approach is selected using either nonreplicated or replicated designs, methods appropriate to the study design should be used to estimate sample sizes. The number of subjects for BE studies based on either the population or individual BE approach can be estimated by simulation if analytical approaches for estimation are not available.

Sponsors should enter a sufficient number of subjects in the study to allow for dropouts. Because replacement of subjects during the study could complicate the statistical model and analysis, dropouts generally should not be replaced. Sponsors who wish to replace dropouts during the study should indicate this intention in the protocol. The protocol should also state whether samples from replacement subjects, if not used, will be assayed. If the dropout rate is high and sponsors wish to add more subjects, a modification of the statistical analysis may be recommended. Additional subjects should not be included after data analysis unless the trial was designed from the beginning as a sequential or group sequential design.

STATISTICAL ANALYSIS

The following sections provide recommendations on statistical methodology for assessment of average, population, and individual BE.

Logarithmic Transformation

General Procedures

This guidance recommends that BE measures (e.g., AUC and C_{max}) be log transformed using either common logarithms to the base 10 or natural logarithms. The choice of common or natural logs should be consistent and should be stated in the study report. The limited sample size in a typical BE study precludes a reliable determination of the distribution of the dataset. Sponsors and/or applicants are not encouraged to test for normality of error distribution after log transformation, nor should they use normality of error distribution as a reason for carrying out the statistical analysis on the original scale. Justification should be provided if sponsors or applicants believe that their BE study data should be statistically analyzed on the original rather than on the log scale.

Clinical Rationale

The FDA Generic Drugs Advisory Committee recommended in 1991 that the primary comparison of interest in a BE study is the ratio, rather than the difference, between average parameter data from the *T* and *R* formulations. Using logarithmic transformation, the general linear statistical model employed in the analysis of BE data allows inferences about the difference between the two means on the log scale, which can then be retransformed into inferences about the ratio of the two averages (means or medians) on the original scale. Logarithmic transformation thus achieves a general comparison based on the ratio rather than the differences.

PK Rationale

Westlake observed that a multiplicative model is postulated for PK measures in BA/BE studies (i.e., AUC and C_{max}, but not T_{max}). Assuming that elimination of the drug is first order and only occurs from the central compartment, the following equation holds after an extravascular route of administration:

$$\mathrm{AUC}_{0-\infty} = \frac{FD}{\mathrm{CL}} \tag{22}$$

$$= FD/(VK_e) \tag{23}$$

where F is the fraction absorbed, D is the administered dose, and FD is the amount of drug absorbed. CL is the clearance of a given subject that is the product of the apparent volume of distribution (V) and the elimination rate constant (K_e). [Note that a more general equation can be written for any multicompartmental model as

$$\mathrm{AUC}_{0-\infty} = \frac{FD}{V_{d\beta}\lambda_n} \tag{24}$$

where $V_{d\beta}$ is the volume of distribution relating drug concentration in plasma or blood to the amount of drug in the body during the terminal exponential phase, and λ_n is the terminal slope of the concentration–time curve.]

The use of AUC as a measure of the amount of drug absorbed involves a multiplicative term (CL) that might be regarded as a function of the subject. For this reason, Westlake contends that the subject effect is not additive if the data are analyzed on the original scale of measurement.

Logarithmic transformation of the AUC data will bring the CL (VK_e) term into the following equation in an additive fashion:

$$\ln \mathrm{AUC}_{0-\infty} = \ln F + \ln D - \ln V - \ln K_e \tag{25}$$

Similar arguments were given for C_{max}. The following equation applies for a drug exhibiting one compartmental characteristics:

$$C_{max} = (FD/V)x\, e^{-k_e T_{max}} \tag{26}$$

where again F, D, and V are introduced into the model in a multiplicative manner. However, after logarithmic transformation, the equation becomes

$$\ln C_{max} = \ln F + \ln D - \ln V - K_e T_{max} \quad (27)$$

Thus, log transformation of the C_{max} data also results in the additive treatment of the V term.

Presentation of Data

The drug concentration in biological fluid determined at each sampling time point should be furnished on the original scale for each subject participating in the study. The PK measures of systemic exposure should also be furnished on the original scale. The mean, standard deviation, and coefficient of variation for each variable should be computed and tabulated in the final report.

In addition to the arithmetic mean and associated standard deviation (or coefficient of variation) for the T and R products, geometric means (antilog of the means of the logs) should be calculated for selected BE measures. To facilitate BE comparisons, the measures for each individual should be displayed in parallel for the formulations tested. In particular, for each BE measure, the ratio of the individual geometric mean of the T product to the individual geometric mean of the R product should be tabulated for each subject. The summary tables should indicate in which sequence each subject received the product.

Data Analysis

ABE

Overview

Parametric (normal theory) methods are recommended for the analysis of log-transformed BE measures. For ABE using the criterion stated in equation (2) or (3), the general approach is to construct a 90% confidence interval for the quantity $\mu_T - \mu_R$ and to reach a conclusion of ABE if this confidence interval is contained in the interval $[-\theta_A, \theta_A]$. Due to the nature of normal theory confidence intervals, this is equivalent to carrying out TOST of hypothesis at the 5% level of significance.

The 90% confidence interval for the difference in the means of the log-transformed data should be calculated using methods appropriate to the experimental design. The antilogs of the confidence limits obtained constitute the 90% confidence interval for the ratio of the geometric means between the T and R products.

Nonreplicated Crossover Designs

For nonreplicated crossover designs, this guidance recommends parametric (normal theory) procedures to analyze log-transformed BA measures. General linear model procedures available in PROC GLM in SAS or equivalent software are preferred, although linear mixed effects model procedures can also be indicated for analysis of nonreplicated crossover studies.

For example, for a conventional two-treatment, two-period, two-sequence (2×2) randomized crossover design, the statistical model typically includes factors accounting for the following sources of variation: sequence, subjects nested in sequences, period, and treatment. The *Estimate* statement in SAS PROC GLM, or equivalent statement in other software, should be used to obtain estimates for the adjusted differences between treatment means and the standard error associated with these differences.

Replicated Crossover Designs

Linear mixed effects model procedures, available in PROC MIXED in SAS or equivalent software, should be used for the analysis of replicated crossover studies for ABE. The following illustrates an example of program statements to run the ABE analysis using PROC MIXED in SAS version 6.12, with SEQ, SUBJ, PER, and TRT identifying sequence, subject, period, and treatment variables, respectively, and Y denoting the response measure [e.g., log(AUC), log(C_{max})] being analyzed.

```
PROC MIXED;
CLASSES SEQ SUBJ PER TRT;
MODEL Y=SEQ PER TRT/DDFM=SATTERTH;
RANDOM TRT/TYPE=FA0(2) SUB=SUBJ G;
REPEATED/GRP=TRT SUB=SUBJ;
ESTIMATE 'T versus R' TRT 1 -1/CL ALPHA=0.1;
```

The Estimate statement assumes that the code for the *T* formulation precedes the code for the *R* formulation in sort order (this would be the case, for example, if *T* were coded as 1 and *R* were coded as 2). If the *R* code precedes the *T* code in sort order, the coefficients in the Estimate statement would be changed to −1 1.

In the *Random* statement, TYPE=FA0(2) could possibly be replaced by TYPE=CSH. This guidance recommends that TYPE=UN not be used, as it could result in an invalid (i.e., not nonnegative definite) estimated covariance matrix.

Additions and modifications to these statements can be made if the study is carried out in more than one group of subjects.

Another statistical software "EquivTestPK" (Statistical Solutions, Saugus, Massachusetts, U.S.A) has been designed and developed with the aim of making the actual execution of equivalence testing procedures easier for a typical final user (e.g., a researcher in a pharmaceutical company), while at the same time ensuring both a high level of statistical competency and access to other powerful features (e.g., import formats, other statistical procedures) of the SAS software. Although SAS GLM and MIXED procedures can be used for "standard" BE testing (e.g., 90% confidence intervals and TOST), their use is not straightforward because the test results have to be calculated (using appropriate formulas) from SAS procedures outputs. Also, it is usually necessary to first examine the results from the model with carryover effects (in case of crossover design), and then (in the absence of significant carryover effects) from the model without carryover effects. Furthermore, reporting on BE studies require that some standard tables, figures, and listings (TFL) (e.g., means, CV, ratios, estimates of inter- and intrasubject variability, etc.) be supplied in addition to the BE test(s) results. These usually require that appropriate manipulations and transformations be applied to the data before TFLs are made. The purpose of EquivEasy application is

- to raise the likelihood of proper reporting on BE studies [for data from two-treatment, two-period crossover design and 3×3 crossover (Williams) design],
- to minimize the errors in report preparation (increased quality),
- to minimize the maximum time required for studies (increased efficiency),
- to reduce the need for in-house SAS expertise (i.e., so as to simplify use and to reduce training costs),
- to maximize the uniformity of reporting (standardization), and
- to minimize additional validation costs by using prevalidated SAS Institute software procedures wherever possible.

Parallel Designs

For parallel designs, the confidence interval for the difference of means in the log scale can be computed using the total between-subject variance. As in the analysis for replicated designs, equal variances should not be assumed.

Population BE

Overview

Analysis of BE data using the population approach should focus first on estimation of the mean difference between the *T* and *R* for the log-transformed BA measure and estimation of the total variance for each of the two formulations. This can be done using relatively simple unbiased estimators such as the method of moments (MM). After the estimation of the mean difference and the variances has been completed, a 95% upper confidence bound for the PBC can be

obtained, or equivalently a 95% upper confidence bound for a linearized form of the PBC can be obtained. Population BE should be considered to be established for a particular log-transformed BA measure if the 95% upper confidence bound for the criterion is less than or equal to the BE limit, θ_P, or equivalently if the 95% upper confidence bound for the linearized criterion is less than or equal to zero.

To obtain the 95% upper confidence bound of the criterion, intervals based on validated approaches can be used. The procedure involves the computation of a test statistic that is either positive (does not conclude population BE) or negative (concludes population BE).

Consider the following statistical model which assumes a four-period design with equal replication of T and R in each of s sequences with an assumption of no (or equal) carryover effects (equal carryovers go into the period effects)

$$Y_{ijld} = \mu_k + \gamma_{ild} + \delta_{ijk} + \varepsilon_{jld} \tag{28}$$

where $i=1,\ldots s$ indicates sequence, $j=1,\ldots n_i$ indicates subject within sequence i, $k=R$, T indicates treatment, $l=1$, 2 indicates replicate on treatment k for subjects within sequence Y_{ijkl} is the response of replicate l on treatment k for subject j in sequence i, Y_{ijkl} represents the fixed effect of replicate l on treatment k in sequence i, δ_{ijk} is the random subject effect for subject j in sequence i on treatment k, and ε_{ijkl} is the random error for subject j within sequence i on replicate l of treatment k. The ε_{ijkl}'s are assumed to be mutually independent and identically distributed as $\varepsilon_{ijkl} \sim N(0, \sigma^2_{Wk})$ for $i=1,\ldots s$, $j=1,\ldots n_i$, $k=R$, T, and $l=1$, 2. Also, the random subject effects

$$\delta_{ij} = (\mu_R + \delta_{ijR},\ \mu_T + \delta_{ijT})' \tag{29}$$

are assumed to be mutually independent and distributed as

$$\delta_{ij} \sim N_2\left[\begin{pmatrix}\mu_R\\ \mu_T\end{pmatrix}, \begin{pmatrix}\sigma^2_{BR} & p\sigma_{BT}\sigma_{BR}\\ p\sigma_{BT}\sigma_{BR} & \sigma^2_{BT}\end{pmatrix}\right] \tag{30}$$

The following constraint is applied to the nuisance parameters to avoid overparameterization of the model for $k=R$, T:

$$\sum_{i=1}^{s}\sum_{l=1}^{2}\gamma_{ild} = 0 \tag{31}$$

This statistical model assumes $s\times p$ location parameters (where p is the number of periods) that can be partitioned into t treatment parameters and $sp-t$ nuisance parameters. This produces a saturated model. The various *nuisance* parameters are estimated in this model, but the focus is on the parameters needed for population BE. In some designs, the sequence and period effects can be estimated through a reparametrization of the nuisance effects. This model definition can be extended to other crossover designs.

Linearized Criteria

$$\text{Reference scaled: } \eta_1 = (\mu_T - \mu_R)^2 + (\sigma^2_{TT} - \sigma^2_{TR}) - \theta_P\sigma^2_{TR} < 0 \tag{32}$$

$$\text{Constant scaled: } \eta_2 = (\mu_T - \mu_R)^2 + (\sigma^2_{TT} - \sigma^2_{TR}) - \theta_P\sigma^2_{T_0} < 0 \tag{33}$$

Estimating the Linearized Criteria

The estimation of the linearized criteria depends on study designs. The remaining estimation and confidence interval procedures assume a four-period design with equal replication of T and R in each of s sequences. The reparametrizations are defined as

$$U_{\mathrm{T}ij} = \frac{1}{2}\left(Y_{ij\mathrm{T}_1} + Y_{ij\mathrm{T}_2}\right) \tag{34}$$

$$U_{\mathrm{R}ij} = \frac{1}{2}\left(Y_{ij\mathrm{R}_1} + Y_{ij\mathrm{R}_2}\right) \tag{35}$$

$$V_{\mathrm{T}ij} = \frac{1}{\sqrt{2}}\left(Y_{ij\mathrm{T}_1} + Y_{ij\mathrm{T}_2}\right) \tag{36}$$

$$V_{\mathrm{R}ij} = \frac{1}{\sqrt{2}}\left(Y_{ij\mathrm{R}_1} + Y_{ij\mathrm{R}_2}\right) \tag{37}$$

$$I_{ij} = Y_{ij\mathrm{T}\cdot} - Y_{ij\mathrm{R}\cdot}, \tag{38}$$

for $i=1,\ldots,s$ and $j=1,\ldots,n_i$, where

$$Y_{ij\mathrm{T}_1} = \frac{1}{2}\left(Y_{ij\mathrm{T}_1} + Y_{ij\mathrm{T}_2}\right) \quad \text{and} \quad Y_{ij\mathrm{R}_1} = \frac{1}{2}\left(Y_{ij\mathrm{R}_1} + Y_{ij\mathrm{R}_2}\right).$$

Compute the formulation means pooling across sequences

$$\hat{\mu}_k = \frac{1}{s}\sum_{i=1}^{s} \bar{Y}_{i.k.}, \qquad k = R, T \quad \text{and} \quad \hat{\Delta} = \hat{\mu}_{\mathrm{T}} - \hat{\mu}_{\mathrm{R}}$$

where

$$\bar{Y}_{i.k.} = \frac{1}{n_i}\sum_{j=1}^{n_i}\frac{1}{2}\sum_{l=1}^{2} Y_{ijld}.$$

Compute the variances of U_{Tij}, $U_{\mathrm{R}ij}$, $V_{\mathrm{T}ij}$, and $V_{\mathrm{R}ij}$, pooling across sequences, and denote these variance estimates by $\mathrm{MU_T}$, $\mathrm{MU_R}$, $\mathrm{MV_T}$, and $\mathrm{MV_R}$, respectively. Specifically,

$$\mathrm{MU_T} = \frac{1}{n_{\mathrm{U_T}}}\sum_{i=1}^{s}\sum_{j=1}^{n_i}(U_{\mathrm{T}ij} - \bar{U}_{\mathrm{T}i})^2 \tag{39}$$

$$\mathrm{MV_T} = \frac{1}{n_{\mathrm{V_T}}}\sum_{i=1}^{s}\sum_{j=1}^{n_i}(V_{\mathrm{T}ij} - \bar{V}_{\mathrm{T}i})^2 \tag{40}$$

$$\mathrm{MU_R} = \frac{1}{n_{\mathrm{U_R}}}\sum_{i=1}^{s}\sum_{j=1}^{n_i}(U_{\mathrm{R}ij} - \bar{U}_{\mathrm{R}i})^2 \tag{41}$$

$$\mathrm{MV_R} = \frac{1}{n_{\mathrm{V_R}}}\sum_{i=1}^{s}\sum_{j=1}^{n_i}(V_{\mathrm{R}ij} - \bar{V}_{\mathrm{R}i})^2$$

$$n_{\mathrm{I}} = n_{\mathrm{U_T}} = n_{\mathrm{U_R}} = n_{\mathrm{V_T}} = n_{\mathrm{V_R}} = \left(\sum_{i=1}^{s} n_i\right) - s \tag{42}$$

Then, the linearized criteria are estimated by

$$\text{Reference scaled: } \eta_1 = \hat{\Delta}^2 + \mathrm{MU_T} + 0.5\,\mathrm{MV_T} - (1+\theta_{\mathrm{P}})[\mathrm{MU_R} + 0.5\,\mathrm{MV_R}] \tag{43}$$

$$\text{Constant scaled: } \eta_2 = \hat{\Delta}^2 + \mathrm{MU_T} + 0.5\,\mathrm{MV_T} - (1)[\mathrm{MU_R} + 0.5\,\mathrm{MV_R}] - \theta_{\mathrm{P}}\sigma_{\mathrm{T}_0} \tag{44}$$

95% Upper Confidence Bounds for Criteria

The table below illustrates the construction of a $(1-\alpha)$ level upper confidence bound based on the two-sequence, four-period design, for the reference-scaled criterion, $\hat{\eta}_1$. Use $\alpha=0.05$ for a 95% upper confidence bound.

H_q = confidence bound	E_q = point estimate	$U_q = (H_q - E_q)^2$
$H_D = (\lvert\hat{\Delta}\rvert + t_{1-\alpha,\ n-s}(1/s^2 \sum_{i=1}^{s} n_i^{-1} M_1)^{1/2})^2$	$E_D = \hat{\Delta}^2$	U_D
$H_1 = (n-s)E_1/(\chi^2 n-s)$	$MU_T = E_1$	U_1
$H_2 = (n-s)E_2/(\chi^2 n-s)$	$0.5MV_T = E_2$	U_2
$H_3 rs = (n-s)E_3 rs/(\chi^2 n-s,\ 1-\alpha)$	$-(1+\theta_p)MU_R = E_3 rs$	$U_3 rs$
$H_4 rs = (n-s)E_4 rs/(\chi^2 n-s,\ 1-\alpha)$	$-(1+\theta_p)MU_R = E_3 rs$	$U_4 rs$
$H_{\eta_1} = \sum E_q + (\sum U_q)^{1/2}$		

$H_{\eta_1} = \sum E_q + (\sum U_q)^{1/2}$ is the upper 95% confidence bound two for $\hat{\eta}_1$. Note $n = \sum_{i=1}^{s} n_i$, where s is the number of sequences, n_i is the number of subjects per sequence, and $\chi^2\alpha,n-s$ is from the cumulative distribution function of the chi-square distribution with $n-s$ degrees of freedom, i.e., $\Pr(\chi^2_{n-s} \le \chi^2_{\alpha,n-s}) = \alpha$. The confidence bound for $\hat{\eta}_2$ is computed similarly, adjusting the constants associated with the variance components where appropriate (in particular, the constant associated with MU_R and MV_R).

H_q = confidence bound	E_q = point estimate	$U_q = (H_q - E_q)^2$
$H_D = (\lvert\hat{\Delta}\rvert + t_{1-\alpha,\ n-s}(1/s^2 \sum_{i=1}^{s} n_i^{-1} M_1)^{1/2})^2$	$E_D = \hat{\Delta}^2$	U_D
$H_1 = (n-s)E_1/(\chi^2 n-s)$	$MU_T = E_1$	U_1
$H_2 = (n-s)E_2/(\chi^2 n-s)$	$0.5 \cdot MV_T = E_2$	U_2
$H_3 cs = (n-s)E_3 cs/(\chi^2 n-s,\ 1-\alpha)$	$-1 \cdot MU_R = E_3 cs$	$U_3 cs$
$H_4 cs = (n-s)E_4 cs/(\chi^2 n-s,\ 1-\alpha)$	$-0.5 \cdot MV_R = E_4 cs$	$U_4 cs$
$H_{\eta_2} = \sum E_q - \theta_p \sigma^2_{T_0} + (\sum U_q)^{1/2}$		

Using the mixed-scaling approach, to test for population BE, compute the 95% upper confidence bound of either the reference-scaled or constant-scaled linearized criterion. The selection of either reference-scaled or constant-scaled approach depends on the study estimate of total standard deviation of the reference product [estimated by $(MU_R + 0.5 \cdot MV_R)^{1/2}$ in the four-period design]. If the study estimate of standard deviation is $\le \sigma_{T_0}$, the constant-scaled criterion and its associated confidence interval should be computed. Otherwise, the reference-scaled criterion and its confidence interval should be computed. The procedure for computing each of the confidence bounds is described above. If the upper confidence bound for the appropriate criterion is negative or zero, conclude population BE. If the upper bound is positive, do not conclude population BE.

Nonreplicated Crossover Designs

For nonreplicated crossover studies, any available method (e.g., SAS PROC GLM or equivalent software) can be used to obtain an unbiased estimate of the mean difference in log-transformed BA measures between the *T* and *R* products. The total variance for each formulation should be estimated by the usual sample variance, computed separately in each sequence, and then pooled across sequences.

Replicated Crossover Designs

For replicated crossover studies, the approach should be the same as for nonreplicated crossover designs, but care should be taken to obtain proper estimates of the total variances. One approach is to estimate the within- and between-subject components separately, as for individual BE (see Section VI.B.3), and then sum them to obtain the total variance. The method for the upper confidence bound should be consistent with the method used for estimating the variances.

Parallel Designs

The estimate of the means and variances from parallel designs should be the same as for nonreplicated crossover designs. The method for the upper confidence bound should be modified to reflect independent rather than paired samples and to allow for unequal variances.

Individual BE

Analysis of BE data using an individual BE approach (Section IV.C) should focus on estimation of the mean difference between T and R for the log-transformed BA measure, the SFI variance, and the within-subject variance for each of the two formulations. For this purpose, we recommend the MM approach.

To obtain the 95% upper confidence bound of a linearized form of the IBC, intervals based on validated approaches can be used. The procedure involves the computation of a test statistic that is either positive (does not conclude individual BE) or negative (concludes individual BE).

Consider the following statistical model that assumes a four-period design with equal replication of T and R in each of s sequences with an assumption of no (or equal) carryover effects (equal carryovers go into the period effects)

$$Y_{ijkl} = \mu_k + \gamma_{ikl} + \delta_{ijk} + \varepsilon_{ijkl} \tag{45}$$

where $i=1,\ldots s$ indicates sequence, $j=1,\ldots n_i$ indicates subject within sequence i, $k=R$, T indicates treatment, $l=1$, 2 indicates replicate on treatment k for subjects within sequence i. Y_{ijkl} is the response of replicate l on treatment k for subject j in sequence i, γ_{ild} represents the fixed effect of replicate l on treatment k in sequence i, δ_{ijk} is the random subject effect for subject j in sequence i on treatment k, and ε_{ijld} is the random error for subject j within sequence i on replicate l of treatment k. The ε_{ijld}'s are assumed to be mutually independent and identically distributed as

$$\varepsilon_{ijkl} \sim N(0, \sigma^2_{\mathrm{W}k}) \tag{46}$$

for $i=1,\ldots s$, $j=1,\ldots n_i$, $k=R$, T, and $l=1$, 2. Also, the random subject effects $\delta_{ij} = (\mu_{\mathrm{R}} + \delta_{ij\mathrm{R}},\ \mu_{\mathrm{T}} + \delta_{ij\mathrm{T}})'$ are assumed to be mutually independent and distributed as

$$\delta_{ij} \sim N_2\left[\begin{pmatrix}\mu_{\mathrm{R}}\\ \mu_{\mathrm{T}}\end{pmatrix}, \begin{pmatrix}\sigma^2_{\mathrm{BR}} & p\sigma_{\mathrm{BT}}\sigma_{\mathrm{BR}}\\ p\sigma_{\mathrm{BT}}\sigma_{\mathrm{BR}} & \sigma^2_{\mathrm{BT}}\end{pmatrix}\right] \tag{47}$$

The following constraint is applied to the nuisance parameters to avoid overparameterization of the model for $k=R,T$:

$$\sum_{i=1}^{s}\sum_{l=1}^{2}\gamma_{ild} = 0 \tag{48}$$

This statistical model proposed by Chinchilli and Esinhart assumes $s\times p$ location parameters (where p is the number of periods) that can be partitioned into t treatment parameters and $sp-t$ nuisance parameters. This produces a saturated model. The various *nuisance* parameters are estimated in this model, but the focus is on the parameters needed for individual BE. In some designs, the sequence and period effects can be estimated through a reparametrization of the nuisance effects.

This model definition can be extended to other crossover designs.

Linearized Criteria

Reference scaled:

$$\eta_1 = (\mu_{\mathrm{T}} - \mu_{\mathrm{R}})^2 + \sigma^2_{\mathrm{D}} + (\sigma^2_{\mathrm{WT}} - \sigma^2_{\mathrm{WR}}) - \theta_1\sigma^2_{\mathrm{WR}} < 0 \tag{49}$$

Constant scaled:

$$\eta_2 = (\mu_{\mathrm{T}} - \mu_{\mathrm{R}})^2 + \sigma^2_{\mathrm{D}} + (\sigma^2_{\mathrm{WT}} - \sigma^2_{\mathrm{WR}}) - \theta_1\sigma^2_{\mathrm{W}_0} < 0 \tag{50}$$

Estimating the Linearized Criteria

The estimation of the linearized criteria depends on study designs. The remaining estimation and confidence interval procedures assume a four-period design with equal replication of T and

R in each of s sequences. The reparametrizations are defined as

$$I_{ij} = Y_{ij\mathrm{T}\cdot} - Y_{ij\mathrm{R}\cdot} \tag{51}$$

$$T_{ij} = Y_{ij\mathrm{T}_1} - Y_{ij\mathrm{T}_2} \tag{52}$$

$$R_{ij} = Y_{ij\mathrm{R}_1} - Y_{ij\mathrm{R}_2} \tag{53}$$

for $i=1,\ldots,s$ and $j=1,\ldots,n_i$, where

$$Y_{ij\mathrm{T}_1} = \frac{1}{2}\left(Y_{ij\mathrm{T}_1} + Y_{ij\mathrm{T}_2}\right) \quad \text{and} \quad Y_{ij\mathrm{R}_1} = \frac{1}{2}\left(Y_{ij\mathrm{R}_1} + Y_{ij\mathrm{R}_2}\right).$$

Compute the formulation means, and the variances of I_{ij}, T_{ij}, and R_{ij}, pooling across sequences, and denote these variance estimates by M_{I}, M_{T}, and M_{R}, respectively, where

$$\hat{\mu}_k = \frac{1}{s}\sum_{i=1}^{s} \bar{Y}_{i.k.},\ k = R, T \quad \text{and} \quad \hat{\Delta} = \hat{\mu}_{\mathrm{T}} - \hat{\mu}_{\mathrm{R}}$$

$$\bar{Y}_{i.k.} = \frac{1}{n_i}\sum_{j=1}^{n_i}\frac{1}{2}\sum_{l=1}^{2} Y_{ijld}$$

$$M_{\mathrm{I}} = \hat{\sigma}_{\mathrm{I}}^2 = \frac{1}{n_{\mathrm{I}}}\sum_{i=1}^{s}\sum_{j=1}^{n_i}(I_{ij} - \bar{I}_i)^2$$

$$n_{\mathrm{I}} = n_{\mathrm{T}} = n_{\mathrm{R}} = \left(\sum_{i=1}^{s} n_i\right) - s$$

$$M_{\mathrm{T}} = \hat{\sigma}_{\mathrm{WT}}^2 = \frac{1}{2n_{\mathrm{T}}}\sum_{i=1}^{s}\sum_{j=1}^{n_i}(T_{ij} - \bar{T}_i)^2$$

$$M_{\mathrm{R}} = \hat{\sigma}_{\mathrm{WR}}^2 = \frac{1}{2n_{\mathrm{R}}}\sum_{i=1}^{s}\sum_{j=1}^{n_i}(R_{ij} - \bar{R}_i)^2.$$

Then, the linearized criteria are estimated by

Reference scaled:

$$\hat{\eta}_1 = \hat{\Delta}^2 + M_1 + 0.5\,M_{\mathrm{T}} - (1.5 + \theta_1)M_{\mathrm{R}} \tag{54}$$

Constant scaled:

$$\hat{\eta}_2 = \hat{\Delta}^2 + M_1 + 0.5\,M_{\mathrm{T}} - 1.5\,M_{\mathrm{R}} - \theta_1\sigma_{\mathrm{W}_0}^2 \tag{55}$$

and the SFI variance component can be estimated by

$$\hat{\sigma}_{\mathrm{D}}^2 = \hat{\sigma}_{\mathrm{I}}^2 - \frac{1}{2}\left(\hat{\sigma}_{\mathrm{WT}}^2 + \hat{\sigma}_{\mathrm{WR}}^2\right) \tag{56}$$

95% Upper Confidence Bounds for Criteria

The table below illustrates the construction of a $(1-\alpha)$ level upper confidence bound based on the two-sequence, four-period design, for the reference-scaled criterion, $\hat{\eta}_1$. Use $\alpha=0.05$ for a 95% upper confidence bound.

H_q = confidence bound	E_q = point estimate	$U_q = (H_q - E_q)^2$
$H_D = (\lvert\hat{\Delta}\rvert + t_{1-\alpha,\, n-s}(1/s^2 \sum_{i=1}^{s} n_i^{-1} M_1)^{1/2})^2$	$E_D = \hat{\Delta}^2$	U_D
$H_I = (n-s)M_I/(\chi^2_{\alpha,n-s})$	$E_I = M_I$	U_I
$H_T = 0.5\,(n-s)M_T/(\chi^2_{\alpha,n-s})$	$E_T = 0.5 \cdot M_T$	U_T
$H_R = -(1.5+\theta_I)(n-s)M_R/\chi^2_{1-\alpha,\, n-s}$	$E_R = -(1.5+\theta_I) \cdot M_R$	U_R
$H_{\eta_1} = \sum E_q + (\sum U_q)^{1/2}$		

where $n = \sum_{i=1}^{s} n_i$, s is the number of sequences, and $\chi^2\alpha,n-s$ is from the cumulative distribution function of the chi-square distribution with $n-s$ degrees of freedom, i.e., Pr $(\chi^2 n-s \leq \chi^2\alpha,n-s) = \alpha$. Then, $H_{\eta_1} = \sum E_q + (\sum U_q)^{1/2}$ is the upper 95% confidence bound for $\hat{\eta}_1$. The confidence bound for $\hat{\eta}_2$ is computed similarly, adjusting the constants associated with the variance components where appropriate (in particular, the constant associated with M_R).

H_q = confidence bound	E_q = point estimate	$U_q = (H_q - E_q)^2$
$H_D = (\lvert\hat{\Delta}\rvert + t_{1-\alpha,\, n-s}(1/s^2 \sum_{i=1}^{s} n_i^{-1} M_1)^{1/2})^2$	$E_D = \hat{\Delta}^2$	U_D
$H_I = (n-s)M_I/(\chi^2_{\alpha,n-s})$	$E_I = M_I$	U_I
$H_T = 0.5\,(n-s)M_T/(\chi^2_{\alpha,n-s})$	$E_T = 0.5 \cdot M_T$	U_T
$H_R = -(1.5)(n-s)M_R/(\chi^2_{1-\alpha,\, n-s})s$	$E_R = -(1.5) \cdot M_R$	U_R
$H_{\eta_1} = \sum E_q - \theta_I \sigma^2_{W_0} + (\sum U_q)^{1/2}$		

Using the mixed-scaling approach, to test for individual BE, compute the 95% upper confidence bound of either the reference-scaled or constant-scaled linearized criterion. The selection of either reference-scaled or constant-scaled criterion depends on the study estimate of within-subject standard deviation of the reference product. If the study estimate of standard deviation is $\leq \sigma_{W_0}$, the constant-scaled criterion and its associated confidence interval should be computed. Otherwise, the reference-scaled criterion and its confidence interval should be computed. The procedure for computing each of the confidence bounds is described above. If the upper confidence bound for the appropriate criterion is negative or zero, conclude individual BE. If the upper bound is positive, do not conclude individual BE.

This guidance recommends that sponsors use either reference scaling or constant scaling at the changeover point. To test for individual BE, compute the 95% upper confidence bounds of both reference-scaled and constant-scaled linearized criteria. The procedure for computing these confidence bounds is described above. If the upper bound of either criterion is negative or zero (either H_{η_1} or H_{η_2}), conclude individual BE. If the upper bounds of both criteria are positive, do not conclude individual BE.

After the estimation of the mean difference and the variances has been completed, a 95% upper confidence bound for the IBC can be obtained, or equivalently a 95% upper confidence bound for a linearized form of the IBC can be obtained. Individual BE should be considered to be established for a particular log-transformed BA measure if the 95% upper confidence bound for the criterion is less than or equal to the BE limit, U_I, or equivalently if the 95% upper confidence bound for the linearized criterion is less than or equal to zero.

The restricted maximum likelihood (REML) method may be useful to estimate mean differences and variances when subjects with some missing data are included in the statistical analysis. A key distinction between the REML and MM methods relates to differences in estimating variance terms. If alternative methods to REML or MM are envisioned, the sponsor is encouraged to discuss it with appropriate CDER review staff prior to submitting their applications.

Variance Estimation

Relatively simple unbiased estimators, the MM or the REML method, can be used to estimate the mean and variance parameters in the individual BE approach. A key distinction between the REML and MM methods relates to differences in estimating variance terms. The REML method estimates each of the three variances, σ^2_D, σ^2_{WR}, σ^2_{WT}, separately and then combines them in the IBC. The REML estimate of σ^2_D is found from estimates of σ^2_{BR}, σ^2_{BT}. The MM approach is to estimate the sum of the variance terms in the numerator of the criterion, $\sigma^2_D + \sigma^2_{WT} - \sigma^2_{WR}$, and does not necessarily estimate each component separately. One consequence of this difference is that the MM estimator of σ^2_D is unbiased but could be negative. The REML approach can also lead to negative estimates, but if the covariance matrix of the random effects is forced to be a proper covariance matrix, the estimate of σ^2_D can be made to be nonnegative. This forced nonnegativity has the effect of making the estimate positively biased and introduces a small amount of conservatism to the confidence bound. The REML method can be used in special cases (e.g., when substantial missing data are present). In addition, the MM approaches have not yet been adapted to models that allow assessment of carryover effects.

Methods for Obtaining Confidence Intervals for Individual and Population BE Criteria

Individual BE Method 1—Constrained REML

Statistical Model

- Mixed effects ANOVA model in natural log scale.
- Subjects within sequence as random effects.
- Within- and between-subject variances allowed to differ by formulation.
- Fixed effects are formulation, period, sequence, and period×sequence interaction (nested within formulation)

Parameter Estimation

- REML estimates of random effects.
- Choose estimation procedure so that the between-subject covariance is nonnegative definite, i.e., so that the correlation does not exceed 1.0. (This is the constraint in "constrained REML." REML without the constraint, similar to Method 2, is possible but has not been evaluated).
- Generalized least-squares estimates of fixed effects (Type III coefficients).

Confidence Intervals

- Ninety-five percent upper confidence bounds using nonparametric percentile bootstrap confidence interval procedure (upper bound of the 90% two-sided confidence interval)
- Use minimum of 1500 (2000 recommended) bootstrap samples that preserve the number of subjects per sequence

Example of an Implementation—SAS

- SAS PROC MIXED version 6.10 for Windows™ 6.09 maintenance release for UNIX, or equivalent or later release (needed for csh covariance structure)
- The following is some SAS code for the above model and four-period designs:
  proc mixed method=reml maxiter=200;
  class formulat subj_id period sequence;
  model lnmetric=formulat period sequence period*sequence(formulat);

```
random formulat/subject=subj_id type=csh;
repeated/group=formulat;
estimate 'T-R' formulat -1 1;
```

- For three-period designs, the simple estimate statement is usually not enough. The coefficients for the estimable function need to be specified.
- Note that the type=un covariance structure is nominally the same as csh for this model (with a 2×2 covariance matrix). However, there are some differences. csh forces the covariance matrix to be nonnegative definite and is the approach that has been evaluated. Un may yield estimates of correlation greater than 1.0 and, hence, estimates of the SFI (σ_D) that are negative. However, the un structure sometimes finds better estimates than csh (in terms of likelihood) when it does find a positive definite covariance matrix.
- Bootstrapping uses SAS macros. (Do not use a BY statement to bootstrap PROC MIXED).

Individual BE Method 2—MM

Parameter Estimation (Complete Data)

- Use MM to obtain unbiased estimates of the components of the criterion, difference of means, sum of variance terms in numerator, the within-subject variance of the reference, and then bootstrap to obtain confidence intervals. The implied estimate of the subject-by-formulation term may then be negative (unconstrained). The between-subject components of variance are not estimated.
- Chinchilli provides estimates of the means.
- Variance estimates are standard unbiased estimates pooled across sequences.
- Formulas depend on design and so are not given here.
- Much simpler to implement than Method 1. For example, implementation in SAS requires only PROCs MEANS, SUMMARY, and TRANSPOSE.
- Compared to Method 1, Method 2 tends to yield larger estimates of the within-subject variances and smaller estimates of the SFI.
- This approach is possible with three-period designs that replicate only the reference. However, evaluation has considered only four-period designs.

Parameter Estimation (Incomplete Data)

- One approach to missing data with Method 2 is to use the methods described above for complete data with the following additions:
  - Any subject missing a formulation metric will not be used in the calculation of intrasubject variance component for that formulation.
  - Any subject with any missing *T* or *R* will not be used in the estimate of SFI.
  - In all cases, the denominator of the variance estimate will be corrected to reflect the number of subjects actually used in calculations for the particular variance component.
- There may be alternate approaches for handling missing data with Method 2. If there are more than minimal missing data, Method 1 is likely preferable to the approach described here for Method 2.

Population BE

Parameter Estimation

- As for individual BE Method 2, obtain unbiased estimates of the components of the criterion, difference of means, sum of variances in numerator, the total variance of the reference, and then bootstrap to obtain confidence intervals.

- Use any available method, such as SAS Proc GLM, to obtain unbiased estimates of the difference of means.
- For standard, two-period, two-sequence crossover designs, the variance estimates are the standard unbiased estimates pooled across sequences.

Some Examples for Individual BE and 95% Upper Confidence Bounds for Θ_S

Allowable upper limit 2.495 ($\varepsilon = 0.05$)	**Constrained REML**		**Method of moments**	
Scaled to	**Reference**	**Constant**	**Reference**	**Constant**
Furosemide	11.293	4.660	10.188	4.638
Verapamil	**2.179**	**1.898**	**1.644**	**1.505**
ac-5-ASA	**1.720**	**1.600**	2.649	**1.079**

Shaded area shows criterion selected by mixed scaling. Figures in bold satisfy the IBC at the 5% level.

Some Examples for Individual BE, Parameter Estimates, and 90% CI using Constrained REML

		Intra-Subject		**SD (σ_w)**	**Inter-Subject**	**SD (σ_B)**	
Dataset	***N***	**Ratio of means (orginal scale)**	**Reference**	**Test/reference ratio$_w$)**	**Reference**	**Test/reference ratio**	**Subject-by-form'n interaction(σ_D)**
Furosemide	8	**98.1**	.242	.986	.059	5.487	.274
		(81.2 – 116.9)	(.108 – .263)	(.543 – 1.813)	(.000 – .157)	(.665 – 6.725)	(.033 – .373)
Verapamil	23	**98.3**	.257	1.248	.533	.904	.051
		(91.1 – 106.0)	(.172 – .278)	(.924 – 1.700)	(.348– - .594)	(.705 – 1.070)	(.005 – .126)
ac-5-ASA	10	115.9	.327	.827	.517	.853	.076
		(106.0 – 127.3)	(.137 – .392)	(.493 – 1.463)	(.000 – .634)	(.635 – 1.058)	(.008 – .147)

Mean ratios in bold satisfy the 80/125 ABE criterion at the 5% level.

MISCELLANEOUS ISSUES

Studies in Multiple Groups

If a crossover study is carried out in two or more groups of subjects (e.g., if for logistical reasons only a limited number of subjects can be studied at one time), the statistical model should be modified to reflect the multigroup nature of the study. In particular, the model should reflect the fact that the periods for the first group are different from the periods for the second group. This applies to all of the approaches (average, population, and individual BE) described in this guidance.

If the study is carried out in two or more groups and those groups are studied at different clinical sites, or at the same site but greatly separated in time (e.g., months apart), questions may arise as to whether the results from the several groups should be combined in a single analysis. Such cases should be discussed with the appropriate CDER review division.

A *sequential* design, in which the decision to study a second group of subjects is based on the results from the first group, calls for different statistical methods and is outside the scope of this guidance. Those wishing to use a sequential design should consult the appropriate CDER review division.

Carryover Effects

Use of crossover designs for BE studies allows each subject to serve as their own control to improve the precision of the comparison. One of the assumptions underlying this principle is that *carryover effects* (also called *residual effects*) are either absent (the response to a formulation administered in a particular period of the design is unaffected by formulations administered in earlier periods) or equal for each formulation and preceding formulation. If the carryover

effects are present in a crossover study and are not equal, the usual crossover estimate of μ_{T-R} could be biased. One limitation of a conventional two-formulation, two-period, two-sequence crossover design is that the only statistical test available for the presence of unequal carryover effects is the sequence test in the analysis of variance (ANOVA) for the crossover design. This is a between-subject test, which would be expected to have poor discriminating power in a typical BE study. Furthermore, if the possibility of unequal carryover effects cannot be ruled out, no unbiased estimate of μ_{T-R} based on within-subject comparisons can be obtained with this design.

For replicated crossover studies, a within-subject test for unequal carryover effects can be obtained under certain assumptions. Typically only first-order carryover effects are considered of concern (i.e., the carryover effects, if they occur, only affect the response to the formulation administered in the next period of the design). Under this assumption, consideration of carryover effects could be more complicated for replicated crossover studies than for nonreplicated studies. The carryover effect could depend not only on the formulation that preceded the current period, but also on the formulation that is administered in the current period. This is called a *direct-by-carryover* interaction. The need to consider more than just *simple* first-order carryover effects has been emphasized. With a replicated crossover design, a within-subject estimate of μ_{T-R} unbiased by general first-order carryover effects can be obtained, but such an estimate could be imprecise, reducing the power of the study to conclude BE.

In most cases, for both replicated and nonreplicated crossover designs, the possibility of unequal carryover effects is considered unlikely in a BE study under the following circumstances:

- It is a single-dose study.
- The drug is not an endogenous entity.
- More than an adequate washout period has been allowed between periods of the study and in the subsequent periods the predose biological matrix samples do not exhibit a detectable drug level in any of the subjects.
- The study meets all scientific criteria (e.g., it is based on an acceptable study protocol and it contains sufficient validated assay methodology).

The possibility of unequal carryover effects can also be discounted for multiple-dose studies and/or studies in patients, provided that the drug is not an endogenous entity and the studies meet all scientific criteria as described above. Under all other circumstances, the sponsor or applicant could be asked to consider the possibility of unequal carryover effects, including a direct-by-carryover interaction. If there is evidence of carryover effects, sponsors should describe their proposed approach in the study protocol, including statistical tests for the presence of such effects and procedures to be followed. Sponsors who suspect that carryover effects might be an issue may wish to conduct a BE study with parallel designs.

Choice of Specific Replicated Crossover Designs

Reasons Unrelated to Carryover Effects

Each unique combination of sequence and period in a replicated crossover design can be called a *cell* of the design. For example, the two-sequence, four-period design recommended in Section V.A.1 has eight cells. The four-sequence, four-period design below has 16 cells.

	Period			
Sequence	**1**	**2**	**3**	**4**
1	T	R	R	T
2	R	T	T	R
3	T	T	R	R
4	R	R	T	T

The total number of degrees of freedom attributable to comparisons among the cells is just the number of cells minus one (unless there are cells with no observations).

The fixed effects that are usually included in the statistical analysis are sequence, period, and treatment (i.e., formulation). The number of degrees of freedom attributable to each fixed effect is generally equal to the number of levels of the effect minus one. Thus, in the case of the two-sequence, four-period design recommended in Section V.A.1, there would be $2-1=1$ degree of freedom due to sequence, $4-1=3$ degrees of freedom due to period, and $2-1=1$ degree of freedom due to treatment, for a total of $1+3+1=5$ degrees of freedom due to the three fixed effects. Because these five degrees of freedom do not account for all seven degrees of freedom attributable to the eight cells of the design, the fixed effects model is not *saturated*. There could be some controversy as to whether a fixed effects model that accounts for more or all of the degrees of freedom due to cells (i.e., a more saturated fixed effects model) should be used. For example, an effect for sequence-by-treatment interaction might be included in addition to the three *main effects*—sequence, period, and treatment. Alternatively, a sequence-by-period interaction effect might be included, which would fully saturate the fixed effects model.

If the replicated crossover design has only two sequences, use of only the three main effects (sequence, period, and treatment) in the fixed effects model or use of a more saturated model makes little difference to the results of the analysis, provided there are no missing observations and the study is carried out in one group of subjects. The least-squares estimate of $\sigma_T-\sigma_R$ will be the same for the main effects model and for the saturated model. Also, the MM estimators of the variance terms in the model used in some approaches to assessment of population and individual BE, which represent within-sequence comparisons, are generally fully efficient regardless of whether the main effects model or the saturated model is used.

If the replicated crossover design has more than two sequences, these advantages are no longer present. Main effects models will generally produce different estimates of μ_{T-R} than saturated models (unless the number of subjects in each sequence is equal), and there is no well-accepted basis for choosing between these different estimates. Also, MM estimators of variance terms will be fully efficient only for saturated models, while for main effects models fully efficient estimators would have to include some between-sequence components, complicating the analysis. Thus, use of designs with only two sequences minimizes or avoids certain ambiguities due to the method of estimating variances or due to specific choices of fixed effects to be included in the statistical model.

Reasons Related to Carryover Effects

One of the reasons to use the four-sequence, four-period design described above is that it is thought to be optimal if carryover effects are included in the model. Similarly, the two-sequence, three-period design is thought to be optimal among three-period replicated crossover designs. Both of these designs are *strongly balanced for carryover effects*, meaning that each treatment is preceded by each other treatment *and itself* an equal number of times.

	Period		
Sequence	**1**	**2**	**3**
1	T	R	R
2	R	T	T

With these designs, no efficiency is lost by including *simple* first-order carryover effects in the statistical model. However, if the possibility of carryover effects is to be considered in the statistical analysis of BE studies, the possibility of direct-by-carryover interaction should also be considered. If direct-by-carryover interaction is present in the statistical model, these favored designs are no longer optimal. Indeed, the TRR/RTT design does not permit an unbiased within-subject estimate of $\mu_T-\mu_R$ in the presence of general direct-by-carryover interaction.

The issue of whether a purely main effects model or a more saturated model should be specified, as described in the previous section, also is affected by possible carryover effects. If carryover effects, including direct-by-carryover interaction, are included in the statistical model, these effects will be partially confounded with sequence-by-treatment interaction in four-sequence or six-sequence replicated crossover designs, but not in two-sequence designs.

In the case of the four-period and three-period designs recommended in Section V.A.1, the estimate of $\mu_T - \mu_R$, adjusted for first-order carryover effects including direct-by-carryover interaction, is as efficient or more efficient than for any other two-treatment replicated crossover designs.

Two-Period Replicated Crossover Designs

For the majority of drug products, two-period replicated crossover designs such as the Balaam design (which uses the sequences TR, RT, TT, and RR) should be avoided for individual BE because subjects in the TT or RR sequence do not provide any information on SFI. However, the Balaam design may be useful for particular drug products (e.g., a long half-life drug for which a two-period study would be feasible but a three- or more period studies would not).

Outlier Considerations

Outlier data in BE studies are defined as subject data for one or more BA measures that are discordant with corresponding data for that subject and/or for the rest of the subjects in a study. Because BE studies are usually carried out as crossover studies, the most important type of subject outlier is the within-subject outlier, where one subject or a few subjects differ notably from the rest of the subjects with respect to a within-subject *T*–*R* comparison. The existence of a subject outlier with no protocol violations could indicate one of the following situations.

Product Failure

Product failure could occur, for example, when a subject exhibits an unusually high or low response to one or the other of the products because of a problem with the specific dosage unit administered. This could occur, for example, with a sustained and/or delayed release dosage form exhibiting dose dumping or a dosage unit with a coating that inhibits dissolution.

SFI Interaction

An SFI could occur when an individual is representative of subjects present in the general population in low numbers, for whom the relative BA of the two products is markedly different than for the majority of the population, and for whom the two products are not bioequivalent, even though they might be bioequivalent in the majority of the population.

In the case of product failure, the unusual response could be present for either the T or R product. However, in the case of a subpopulation, even if the unusual response is observed on the *R* product, there could still be concern for lack of interchangeability of the two products. For these reasons, deletion of outlier values is generally discouraged, particularly for nonreplicated designs. With replicated crossover designs, the *retest* character of these designs should indicate whether to delete an outlier value or not. Sponsors or applicants with these types of datasets may wish to review how to handle outliers with appropriate review staff.

Discontinuity

The mixed-scaling approach has a discontinuity at the changeover point, σ_{W_0} (IBC) or σ_{T_0} (PBC), from constant to reference scaling. For example, if the estimate of the within-subject standard deviation of the reference is just above the changeover point, the confidence interval will be wider than just below. In this context, the confidence interval could pass the predetermined BE limit if the estimate is just below the boundary and could fail if just above. This guidance

recommends that sponsors applying the individual BE approach may use either reference scaling or constant scaling at either side of the changeover point.

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APPENDIX A: THE SAS GLM PROCEDURE

Overview

PROC GLM is for complete data (subjects completed all treatment periods), whereas PROC MIXED is for incomplete (some data are missing), irrespective of the number of treatments. GLM uses an exact solution, whereas MIXED is iterative; when complete data are available, both can be used but GLM is faster.

The GLM procedure uses the method of least squares to fit general linear models. Among the statistical methods available in PROC GLM are regression, ANOVA, analysis of covariance, multivariate ANOVA (MANOVA), and partial correlation. PROC GLM analyzes data within the framework of general linear models. PROC GLM handles models relating one or several continuous dependent variables to one or several independent variables. The independent variables may be either *classification* variables, which divide the observations into discrete groups, or *continuous* variables. Thus, the GLM procedure can be used for many different analyses, including

- simple regression
- multiple regression
- ANOVA, especially for unbalanced data
- analysis of covariance
- response surface models
- weighted regression
- polynomial regression
- partial correlation
- MANOVA
- repeated measures analysis of variance

As described previously, PROC GLM can be used for many different analyses and has many special features not available in other SAS procedures. The following procedures perform some of the same analyses as PROC GLM:

ANOVA—Performs ANOVA for balanced designs. The ANOVA procedure is generally more efficient than PROC GLM for these designs.
MIXED—Fits mixed linear models by incorporating covariance structures in the model fitting process. Its RANDOM and REPEATED statements are similar to those in PROC GLM but offer different functionalities.
NESTED—Performs ANOVA and estimates variance components for nested random models. The NESTED procedure is generally more efficient than PROC GLM for these models.
NPAR1WAY—Performs nonparametric one-way analysis of rank scores. This can also be done using the RANK procedure and PROC GLM.
REG—Performs simple linear regression. The REG procedure allows several MODEL statements and gives additional regression diagnostics, especially for detection of collinearity. PROC REG also creates plots of model summary statistics and regression diagnostics.
RSREG—Performs quadratic response surface regression, and canonical and ridge analysis. The RSREG procedure is generally recommended for data from a response surface experiment.
TTEST—Compares the means of two groups of observations. Also, tests for equality of variances for the two groups are available. The TTEST procedure is usually more efficient than PROC GLM for this type of data.
VARCOMP—Estimates variance components for a general linear model.

The following statements are available in PROC GLM.

PROC GLM <options>;
CLASS variables;
MODEL dependents=independents </options>;
ABSORB variables;
BY variables;
FREQ variable;
ID variables;
WEIGHT variable;
CONTRAST 'label' effect values <…effect values> </options>;
ESTIMATE 'label' effect values <… effect values> </options>;
LSMEANS effects </options>;
MANOVA <test-options> </detail-options>;
MEANS effects </options>;
OUTPUT <OUT=SAS-data-set>
Keywords=names <… keyword=names> </option>;
RANDOM effects </options>;
REPEATED factor-specification </options>;
TEST <H=effects> E=effect </options>;

Although there are numerous statements and options available in PROC GLM, many applications use only a few of them. To use PROC GLM, the PROC GLM and MODEL statements are required. You can specify only one MODEL statement (in contrast to the REG procedure, for example, which allows several MODEL statements in the same PROC REG run). If your model contains classification effects, the classification variables must be listed in a CLASS statement, and the CLASS statement must appear before the MODEL statement. In addition, if you use a CONTRAST statement in combination with a MANOVA, RANDOM, REPEATED, or TEST statement, the CONTRAST statement must be entered first in order for the contrast to be included in the MANOVA, RANDOM, REPEATED, or TEST analysis (Table A.1).

TABLE A.1 Statements in the GLM Procedure

Statement	Description
ABSORB	Absorbs classification effects in a model
BY	Specifies variables to define subgroups for the analysis
CLASS	Declares classification variables
CONTRAST	Constructs and tests linear functions of the parameters
ESTIMATE	Estimates linear functions of the parameters
FREQ	Specifies a frequency variable
ID	Identifies observations on output
LSMEANS	Computes least-squares (marginal) means
MANOVA	Performs a multivariate ANOVA
MEANS	Computes and optionally compares arithmetic means
MODEL	Defines the model to be fit
OUTPUT	Requests an output dataset containing diagnostics for each observation
RANDOM	Declares certain effects to be random and computes expected mean squares
REPEATED	Performs multivariate and univariate repeated measures ANOVA
TEST	Constructs tests using the sum of squares for effects and the error term you specify
WEIGHT	Specifies a variable for weighting observations

PROC GLM for Unbalanced ANOVA

ANOVA typically refers to partitioning the variation in a variable's values into variation between and within several groups or classes.

TABLE A.2 A 2x2 ANOVA Model

	A	
B	**1**	**2**
1	12	20
	14	18
	11	17
2	9	

```
title 'Analysis of Unbalanced 2-by-2 Factorial';
data exp;
input A $ B $ Y @@;
datalines;
A1 B1 12 A1 B1 14 A1 B2 11 A1 B2 9
A2 B1 20 A2 B1 18 A2 B2 17;
```

Note that there is only one value for the cell with A = 'A2' and B = 'B2'. Since one cell contains a different number of values from the other cells in the table, this is an unbalanced design (Table A.2).

The following PROC GLM invocation produces the analysis.

```
proc glm;
class A B;
model Y=A B A*B;
run;
```

Both treatments are listed in the CLASS statement because they are classification variables. A*B denotes the interaction of the effects A and B.

TABLE A.3 Class Level Information

Analysis of unbalanced 2 × 2 factorial

The GLM procedure

Class level information		
Class	**Levels**	**Values**
A	2	A1 A2
B	2	B1 B2

Number of observations	7

Table A.3 displays information about the classes as well as the number of observations in the dataset. Table A.4 shows the ANOVA table, simple statistics, and tests of effects.

TABLE A.4 ANOVA Table and Tests of Effects

Analysis of unbalanced 2 × 2 factorial

The GLM procedure

Dependent variable: *Yv*

Source	DF	Sum of squares	Mean square	F-value	Pr > F
Model	3	91.71428571	30.57142857	15.29	0.0253
Error	3	6.00000000	2.00000000		
Corrected total	6	97.71428571			

R^2	Coeff Var	Root mean square error	Y mean
0.938596	9.801480	1.414214	14.42857

Source	DF	Type I SS	Mean square	F-value	Pr > F
A	1	80.04761905	80.04761905	40.02	0.0080
B	1	11.26666667	11.26666667	5.63	0.0982
A*B	1	0.40000000	0.40000000	0.20	0.6850

Source	DF	Type III SS	Mean square	F- value	Pr > F
A	1	67.60000000	67.60000000	33.80	0.0101
B	1	10.00000000	10.00000000	5.00	0.1114
A*B	1	0.40000000	0.40000000	0.20	0.6850

The degrees of freedom may be used to check your data. The model degrees of freedom for a 2×2 factorial design with interaction are $(ab-1)$, where a is the number of levels of A and b is the number of levels of B; in this case, $(2\times2-1)=3$. The corrected total degrees of freedom are always one less than the number of observations used in the analysis; in this case, $7-1=6$.

The overall F-test is significant ($F=15.29$, $p=0.0253$), indicating strong evidence that the means for the four different A×B cells are different. You can further analyze this difference by examining the individual tests for each effect.

Four types of estimable functions of parameters are available for testing hypotheses in PROC GLM. For data with no missing cells, the Type III and Type IV estimable functions are the same and test the same hypotheses that would be tested if the data were balanced. Type I and Type III sum of squares are typically not equal when the data are unbalanced; Type III sum of squares are preferred in testing effects in unbalanced cases because they test a function of the underlying parameters that is independent of the number of observations per treatment combination.

According to a significance level of 5% ($\alpha=0.05$), the A*B interaction is not significant ($F=0.20$, $p=0.6850$). This indicates that the effect of A does not depend on the level of B and vice versa. Therefore, the tests for the individual effects are valid, showing a significant A effect ($F=33.80$, $p=0.0101$) but no significant B effect ($F=5.00$, $p=0.1114$).

APPENDIX B: BE TESTING SOFTWARE

ABSPLOTS	Lotus 123 spreadsheet for Wagner–Nelson calculations
acslXtreme	Physiologically based PK (PBPK) and pharmacodynamic (PD) simulation software
acslXtreme PK toolkit	PBPK and PD effects with the acslXtreme PK toolkit
ADAPT II	Supplied as FORTRAN code for VAX VMS, MS DOS, and SUN UNIX system. This program performs simulations, nonlinear regression, and optimal sampling. Includes extended least squares and Bayesian optimization. Models can be expressed as integrated or differential equations using FORTRAN statements
ATIS	Nonlinear least squares
AUC-RPP	Noncompartmental evaluation of PK parameters
BIOEQV52, BIOPAR40, and BIOEQNEW	BE calculations including statistical power. Reference Wijnand H.P. 1994 Updates of BE programs (including statistical power approximated by Student's *t*-test, Computer Methods Programs Biomedicine 42, 275–281)
Biokmod	A Mathematica toolbox for solving systems of differential equations, fitting coefficients, convolution, and more, with applications for modeling linear and nonlinear systems
BIOPAK	Statistical analysis package for BA/BE studies
BOOMER/MULTI-FORTE	Supplied as compiled programs for Macintosh (including PowerMac), MS DOS, and VAX VMS systems. This program performs simulations and nonlinear regression. Includes Bayesian optimization. Models, integrated or differential equations, can be expressed as a sequence of parameters (BOOMER) or using FORTRAN statements (MULTI-FORTE)
CSTRIP	Polyexponential stripping
CXT	(CompleX Tools for Linear Dynamic System Analysis) from BIO-LAB Bratislava uses the frequency response method to model PK and/or PD data
Cyber Patient	Multimedia PK simulation program that can be used for development and presentation of problem-solving case studies
EASYFIT	Analysis of compartmental models
EDFAST	Fitting and simulating linear PK models
EquivEasy	Modeling and BE testing program—an interface for SAS PROC GLM and other modules; excellent choice for a CFR compliant software
GastroPlus™	Simulates absorption and PBPK for orally dosed drugs
INTELLIPHARM PK	Simulates drug dissolution, absorption, and PK
JavaPK for Desktop	Bayesian individualized PK–PD parameters estimation (UDBM) for analyzing batch input data. Users can define their own model with population PK–PD parameters using a single-dose, integral equation (for the multiple-dosed) or a steady state integral equation
JGuiB	Includes three most commonly used functions of Boomer in PK–PD modeling: normal fitting, simulation, and Bayesian estimation
KINBES	BA and rate of drug absorption by various methods such as numerical deconvolution and web logic server (WLS)-reconvolution. Also featuring a number of statistical tests on BE (e.g., ANOVA, FDA 75/75 rule, etc.)

(Continued)

APPENDIX B: BE TESTING SOFTWARE *(Continued)*

Kinetica	Thermo Electron's PK analysis tool, offers fast high-throughput data analysis for clinical, preclinical, discovery, drug metabolism, and drug delivery settings. This tool standardizes analyses across the organization and minimizes variability between PK analysts and analyses. Together, with EP, it becomes a fully FDA CFR 21, part 11 compliant PK–PD database (DB) enabling full audit trail from protocol inception to final report
MKMODEL	The program, for MS DOS systems, performs nonlinear least-squares regression with extended least squares. Models can be represented by integrated or differential equations
ModKine	Modeling program with custom features for PK and PD (Windows) from Biosoft
NCOMP	Noncompartmental analysis of PK data
NLMEM SAS/IML macro	The macro is designed for hierarchical nonlinear mixed effects models. The program invokes part of the code contained in the SAS/NLINMIX macro developed by SAS Technical Support and can be considered as an interface for the NLINMIX macro to SAS/IML. The macro runs under SAS system and is an attractive alternative to NONMEM software
NONMEM	The program is provided as FORTRAN source code for UNIX, IBM, and other computers. The program performs nonlinear regression analysis of individual or population data
NPEM	Nonparametric expectation maximization. This is part of the USC PACK collection
NPML	(Nonparametric maximum likelihood estimation procedure) by A. Mallet. ! Reference: Mallet, A. 1986 A maximum likelihood estimation method for random coefficient regression models. Biometrika, 73: 654-656
PCDCON	By W.R. Gillespie (gillespiew@donald.cder.fda.gov) performs deconvolution analysis. This program is available as a compiled program for the IBM PC. Reference: Karol, M., Gillespie, WR., and Veng-Pederson, P. 1991 AAPS Short Course: Convolution, Deconvolution and Linear Systems, AAPS, Washington, D.C., November 17
PDx-IVIVC	Comprehensive toolset for in vitro–in vivo correlation
PDx-Pop	Integrates with NONMEM and other existing software to expedite population modeling and analysis
PH\EDSIM	A universal PK–PD modeling tool that enables the user to create custom-made PK, PD, or PK–PD models in a graphical way without the need for programming. Created models may be used for simulation and fitting purposes
Physiological parameters for PBPK modeling version 1.0 (P3M)	P3M provides a convenient tool for parameterization of PBPK models of interindividual variation
PK functions for Microsoft Excel	By Joel Usansky, Atul Desai, and Diane Tang-Liu. Download the Word document first for a description and installation instructions
PK Simulations	By Guenther Hochhaus
PK Solutions	Is an Excel-based noncompartmental PK software program
PKAnalyst for Windows	Provides the capability of simulation and parameter estimation for PK models
PKBugs	Is an efficient and user-friendly interface for specifying complex population PK–PD models within the widely-used WinBUGS software
POP3CM	A Free Visual Compartmental Population Analysis Program. The program POP3CM provides a graphical user interface for the analysis of a three-compartment model
PopKinetics	PopKinetics is a population analysis program. It is a companion application to SAAM II that uses parametric algorithms, standard two-stage and iterated two-stage, to compute population parameters and their confidence intervals. PopKinetics operates directly on SAAM II study files
SAAM II	Is a compartmental (differential equations) and numerical (algebraic equations) modeling program that can be used in the analysis of PK, PD, and enzyme kinetic studies. It is designed to help researchers easily create models, perform simulations, and fit experimental kinetic data resulting in parameter estimates and their associated errors. SAAM II has a user-friendly graphical user interface which is fully menu driven. Development of SAAM II, at the University of Washington, Seattle, was supported by a research resource grant from the National Institutes of Health. SAAM II is available for PC Windows (Win95/98, NT). The Macintosh version [68030 or higher (with FPU), PowerMac] is still available but no longer supported. Available from the SAAM Institute, info@saam.com, phone (206)729-1315, fax (206)729-7854. A demo version is available on the website

(Continued)

APPENDIX B: BE TESTING SOFTWARE *(Continued)*

SAAM/CONSAM	Is available from L.A. Zech and P.C. Greif, Laboratory of Mathematical Biology, Building 10, Room 4B-56, NIH/NCI, Bethesda, MD 20892. Internet: zech@ncifcrf.gov. The program is provided as compiled programs for VAX VMS and MS DOS computers. The program performs nonlinear regression in batch (SAAM) or conversational mode (CONSAM). The SAAM/CONSAM programs are kindly provided by the USPHS/NIH/DRR-NHLBI-NCI joint development project. Reference
SAS	Most important, yet difficult-to-use software; PROC GLM is the preferred tool by the FDA.
Simcyp	Includes a fully automated whole-body PBPK model that incorporates enzyme kinetic data from routine in vitro studies
TopFit	This MS DOS program performs noncompartmental and model based analyses
WinNonlin	Provides an easy-to-use Windows application for PK, PK–PD, and noncompartmental analysis. WinNonlin includes extensive libraries of PK and PK–PD models, and provides tools for table generation, scripting, and data management
WinNonMix	Is a program for nonlinear mixed effects modeling provided in an interactive and easy-to-use Windows application
WinSAAM	Is a Windows version of the original interactive biological modeling program, CONSAAM, developed in 1980 at NIH
Xpose	Is an R-based model building aid for population analysis using NONMEM. It facilitates dataset checkout, exploration and visualization, model diagnostics, candidate covariate identification, and model comparison

APPENDIX C: A TYPICAL STUDY REPORT
(Courtesy of Gulf Pharmaceutical Industries, Ras Al-Khaimah, United Arab Emirates)

Detailed Statistical Treatment of Cefaclor

Introductory Comment

Note: The tables and figures were not reported to maintain confidentiality of data and are referred here to show that a complete report would include these.

A number of statistical and nonstatistical issues have converged, in recent years, to focus increasing attention on decision making in BE studies.

Although much has been published on the evaluation of data from such studies, very few papers dealt explicitly with the ultimate evaluation step, namely the decision to accept BE of the compared drug formulations.

Unfortunately, the principles that govern the design, conduct, and analysis of equivalence trials are not well understood as they should be. Consequently, such trials often include few subjects or have design biases that tend toward the conclusion of "no significant difference."

Furthermore, the application of hypothesis testing in analyzing and interpreting data from BE trials sometimes compounds the drawing of inappropriate conclusions. Statistical analysis strategies therefore should seek to show the similarity in the conclusions drawn from a range of statistical approaches.

The key concept in BE is the "equivalent therapeutic effect" of equal amounts of the same drug administered in different drug products. The goal is, therefore, to determine whether the test and reference drug products are sufficiently similar to ensure therapeutic equivalence.

Bearing in mind that the basis of BE trials lie in the relationship between blood levels and therapeutic effect(s), only those blood level characteristics that possess some meaningful relation to the therapeutic use of the drug need to be analyzed.

Differentiation between three types of BE is also important, since each requires a specific statistical testing approach.

(A) ABE

BE testing procedures deal mainly with the average or median levels of the specified characteristics used as measures of BA, if the two averages or medians ($\bar{X}$ test and $\bar{X}$ reference) are sufficiently close, the two drug formulations are considered ABE.

(B) Population BE

Among a population of subjects, each drug will have some distribution of BAs. [ABE considers only one aspect of that distribution, namely its mean (or median).] Population BE is a BE criterion that requires the distribution of BAs of the test formulation to be "sufficiently similar" to that of the reference in a certain population. Reports indicate that the intent of BE studies must be the conclusion of population BE. Hence, ABE is, at best, an approximation to the population BE.

(C) Individual BE

Refers to the particular individual's similarity of response to the two formulations. It is easy to recognize that while the average and/or population BE are acceptable, the individual variability may be large enough to bear significance when switching between different drug products is necessary (switchability).

Statistical Data Treatment/Methods and Results

The Design

The experimental design, followed a balanced two-treatment, two-period, two-sequence (2×2) randomized crossover concept. Each subject (nTR=13, nRT=13) received the test (Recocef) and the reference (Ceclor) formulations in turn, with an appropriate washout period between administrations (one week).

The Model

The statistical model, considered the standard 2×2 crossover design and accounted to the following: The model below allows the intrasubject variability to vary from formulation to formulation.

$$*Y_{ijk} = \mu + S(ik) + P(j) + F(j,k) + C(j-1,k) + e_{ijk}$$

where i (subject)=1, $2\eta_R$.
j (period)=in the 2×2; 1 or 2.
k (sequence)=1 (RT), 2 (TR).
μ=overall mean;
S_{ik}=The between-subject effect; the random effect of the ith subject in the kth sequence, in the above case $S(1)\ldots S(2)$ or $S12\ldots S(12)2$ (intersubject variability).
P_j=The fixed effects of the jth period, $j=1$ and 2, $\Sigma F(j,k)=0$
$F(j,k)$=The direct fixed effect of the formulation in the kth sequence which is administered at the jth period, and $\sum F(j,k)=0$.
$C(j-1,k)$=The fixed first-order carryover effect of the formulation in the kth sequence administered at the $(j-1)$the period, where Co,$k=0$ and $\Sigma Cj-1, k=0$.
e_{ijk}=the (within-subject) random error in observing Y_{ijk}, (intrasubject variability).

(The carryover effects can only occur at the second period, e.g., one can define CR as the carryover effect of the reference formulation from the first period to the second at sequence 1.) The above model has included three fixed effects, namely, the period effect, direct drug effect, and carryover effect in addition to the two random effects.

Statistical Interferences for Effects from a Standard

2×2 Crossover Design

One can summarize the fixed effects which occur in each period as follows:

Sequence	Period I	Period II
1 (RT)	$\mu_{11}=\mu+P_1+\mathrm{FR}$	$\mu_{21}=\mu+P_2+\mathrm{FT}+\mathrm{CR}$
2 (TR)	$\mu_{12}=\mu+P_1+\mathrm{FT}$	$\mu_{22}=\mu+P_2+\mathrm{FR}+\mathrm{CT}$

where $U_{jk}=E(Y_{ijk})$, $P_1+P_2=0$, $\mathrm{FR}+\mathrm{FT}=0$, $\mathrm{CR}+\mathrm{CT}=0$

For a BE study, it is usually assumed that (*i*) there is no period effect and (*ii*) there are no carryover effects. Since their presence will certainly increase the complexity of the statistical analysis, it is of interest to perform some preliminary tests for the presence of the period effect and/or carryover effects before a decision on BE is made.

Statistical interferences on these effects will be examined under the model described above, with the following assumptions:

(i) $\{S_{ik}\}$ = The random effect(s) of the *i*th subject in the *k*th sequence are independently and identically distributed (i.i.d.) normal, with mean 0 and variance σ_s^2.*Note*: (σ_s^2 is usually used to explain intersubject variability).

(ii) $\{e_{ijk}\}$ = The within-subject random error in observing Y_{ijk}; i.i.d. normal with mean = 0, and variance σ_t^2, where $t=1, 2$ (no. of formulations to be compared).*Note*: (σ_d^2 is usually used to explain intrasubject variability for the *t*th formulation).

(iii) $\{S_{ik}\}$ and $\{e_{ijk}\}$ are mutually independent.

Under the above assumptions, statistical inferences such as estimation and assessment, confidence interval, and hypotheses testing for the fixed effects have been derived based on the two-sample *T*-statistics.

The results of the PK characteristics are summarized in tables IV-A-1 to 4 and IV-B-1 to 9. ANOVA was carried out using the SAS statistical programs and models as shown in tables IV-A-5 to 8. The data were also used to estimate and separate fixed effects such as (*i*) carryover effect, (*ii*) direct drug effect, and (*iii*) period effect as summarized in the results of the statistical inferences shown in table IV-B-11. A preliminary examination of the data plotted subject profiles for each sequence and sequence-by-period means (figures IV-B-i and ii). (Tables and figures are not provided for simplicity).

Validity of Assumptions and Model Selection

Statistical methods of average BA can be applied to either raw data or log-transformed data. It is important to check the validity of the assumptions outlined below before an appropriate model is chosen. The following are worth investigating:

1. $\{S_{ik}\}$ are i.i.d. normal with mean 0 and variance σ_s^2
2. $\{e_{ijk}\}$ are i.i.d. normal with mean 0 and variance σ_e^2
3. $\{S_{ik}\}$ and $\{e_{ijk}\}$ are mutually independent.

Several tests for the above assumptions using intersubject and intrasubject residuals are discussed.

For the standard 2×2 crossover design, with the above assumptions, the total sum of squares were partitioned into the between-subject sum of squares (SSbetween) and the within-subject sum of squares. The SSbetween were further partitioned into the sum of squares of carryover effects and the sum of squares of intersubject error (SSinter). The within-subject sum of squares were decomposed into sum of squares of formulation effect (SSDrug), sum of squares of period effects (SSperiod), and the sum of squares of intrasubject residuals (SSintra).

For testing the above assumptions, the inter- and intrasubject residuals were used in accordance with the following.

Intrasubject Residuals ($\hat{e}_{ijk}$) and Intersubject Residuals (S_{ik})

The intrasubject residual for subject i within sequence k during period j, denoted by $\hat{e}_{ijk}$, is defined as the difference between the observed response Y_{ijk} and its predicted value $\bar{Y}_{ijk}$. In practice, σ_e^2 is usually unknown but can be estimated unbiasedly by MSintra. One can thus estimate the studentized intrasubject residuals.

Based upon $\{\hat{e}_{ilk}\}$ and $\{\bar{Y}_{ilk}\}$ when $i=1,2\ldots, nk$ and $k=1, 2$, the above assumptions have been examined in terms of the normal probability plot of $\{\bar{Y}_{ilk}\}$, which was used to examine the normality assumption on the intrasubject variability (e_{ijk}), while the residual plot between $\{\hat{e}_{ilk}\}$ and $\{\bar{Y}_{ilk}\}$ was used to examine the adequacy of the model. It is worth noting that the latter can provide preliminary information on potential outlying data. The above was also separated for intersubject residuals as illustrated in figures IV-B-iii to xi.

Statistical Methods for Average BA

To claim ABE, the ± 20 rule requires that the ratio of the two true formulation means for AUC and C_{max} be within (80%, 120%) limits; alternatively the difference $(\mu_T-\mu_R)$ is within ± 20 (%) of μ_R. The FDA requires that the BE be asserted using the limits $\in$(80%, 120%) with 90% assurance.

Along the above line, several methods have been proposed including (*i*) the confidence interval approach, (*ii*) the method of interval hypotheses testing, (*iii*) the Bayesian approach, and (*iv*) nonparametric methods.

The Confidence Interval Approach

Several methods for constructing a 90% confidence interval for (μ_T/μ_R) have been proposed under a raw data model. Among others the following have been included:

1. The classical confidence interval which is also known as the shortest confidence interval.
2. Westlake's symmetric confidence interval.
3. Confidence interval for $(\mu_T-\mu_R)$ based on Filler's theorem.
4. Chow and Shoa's joint confidence region for (μ_T, μ_R).

The Classical (Shortest) Confidence Interval

Let $\bar{Y}_T$ and $\bar{Y}_R$ be the respective least-squares means for the test and reference formulations, which can be obtained from the sequence-by-period means.

The classical $(1-2\alpha)\times 100$ (%) confidence interval can then be obtained based on the following T-statistic:

$$T = \frac{(\bar{Y}_T-\bar{Y}_R)-(\mu_T-\mu_R)}{\sigma_d^2\sqrt{\frac{1}{n_1}+\frac{1}{n_2}}}$$

Under normality assumptions, T follows a central Student's t distribution with degrees of freedom, n_1+n_2-2.

Thus, the classical $(1-2\alpha)$ 100% confidence interval for (μ_T/μ_R) by dividing by $\bar{Y}_R$, i.e.,

$$L_2 = (L_1/\bar{Y}_R + 1)\times 100\,(100\%)$$

$$U_2 = (U_1/\bar{Y}_R + 1)\times 100\,(100\%)$$

Let θ_L and θ_U be the respective lower and upper equivalence limits for the difference, whereas $(\mu_T-\mu_R)$ δ_L and δ_U be the respective lower and upper equivalence limits for the ratio; hence, we may conclude BE if

$$(L_1, U_1)\in(\theta_L, \theta_U)\in\left(-0.2_{\mu_R}, 0.2_{\mu_R}\right)$$

or

$$(L_2, U_2)\in(\delta_L, \delta_U)\in(80\% = \delta_L,\ 120\% = \delta_\mu)$$

The classical shortest confidence interval for $AUC_{0\to\infty}$ and C_{max} values are summarized in table IV-B-12.

Westlake's Symmetrical Confidence Interval

When the data have been logarithmically transformed, particularly for $AUC_{0\to\infty}$ and C_{max}, application of Westlake's confidence interval approach leads to a confidence interval for (μ_T/μ_R), which is symmetrical about unity.

The "effective" length of the confidence interval, which is not $(C_1 - C_2)$ but rather $2k$, where k is the maximum of $|C_1|$, $|C_2|$ was evaluated. Westlake's approach decreased the maximum of $|C_1|$, $|C_2|$ until (C_1) was equal (C_2). This ensured a shorter "effective" confidence interval.

Assuming that the normality assumption is obeyed and a 90% confidence interval of the following type results:

$$K_2 \le \frac{[(\bar{X}_R - \bar{X}_T)(\mu_R - \mu_T)]}{\sqrt{2S^2/n}} \le K_1$$

After rearrangement, one obtains

$$K_2\sqrt{2S^2/n} - (\bar{X}_R - \bar{X}_T) \le \mu_T - \mu_R \le K_1\sqrt{2S^2/n} - (\bar{X}_R - \bar{X}_T)$$

where k_1 and k_2 are chosen so that

$$(K_1 + K_2)\sqrt{2S^2/n} = 2(\bar{X}_R - \bar{X}_T) = 2$$

Hence, if

$$\int_{K_2}^{K_1} f(x)\mathrm{d}x = (1-\alpha)$$

where $\int$ is the probability density function of a corresponding t distribution.

The lower and upper limits of the confidence interval can be evaluated such that

$$\text{lower} = \exp\left[K_2\sqrt{2S^2/n} - (\bar{X}_R - \bar{X}_T)\right]$$
$$\text{upper} = \exp\left[K_1\sqrt{2S^2/n} - (\bar{X}_R - \bar{X}_T)\right]$$

The results obtained from Westlake's approach to obtain symmetrical confidence interval is shown in table IV-B-13.

In applying the above approach, the following points are worth noting:

1. The effective length of the confidence interval decreased compared with the conventional nonsymmetrical approach for calculating the confidence interval.
2. The confidence coefficient can be shown to be always $\sim$0.95.
3. Data must be logarithmically transformed. After transformation, the above method leads to calculating a confidence interval for (μ_R/μ_T) which is symmetrical about unity.

The Methods of Interval Hypothesis

Assessment of BE is also based on the comparison of BA profiles between formulations. However, in practice, it is recognized that no two formulations will have exactly the same BA profiles. Therefore, if the profiles of the two formulations differ by less than a (clinically) meaningful limit, the profiles of the two formulations may be considered equivalent.

Based on the above idea, Schuirmann introduced the interval hypotheses for assessing BE.

Schuirmann's TOST Procedure

The Method of Interval Hypothesis

The TOST consists of decomposing the interval hypothesis H_0 and H_1 into two sets of one-sided hypothesis.

$$H_{01} : \mu_T - \mu_R \leq \theta_1 = -0.20\ \mu_R \quad \text{(The lower tail)}$$
$$H_{11} : \mu_T - \mu_R > \theta_1 = -0.20\ \mu_R$$

and

$$H_{02} : \mu_T - \mu_R \geq \theta_2 = (0.20\ \mu_R) \quad \text{(The upper tail)}$$
$$H_{12} : \mu_T - \mu_R < \theta_2 = (0.20\ \mu_R)$$

The TOST procedure consists of rejecting the interval hypothesis H_0 and thus concluding BE, if and only if both H_{01} and H_{02} are rejected simultaneously at a chosen nominal level of significance α.

Under the normality assumption, the two sets of one-sided hypotheses will be tested with ordinary one-sided *t*-tests. For a balanced study, equivalence is concluded if

$$t_1 = \frac{(\overline{X}_T - \overline{X}_R) - \theta_1}{S\sqrt{2/n}} \geq t_{1-\alpha(\nu)}$$

and

$$t_2 = \frac{\theta_2 - (\overline{X}_T - \overline{X}_R)}{S\sqrt{2/n}} \geq t_{1-\alpha(\nu)}$$

where

S = the square root of the "error" mean square from the crossover design ANOVA.
$t_{1-\alpha(\nu)}$ = the point that isolates the probability α in the upper tail of the Student's t distribution with υ degrees of freedom.
$[\theta_1,\theta_2]$ = equivalence interval, known numbers expressed as proportions of the unknown reference average μ_R.
$\left.\begin{matrix}\theta_1 = -0.20\mu_R \\ \theta_2 = +0.20\mu_R\end{matrix}\right\}$ The common "$\pm$20%" criteria.

In applying the TOST procedure, the following points are worth noting:

1. Data arise from a normal distribution.
2. The within-subject variances of the test and reference products are identical.
3. The TOST procedures depends on the choice of the nominal level of significance (α)
4. On the logarithmic scale, the hypothesis can be restated as

$$H_0 : \frac{\mu_T}{\mu_R} \leq 0.810 \quad \text{or} \quad \frac{\mu_R}{\mu_R} \geq 1.20$$
$$H_1 : 0.80 < \frac{\mu_T}{\mu_R} < 1.20$$

$$H_0' : \eta_T - \eta_R \leq \log(0.8) \quad \text{or} \quad \eta_T - \eta_R \geq \log(1.25)$$

$$H_1' : \log(0.8) < \eta_T - \eta_R < \log 1.20$$

Results of the statistical treatment corresponding to Schuirmann's TOST procedure are shown in table IV-B-14.

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7 Physicochemical Properties Affecting Bioequivalence

BACKGROUND

Chemical equivalence is relatively easy to establish; the gulf between the chemical equivalence of a multisource drug product and its bioequivalence is attributed mainly to the differences in the physical characteristics of the active drug, the choice and characteristics of excipients, the specification of the drug delivery system, and also how the drug is tested for bioequivalence. It is thus crucial for the formulators to be keenly aware of the subtleties in the physical and chemical properties that may lead to substantial differences in the bioequivalence of the tested dosage forms.

Drugs should be capable of existing either in a molecular dispersion such as solutions or in an aggregate state such as tablets, capsules, suspensions, etc. that are readily rendered into finer state of dispersion and dissolution. Regardless of the stage of aggregation in the final formulation, the active pharmaceutical ingredient must be released from the drug delivery system as the first step, and dissolve in an aqueous environment; this will then be followed possibly by one or more transfers across nonaqueous barriers. Whereas the design of drug delivery systems can alter release characteristics to some extent, the basic permeation characteristics remain an innate property based on the physicochemical nature of the drug.

Drug absorption depends on the release of the drug substance from the drug product (dissolution), the solubility, and the permeability across the gastrointestinal tract. The release characteristics of a drug delivery system are often determined by the manufacture of the product and are highly affected by drug solubility, which also affects dissolution rates. The release step is followed by dissolution of the active ingredient.

Absorption of drugs from the various cavities in human body follows certain general principles; for example, a drug must be present in a solution (monomolecular dispersion) or reasonably dispersed to be absorbed. The ionic forms of a drug are not readily absorbed and similarly the size of drug molecules is often critical. These properties become important since a drug molecule must traverse through several biologic barriers, both aqueous and nonaqueous; these barriers exist to protect our body from the noxious agents that can be toxic to our body. A compound highly soluble in water or highly insoluble in water would not be able to penetrate the deeper tissues and thus rendered ineffective. Neutral compounds without any polarizable centers often prove pharmacologically inert; take for example, the fluorinated hydrocarbons like perfluorodecalin—a hexane structure with full fluorination. Fluorine is so highly electronegative that it pulls the electrons from the parent structure making it an inert compound. Interactions at the site of action are often electrically driven and as a result, it is more likely that we will discover a compound that has weak acid or base properties as an active entity, which is more subject to variability in bioavailability and bioequivalence because of the physicochemical interactions with milieu interior.

CHEMICAL PROPERTIES

Ionization

Chemical moieties are known to attract to each other and under appropriate conditions, disassociate; when this process is driven by the electrical charges on the components of the moiety, this phenomenon, known as ionization. The physicochemical properties of dissociated species differ significantly from the undissociated species and form a basis not only of the physicochemical stability but also of physiologic activity of molecules and ions. Acids give rise to excess of H^+ in aqueous solution whereas a base gives rise to excess of OH^- in solution (Brønsted–Lowry theory). A more general theory of acids and bases is the Lewis theory wherein when an H^+ ion combines with an OH^- ion to form water. The pair of electrons that go into the new covalent bond are donated by the OH^- ion and thus the Lewis theory argues that any substance that can act as an electron-pair donor is a Lewis base (such as the OH^- ion). Figure 1 shows the inverse relationship that exists between pK_a and pK_b values of typical acids and bases.

The strongest acids appear on the left side of the figure; the strongest bases on the right side of the figure. Any base can deprotonate any acid on the left side of it, a weaker base. Acetic acid, a weak acid will ionize (or get deprotonated) water, methanol, or ammonia.

Henderson–Hasselbach Equation

At a given temperature the thermodynamic ionization constants are independent of concentration, and at a pH value equal to pK_a the activity of ionized and neutral forms is equal. In many measurement techniques we measure concentration rather than activity, such as in the use of spectroscopic methods. In such instance:

$$K_c a = [H^+ +][A^-]/[HA] \quad (1)$$

where values in brackets are observed concentration from spectroscopic measurements based on the Beer–Lambert law. The "Thermodynamic" Ionization Coefficient is related to the "Concentration" Ionization Coefficient by:

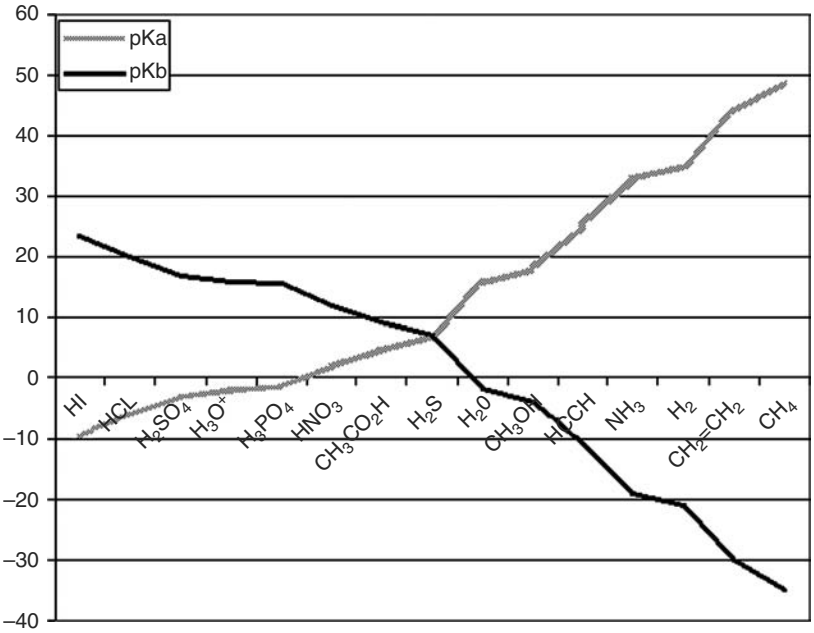

FIGURE 1 Typical Brønsted acids and their conjugate bases.

$$K_a = K_c a\left(\frac{f_A f_H}{f_{HA}}\right) \tag{2}$$

where f is the activity coefficient.

The pK_a values are also temperature dependent, often in a nonlinear and unpredictable way. Samples measured by potentiometry are therefore held at a constant temperature bath and therefore pK_a value should be quoted at a specific temperature. Often a temperature of 25°C is chosen to reflect room temperature whereas this may be quite different from the body temperature.

The Henderson–Hasselbach equation defines the relationship between ionization and pH; it is understood in equation (3). This equation relates the pK_a to the pH of the solution and the relative concentrations of the dissociated and undissociated parts of a weak acid:

$$\text{pH} = \text{p}K_a + \log[A^-]/[HA] \tag{3}$$

or

$$\text{pH} = \text{p}K_a + \log[\text{salt}]/[\text{acid}] \tag{4}$$

where $[A^-]$ is the concentration of the dissociated species and [HA] is the concentration of the undissociated species. This equation can be manipulated into the form given by equation (4) to yield the percentage of a compound that will be ionized at any particular pH.

$$\%\ \text{ionised} = \frac{100}{1 + 10^{[\text{charge}(\text{pH}-\text{p}K_a))]}} \tag{5}$$

One simple point to note about equation (5) is that 50% dissociation (or ionization) pK_a = pH. It should also be noted that, usually, pK_a values are preferred for bases instead of pK_b values (pK_w = pK_a + pK_b). As a result, the extent of ionization of a compound will depend on the pH of medium. Figure 2 shows pH values of common fluids.

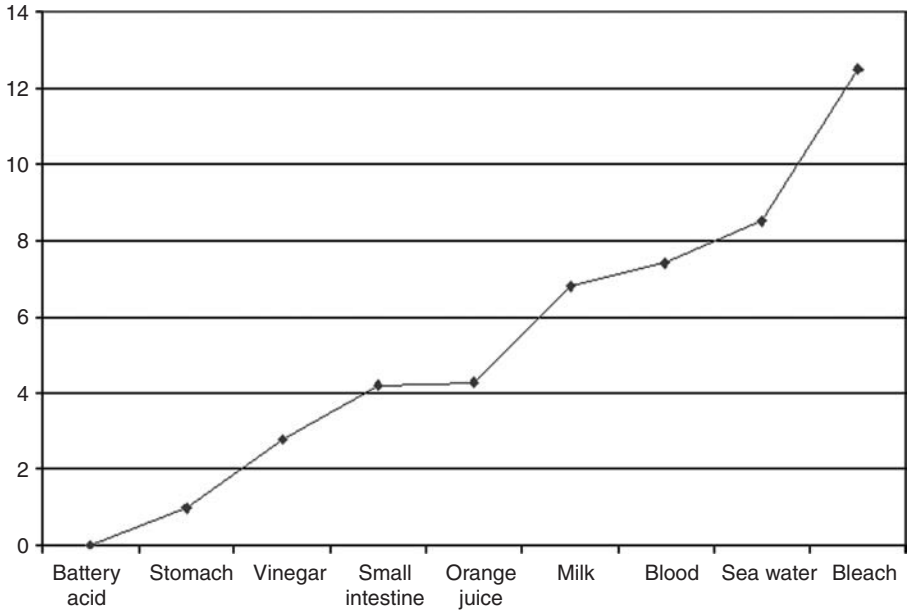

FIGURE 2 pH of common fluids.

Partitioning

The partition coefficient is a measure of the extent a substance partitions between two phases, generally an oil phase and an aqueous phase. This ratio is often expressed as log P (logarithm of partition ratio). Both pK_a and log P measurements are useful parameters in understanding the dissolution and absorption behavior of drug molecules. The pK_a will determine the species of molecules, which is likely to be present at the site of absorption and how quickly or completely the species would cross a large number of transport barriers in the body, regardless of the route of administration.

Partition coefficient is a ratio of the concentration in two immiscible solvents.

$$\text{Partition Coefficient, } P = \left(\frac{\text{Organic}}{\text{Aqueous}}\right) \tag{6}$$

where the values in brackets describe measured concentrations.

$$\text{Log } P = \log 10 \text{ (Partition Coefficient)} \tag{7}$$

In practical terms, the uncharged or neutral molecule exists for bases >2 pK_a units above the pK_a and for acids >2 pK_a units below. In practice the log P will vary according to the conditions under which it is measured and the choice of partitioning solvent.

It is worth noting that this is a logarithmic scale, therefore, a log $P=0$ means that the compound is equally soluble in water and in the partitioning solvent. If the compound has a log $P=5$, then the compound is 100,000 times more soluble in the partitioning solvent. A log $P=-2$ means that the compound is 100 times more soluble in water, i.e., it is quite hydrophilic.

Log P values have been studied in approximately 100 organic liquid–water systems. Since it is virtually impossible to determine log P in a realistic biologic medium, the octanol water system has been widely adopted as a model of the lipid phase. Whilst there has been much debate about the suitability of this system, it is most widely used in pharmaceutical studies. Octanol and water are immiscible, but some water does dissolve in octanol in a hydrated state. This hydrated state contains 16 octanol aggregates, with the hydroxyl head groups surrounded by trapped aqueous solution. Lipophilic (unionized) species dissolve in the aliphatic regions.

Generally, compounds with log P values between 1 and 3 show good absorption, whereas those with log P values greater than 6 or less than 3 often have poor transport characteristics. Highly nonpolar molecules have a preference to reside in the lipophilic regions of membranes, and very polar compounds show poor bioavailability because of their inability to penetrate membrane barriers. Thus, there is a parabolic relationship between log P and transport, i.e., candidate drugs that exhibit a balance between these two properties will probably show the best oral bioavailability.

Distribution Coefficient

The partition coefficient refers to the intrinsic lipophilicity of the drug, in the context of the equilibrium of unionized drug between the aqueous and organic phases. If the drug has more than one ionization center, the distribution of species present will depend on the pH. The concentration of the ionized drug in the aqueous phase will therefore have an effect on the overall observed partition coefficient. This leads to the definition of the distribution coefficient (log D) of a compound, which takes into account the dissociation of weak acids and bases.

Since in the aqueous phase, the total concentration may comprise both ionized and unionized forms the distribution is given as:

$$\text{Distribution Coefficient, } D = [\text{Unionised}](\text{o})/[\text{Unionised}](\text{aq}) + [\text{Ionised}](\text{aq}) \tag{8}$$

$$\text{Log } D = \log 10 \text{ (Distribution Coefficient)} \tag{9}$$

Log D is related to log P and the pK_a by the following equations:

$$\text{Log } D \text{ (pH)} = \log P - \log[1 + 10^{(\text{pH}-\text{p}K_a)}] \text{ for acids} \tag{10}$$

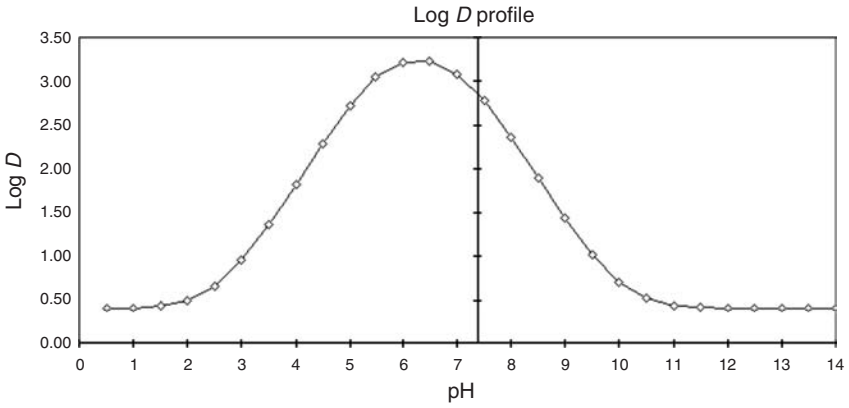

FIGURE 3 Log *D* profile of an acid, $pK_a = 8$.

$$\text{Log } D_{(\text{pH})} = \log P - \log[1 + 10^{(\text{p}K_a - \text{pH})}] \text{ for bases} \tag{11}$$

Log *D* is the log distribution coefficient at a particular pH. This is not constant and will vary according to the protogenic nature of the molecule. Log *D* at pH 7.4 is often quoted to give an indication of the lipophilicity of a drug at the pH of blood plasma. Figures 3–5 show the distribution profiles of various acids and bases.

It is important to understand that the species that partition are primarily the neutral molecules or molecules that appear neutral through interaction such as ion-pairing which allows transport of ionic species and thus complicating the calculations of log *P* and log *D*.

Besides projecting the solubility, the log *P* value has several important applications providing greater insight into how the molecule will cross various biologic barriers and hence proves effective as a prospective new lead compound. In general, where passive absorption is assumed, the log *P* can be related to various fixed value ranges (Fig. 6).

Generally, a low log *P* (below 0) is desirable for injectable products whereas a medium (0–3) range is suitable for oral administration; transdermal administration requires a higher

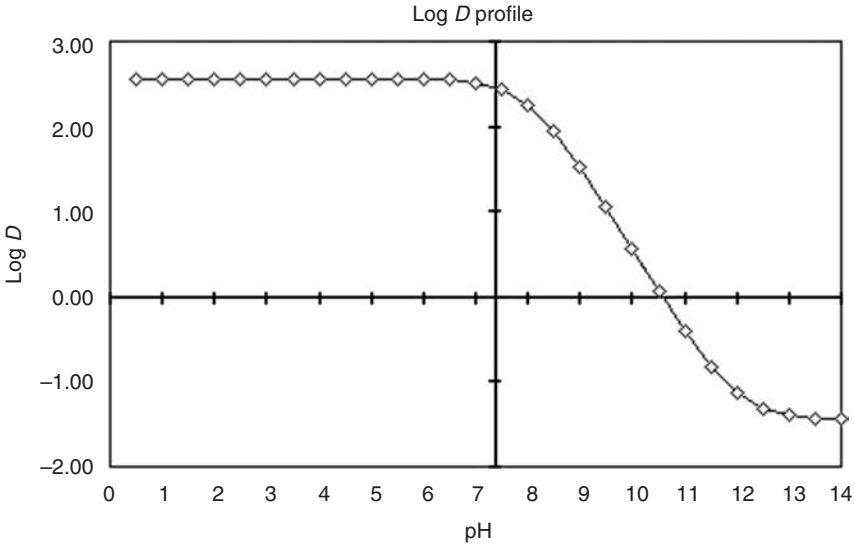

FIGURE 4 Log *D* profile of a base, $pK_a = 8$.

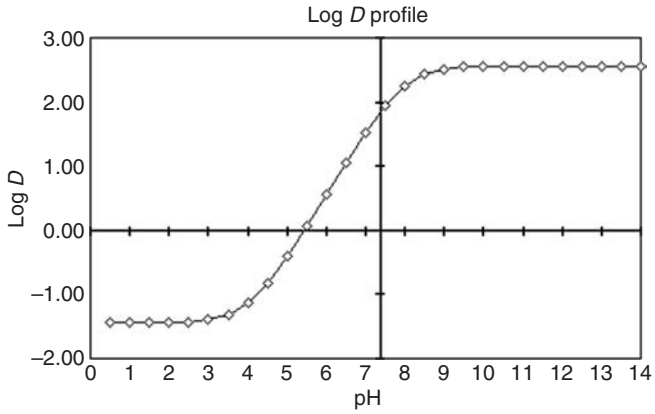

FIGURE 5 Log *D* profile of a zwitter ion (base), pK_a (base) = 5.6, and pK_a (acid) = 7.0.

value (3–4) but once we reach in the range of 4 to 7, we risk accumulation of drug I in the body fat that can prove toxic due to accumulation of drug in multiple dosing situations. The renal clearance of drugs with log *D* (measured at pH 7.4) above zero will decrease renal clearance and increase metabolic clearance; the pK_a of drugs also plays an important role here as highly ionized drugs are kept out of cells and thus out of systemic toxicity; generally, a pK_a of 6 to 8 will be most optimal for transport across various biologic membranes.

When making a choice, generally a drug with lowest log *P* will be desirable; however, that may require making a choice between a high- versus a low-molecular weight molecule; it is known that high-molecular weight drugs are generally more allergenic. The goal should be to achieve a minimum hydrophobicity using a combination of log *P*, pK_a and molecular size. The principle of minimum hydrophobicity keeps the drugs out of central nervous system that may produce side effects like depression, etc., which means that most molecules should have a log *P* lower than 2.0; this technique was used in the design of the newer generation of non-sedative

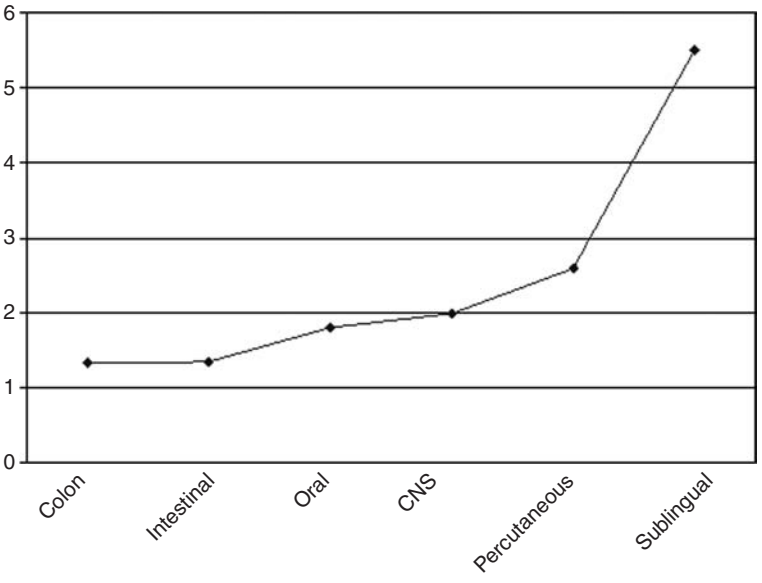

FIGURE 6 Optimal Log *P* values for absorption from various sites of administration.

antihistamines. A very high lipophilicity should also be avoided because of adverse effects on protein binding and drug absorption, including solubility.

Chemical Structure and Form

The chemical form significantly affects dissolution. Chemical modifications can involve changing the chemical structure of a drug to a form, which is significantly different from the active drug entity. This form can, however, provide a similar therapeutic response since within the body it breaks down into the active entity. Ideally, a drug molecule should have sufficient aqueous solubility for dissolution; an optimum oil/water partition coefficient to provide diffusion through several bilipid layers; and stable chemical groups which will interact with the receptor site. Such an ideal molecule does not usually exist in nature, and so chemical modifications are generally directed toward that part of the molecule, which is responsible for the hindrance in the overall absorption process. For example, it is desirable to restrict the absorption of a sulfonamide if it is to provide a local action in the gastrointestinal tract. This can be achieved by the synthesis of chemical forms such as succinylsulfathiazole, phthalylsulfathiazole, phthalylsulfacetamide, and salazosulfapyridine, with a free carbonic acid structure which can ionize in the gut. These chemical modificaions, which lead to an ionized species, decrease the lipid–water partition coefficient sufficiently to restrict the absorption of the sulfonamides. The antibacterial activity is unfolded when the amide links are broken down by hydrolysis, thus releasing the free and active sulfonamide structures. The aqueous solubility of drugs can be increased by modifications, such as sulfacarboxychysoidine, and so on, a sulfonamide designed on the basis of insight gained with prontosil and pontosil rubrum. The introduction of dicarbonic acid and sugars into the chemical structure increases the aqueous solubility of tuberculostatic, thiosemicarbazone, and isonicotinic acid hydrazide, erythromycin, and chloramphenicol also provide increased aqueous solubility, Table 1 lists several examples of drugs whose water has solubility been increased as a result of chemical modifications. On the other hand, a decrease in the ionization will result in better absorption, as demonstrated for ganglionic blocking agents of the onium type such as hexamethonium. By switching to tertiary amines, such as mecamylamine and pempidine, one obtains drugs that are more steadily and completely absorbed.

Lipophilizing Modifications

Increasing lipid solubility through chemical modifications is exemplified by doxycycline, a derivative of tetracycline. This compound is more efficiently absorbed from the intestine than tetracycline partly because of better lipid solubility and partly because of a decreased tendency to form poorly soluble complexes with calcium and phosphate. This facilitated absorption decreases the risk of disturbances in the intestinal flora and intestinal superinfection. Chemical

TABLE 1 Examples of Solubility-Increasing Modifications to Drugs

Drug	High water soluble form
Tetracycline	Rolitetracycline, piperacillin, tetralysine
Theophylline	Diprophylline
Theobromin	Isobromin
Prednisolone	Soludacortin, ultracorten, corticosol
Deoxycortone	Docaquosum, diethylstilbestrol, idroestril
Testosterone	Testosterone phosphate
Sulfanilamide	Glucosyl sulfanilamide
Menadiol	Menadiol diphosphate, menadiol disulfate
Tocopherol	Tocopheryl hemisuccinate
Chloramphenicol	Chloramphenicol hemisuccinate/hemiphthalate
Estriol	Estriol hemisuccinate
Phenetidine	Phenetidine hemisuccinate
Oxazepam	Oxazepam hemisuccinate
Hydroxydione	Hydroxydione dihemisuccinate
Griseofulvin	Succinate/oxime derivatives

TABLE 2 Partition Coefficient and Absorption of Barbiturates

Drug	Percent absorption	Partition coefficient
Barbital	12	0.7
Aprobarbital	17	4.9
Phenobarbital	20	4.8
Butalbital	23	10.5
Butethal	24	11.7
Cyclobarbital	24	13.9
Pentabarbital	30	28.0
Secobarbital	40	50.7
Hexethal	44	100

changes related to lipid solubility and its effect on gastrointestinal absorption are best exemplified by barbiturates, in which an increase in lipid solubility is directly related to absorption from the colon (Tables 2 and 3).

Salt Forms

Many important drugs are weak acids or bases. Salts of acidic or basic drugs have different solubility characteristics and show different bioavailability. Sodium or potassium salts of weak acids dissolve much more rapidly than the corresponding free acids, regardless of the pH of the dissolution medium. The same is usually true of the hydrochloride salts or other strong acid salts of weak bases, such as tetracycline hydrochloride, or atropine sulfate. The salt form of the drug is generally more soluble in an aqueous medium. However, the solubility of the salt depends on the strength and quantity of the counterions; the smaller the counterion the more soluble is the salt. For example, *p*-Amino salicylic acid (PAS) exists in various salt forms and their solubility is given in Table 4.

In comparing the absorption of PAS in humans the salts provide clearly greater absorption than the acid form and the rates of absorption are related to the solubility of the salt form. Novobiocin sodium salt is twice as bioavailable as the calcium salt and 50 times more available than the parent acid.

However, the use of salt forms is not always desirable such as demonstrated for several drugs as listed in Table 5.

One approach to the use of salt formation involves additives, which provide an alkaline pH around the dissolving particles of weakly acidic drugs. This is best exemplified by the buffered aspirin formulation in which the sodium bicarbonate content provides the alkaline pH. Similarly, sodium phosphate also provides and alkaline pH upon its hydrolysis in the gastrointestinal tract.

Combinatorial chemistry offers many advantages including synthesis of larger molecular weight drugs, which are mostly lipophilic; biovailability considerations require converting them to salt forms. This trend is apparent from recent regulatory approvals by Food and Drug Administration (FDA) where more than 50% of new drugs approved have been in salt forms. There are fewer salt-forming species for weak acids than there are for weak bases, and the available information suggests that, in general, alkali metal salts exhibit greater solubility than the corresponding alkaline earth salts. Among cations, the most frequently found ion is

TABLE 3 Examples of Enhanced Lipid Solubility

Erythromycin	Erythromycin estolate
Tetracycline	Doxycycline
6-Azauridine	Triacetyl azaurindine
Lincomycin	Propionate stearate and ethyl carbonate forms
Corticosteroids	Valerate forms for topical use
Nicotinic acid	Ester forms for topical use
Salicylic acid	Ester forms for topical use
Thiamine/other vitamins	Nonquaternary form

TABLE 4 Solubility of PAS as a Function of Its Salt Form

Form	Solubility (mg/mL)
Unionized acid	1.7
Potassium salt	100
Calcium salt	143
Sodium salt	500

Abbreviation: PAS, *p*-amino salicylic acid.

sodium (62%), followed by potassium and calcium (10%); this is followed by zinc and meglumine (3%), lithium, magnesium diethanolamine, benzathine, ethyldiamine, aluminum, chloroprocaine, and choline (in decreasing order of frequency). Among anions, the most frequently used counterion is hydrochloride (almost 50%), followed by sulfate (8%), bromide and chloride (5%), diphosphate, citrate, maleate (3%), iodine mesylate, hydrobromide (2%), acetate, pamoate (1%), isothionate, methylsulfate, salicylate, lactate, methylbromide, nitrate, bitartrate, benzoate, dihydrochloride, gluconate, carbonate, edisylate, mandelate, methylnitrate, subacetate, succinate, benzenesulfonate, calcium edentate, camsylate, edentate, fumarate, glutamate, hydrobromine, napsylate, pantothenate, stearate, gluceptate, bicarbonate, estolate, esylate, glycollylarsenate, hexylresorcinate, lactobionate, maleate, mucate, polygalacturonate, teoclate, triethiodide (in decreasing order of frequency). The choice of counterions is a function of the pK_a of the weak acid involved in the formation of salt. Table 6 lists pK_a values of weak acids that are most frequently used in salt formation.

To form a salt of a basic compound, the pK_a of the salt-forming acid has to be less than or equal to the pK_a of the basic center of the compound as a result, very weak basic compounds having a pK_a of around 2 have a greater range of possibilities for salt formation. Since most drugs are weak bases, it is not surprising that hydrochloride, sulfuric, and toluenesulfonic salts are very common.

PHYSICAL PROPERTIES

Physical properties as affected by solid-state properties can affect the activity of the drug as determined by the rate of delivery. Chemical stability, which is affected by physical properties can be significant. Whereas it is always desirable to enhance chemical stability (a pursuit of the synthetic chemist), modulation of physical properties like reducing hygroscopicity by increasing hydrophobicity of acid, or by moving to carboxylic rather than sulfonic or mineral acid or using acid of higher pK_a to raise pH of solution often provides more stable compounds. Stability is also improved by decreasing solubility and increasing crystallinity by increase of melting. It is important to realize that factors that improve chemical stability often impact adversely on the physical properties. Therefore a fine balance must be achieved when selecting between physical properties of a chemical property modulation.

Stability of the salt could also be an important issue and depending on the pK_a, many properties can change including indirectly related physical characteristics such as volatility (e.g., hydrochloride salts are often more volatile than sulfate salts). Discoloration of salt form of drugs is also prominent for some specific forms as the oxidation reactions (often accompanied by hydrolysis) are a result of factors such as affinity for moisture, surface hydrophobicity, etc.

TABLE 5 Examples Where Salt Form Reduces Dissolution and Bioavailability

Example	Mechanism
Sodium phenobarbital	Swelling of table, retarded disintegration
Aluminum aspirin	Water insoluble aluminum coates the surface
Chlortetracycline hydrochloride	Common ion suppression—excess chloride ions
Sodium heptabarbital	Salt absorbed faster but incompletely due to large crystal precipitation
Sodium warfarin/benzamphetamine pamaote	Surface precipitation of free acid

TABLE 6 pK_a of Common Weak Acids Used in Salt Formation

Acid	pK_a
Acetate	4.76
Ascorbate	4.21
Benzoate	4.20
Besylate	2.54
Citrate	3.13
Fumarate	3.0, 4.4
Gluconate	3.60
Hydrobromide	−8.0
Hydrochloride	−6.1
Malate	3.5, 5.1
Mesylate	1.92
Napsylate	0.17
Oleate	~4.0
Phosphate	2.15, 7.20, 12.38
Succinate	4.2, 5.6
Sulfate	−3.0
Tartrate	3.0, 4.3
Tosylate	−0.51

Hydrolysis of a salt back to the free base may also take place if the pK_a of the base is sufficiently weak.

Crystal Morphology

A crystalline species is defined as a solid that is composed of atoms, ions or molecules arranged in a periodic, three-dimensional pattern. A three-dimensional array is called a lattice as shown in Figure 7. The requirement of a lattice is that each volume, which is called a unit cell is surrounded by identical objects. Three vectors, a, b, and c, are defined in a right-handed sense for a unit cell. However, since three vectors are quite arbitrary a unit cell is described by six scalars, a, b, c, α, β, and γ without directions (Fig. 8). Several kinds of unit cells are possible, for example, if $a=b=c$ and $\alpha=\beta=\gamma=90°$, the unit cell is cubic. It turns out that only seven different kinds of unit cells are necessary to include all the possible lattices. These correspond to the seven crystal systems as shown in Table 7.

The seven different point lattices can be obtained simply by putting points at the corners of the unit cells of the seven crystal systems. However, there are more possible arrangements of points, which do not violate the requirements of a lattice.

A crystalline particle is characterized by definite external and internal structures. Habit describes the external shape of a crystal, whereas polymorphic state refers to the definite arrangement of molecules inside the crystal lattice. Crystallization is invariably employed as the final step for purification of a solid. Use of different solvents and processing conditions may alter the habit of recrystallized particles, besides modifying the polymorphic state of the solid. Subtle changes in crystal habit at this stage can lead to significant variation in raw material

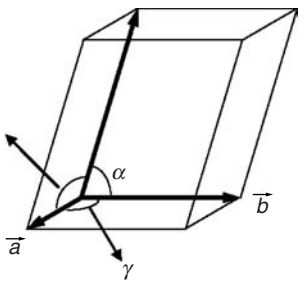

FIGURE 7 Crystal lattice.

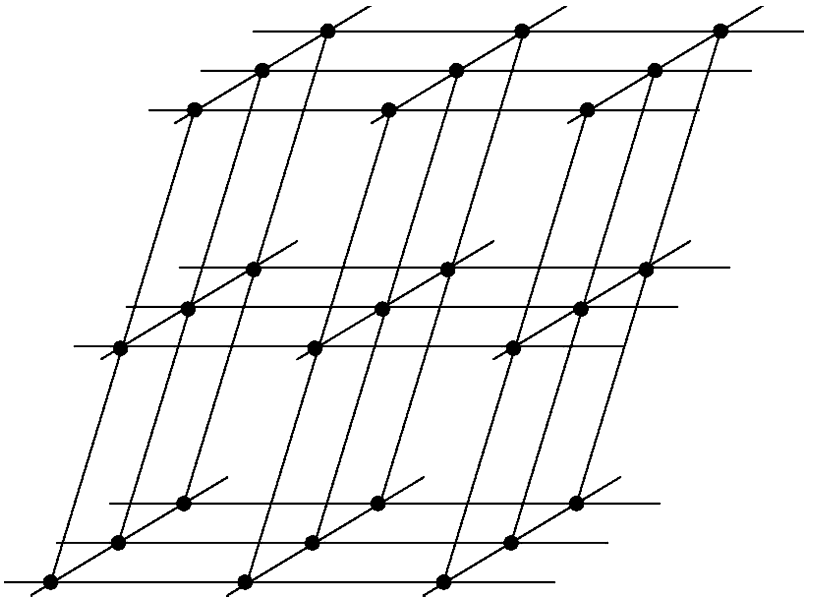

FIGURE 8 Scalars of lattice structure.

characteristics. Furthermore, various indices of dosage form performance such as particle orientation, flowability, packing, compaction, suspension stability, and dissolution can be altered even in the absence of significantly altered polymorphic state. These effects are a result of the physical effect of different crystal habits. In addition, changes in crystal habit accompanied with or without polymorphic transformation during processing or storage can lead to serious implications of physical stability in dosage forms. Therefore to minimize variations in raw material characteristics, to ensure reproducibility of results during preformulation, and to correctly judge the cause of instability and poor performance of a dosage form, it is essential to recognize the importance of changes in crystal surface appearance and habit of pharmaceutical powders.

The crystal habit is also affected by impurities present in the solution crystallizing; often these impurities provide the earliest nucleation of crystal growth and become integral part of the crystal. In some instances presence of impurities inhibit crystal growth as shown when certain dyes or heavy metals are mixed with solutions. If an impurity can adsorb at the growing

TABLE 7 Seven Crystal Systems

Crystal System	Axial lengths and angles
Cubic	$a=b=c$ $\alpha=\beta=\gamma=90^\circ$
Tetragonal	$a=b\neq c$ $\alpha=\beta=\gamma=90^\circ$
Orthorhombic	$a\neq b\neq c$ $\alpha=\beta=\gamma=90^\circ$
Rhombohedral (trigonal)	$a=b=c$ $\alpha=\beta=\gamma\neq90^\circ$
Hexagonal	$a=b\neq c$ $\alpha=\beta=90^\circ$, $\gamma=120^\circ$
Monoclinic	$a\neq b\neq c$ $\alpha=\gamma=90^\circ\neq\beta$
Triclinic	$a\neq b\neq c$ $\alpha\neq\beta\neq\gamma\neq90^\circ$

face, it can significantly alter the course of crystal growth and geometry. The habits bound by plane faces are termed *euhedral* and those with irregularly shaped are called *anhedral*. The symmetry of a crystal is generally studied by using optical goniometer that allows measurement of the angles between the crystal faces. This technique is of use only when good crystals of size >0.05 mm in each direction can be obtained, which is generally not the case.

Polymorphism

Both organic and inorganic pharmaceutical compounds can crystallize into two or more solid forms that have the same chemical composition and is called as polymorphism. Polymorphs have different relative intermolecular and/or interatomic distances as well as unit cells, resulting in different physical and chemical properties such as density, solubility, dissolution rate, bioavailability, etc. When crystal structure contains solvents (or water) these are often called as psudopolymorphs with distinct physical and chemical properties. It is possible for each pseudopolymorph to have many polymorphs. In polymorphism, the crystal lattices formation can take place through two mechanisms: packing polymorphism and conformational polymorphism. Packing polymorphism represents formation of different crystal lattices of conformationally relatively rigid molecules that can be rearranged stably into different three-dimensional structures through different intermolecular mechanisms. When a non-conformationally rigid molecule can be folded into alternative crystal structures the polymorphism is categorized as conformational polymorphism.

Polymorphs and pseudopolymorphs can be also classified as either monotropes or enantiotropes, depending upon whether or not one form can transform reversibly to another. In a monotropic system, Form I does transform to Form II because the transition temperature cannot appear before the melting temperature (Fig. 9, monotropy). In Figure 10 (enantiotropy), Form II is stable over a temperature range below the transition temperature at which two solubility curves meet and Form I is stable above the transition temperature. At the transition temperature, reversible transformation between two forms happens. Figure 11 (enantiotropy with metastable phases) shows the kinetic effects on thermodynamic property of solubility, which shows Ostwald ripening effect. An unstable system does not necessarily transform directly into the most stable state, but into one, which most closely resembles its own, i.e., into another transient state whose formation from the original is accompanied by the smallest loss of free energy.

When the decision on whether two polymorphs are enantiotropes or monotropes need to be made, which is very useful to use the thermodynamic rules developed by Burger and Ramberger tabulated in Table 8.

The stability of polymorphs is thermodynamically related to their free energy. The more stable polymorph has the lower free energy at a given temperature. The above classification of polymorphic substances into monotropic and enantiotropic classes from the view of the lattice theory is not always appropriate. There is a need to explore how the crystal lattice structures of polymorphs are related. At a transition point with the temperature and the pressure fixed, it is possible for interconversion to happen between two polymorphs only in the case that the

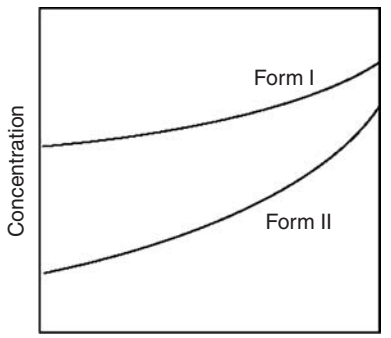

FIGURE 9 Monotropic system as a function of temperature (*x*-axis).

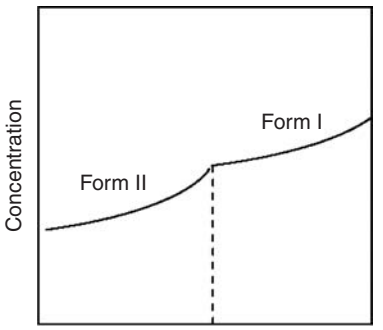

FIGURE 10 Enantiotropic system as a function of temperature (*x*-axis).

structures of the polymorphs are related. If complete rearrangement is required by atoms or molecules during transformation, no point of contact for reversible interconversion exists. Therefore, the existence of enantiotropes or monotropes in thermodynamics and phase theory is corresponding to related or unrelated lattice structures in structural theory. Transformation between polymorphs that have completely different lattice structures exhibits the dramatic changes in properties. The difference in energy between polymorphs is not always considerable as shown with diamond/graphite. In most cases, polymorphs in this category are required to break bonds and rearrange atoms or molecules and, consequently, the polymorphs have a monotropic relation.

For the study of polymorphs that are structurally related firstly the structural relationships between the polymorphs should be established, secondly it should be explained why a particular substance is able to arrange its structural units in two closely related lattices and finally there should be a description of the manner and conditions under which rearrangement of the units from one lattice type to another can happen. For drugs that undergo degradation in the solid state, the physical form of the drug influences degradation. Selection of a polymorph that is chemically more stable is a solution in many cases. Different polymorphs also lead to different morphology, tensile strength and density of powder bed which all contribute to compression characteristics of materials. Some investigation of polymorphism and crystal habit of a drug substance as it relates to pharmaceutical processing is desirable during its preformulation evaluation, especially when the active ingredient is expected to constitute the bulk of the tablet mass.

Various techniques are available for the investigation of the solid state. These include microscopy (including hot-stage microscopy), infrared spectrophotometry, single-crystal X-ray and X-ray powder diffraction (XRPD), thermal analysis, and dilatometry.

Most organic compounds are capable of exhibiting polymorphism because of their complex flexible structure; the window of physicochemical stress that a drug is generally subjected to during manufacturing is at times not able to adduce the differentiation of a drug into its possible polymorphic forms. For example, enantiotropic state is when one polymorph can be reversibly changed into another one by varying the temperature or pressure. One way of

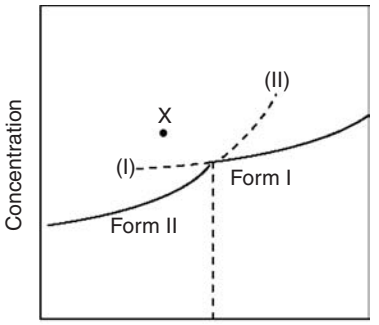

FIGURE 11 Enantiotropic system with metastable phases as a function of temperature (*x*-axis).

TABLE 8 Thermodynamic Rules for Polymorphic Transitions

Enantiotropy	Monotropy
Transition < melting I	Transition > melting I
I stable > transition	I always stable
II stable < transition	—
Transition reversible	Transition irreversible
Solubility I higher < transition	Transition I always lower
Solubility I lower > transition	—
Transition II → I endothermic	Transition II → I exothermic
$\Delta H_f^I < \Delta H_f^{II}$	$\Delta H_f^I > \Delta H_f^{II}$
IR peak I before II	IR peak I after II
Density I < II	Density I > II

Abbreviation: IR, infrared.

assessing whether the solid is a metastable form of the compound is to slurry the compound in a range of solvents. In this way, a solvent-mediated phase transformation may be detected using the usual techniques. The monotropic state exists when the change between the two forms is irreversible. Since all polymorphs are interchangeable, the lowest energy polymorph or the most stable polymorph is often needed to assure consistency in the physicochemical properties; this is necessary for consistency in manufacturing procedures as well as in bioavailability. The right polymorph at time is not necessarily the most stable polymorph; unstable forms like amorphous forms (that are most constrained) are often used because of their higher solubility and often a better bioavailability profile.

The manufacturing factors that may be affected by the choice of a particular polymorphic form include granulation, milling and compression, stability (particularly for semisolid forms), amount of dose delivered in metered inhalers, crystallization from different solvents at different speeds and temperature, precipitation, concentration or evaporation, crystallization from the melt, grinding and compression, lyophilization, and spray drying. In the manufacturing processing, crystallization is a major problem and it can be avoided by a careful study of polymorphic transition particularly in supercritical fluids.

Polymorphism is frequently a function of the type of salt used because the presence of counterions can cause crystallization in different forms leading to widely variable physicochemical properties as described above under the polymorphism description. Generally, salts exhibiting polymorphism should be avoided.

An interesting example of polymorphic structure differentiation is that of HIV protease inhibitors. The HIV protease inhibitors have serious problem in their bioavailability Invirase showed only modest market performance, and it was soon superseded by drugs, such as ritonavir (Norvir®) and indinavir sulfate (Crixivan®) that had better bioavailability. Three years after initial approval, saquinavir was reintroduced in a formulation with sixfold higher oral bioavailability relative to the original product. Ritonavir was originally launched as a semisolid dosage form, in which the waxy matrix contained dispersed drug in order to achieve acceptable oral bioavailability. Two years after its introduction, ritonavir exhibited latent crystal polymorphism which caused the semisolid capsule formulation of Norvir to be removed from the market.

Each polymorph has a certain thermodynamic energy associated with it as a result of strains in the bonds of the lattice structure, and therefore one polymorph may be more stable than the others. At any given temperature and pressure only one crystal form of a drug will be stable, and other forms will convert to this form. When the conversion is relatively slow, the polymorph is said to be metastable. The various polymorphic forms are chemically indistinguishable. However, they differ in physical properties, such as density, melting point, solubility, and dissolution rates. For example, riboflavin exists in several polymorphic forms with a 20-fold difference in their aqueous solubility. Amorphous forms in which no internal crystal structure exists have the highest solubilities, giving the order of dissolution rates for the crystal forms can be arranged as amorphous > metastable > stable forms.

TABLE 9 Effect of Polymorphism on Dosage Form Characteristics

Example	Explanation
Novobiocin	Increased BA from amorphous form, suspension stabilized by methyl cellulose
Sulfathiazole	Increased dissolution from amorphous form conversion stabilized by PEG 400
Lente insulin	Amorphous form for quick absorption, crystalline form giving sustained delivery
Theobroma oil	High melting point form for room temperature stability
Penicillin G	Amorphous form less chemically stable
Chloramphenicol stearate	Amorphous form active
Aspirin, barbital, estrone, sulfonamides, chloramphenicol, chlordiazepoxide, adiphenine, erythromycin, methotrexate, cholesteryl palmitate	Altered bioavailability

Abbreviations: BA, bioavailability; PEG, polyethylene glycol.

It has been suggested that almost 40% of all organic compounds can exist in various polymorphic forms; sometimes in as many as five different forms, as in the case of cortisone acetate; almost 50% of all barbiturates and 70% of steroids exhibit polymorphism.

This premise, however, may not be applicable to all drugs, especially those which are absorbed by an active process, e.g., various vitamins. Table 9 lists effects of polymorphism on drug and dosage form characteristics.

Amorphous Forms

Solid powders wherein there is no particular order of molecules are technically noncrystalline and called amorphous forms. The amorphous forms are formed by vapor condensation, supercooling of a melt, precipitation from solution, and milling and compaction of crystals. These are more like liquids where the molecular interaction has weakened; in most instances there would be some crystalline forms among the amorphous forms as well. This two-state model is described in United States Pharmacopoeia (USP). The amorphous forms are thermodynamically unstable as they have high energy (that went into breaking intermolecular bonds) and as a result they may turn into crystalline form, particularly in suspension dosage forms and even in solid dosage forms wherein atmosphere moisture may serve as nucleation points.

Discovery programs frequently yield amorphous compounds due to time pressures, the methods used to isolate them on small scales, and the increasing complexity of newly discovered molecules. Amorphous compounds carry inherent risks due to their physicochemical nature and as a result very few FDA-approved drugs appear in amorphous forms; examples include Accupril®/Accuretic®, intraconazole, Accolate® (zafirlukast), Viracept® (nelfinavir mesylate), paroxetine. Other drugs that are available in amorphous forms include: celecoxib, amifostine, cefuroxime axetil, cefpodoxime proxetil, and novobiocin. In addition to being physically metastable physical form, amorphous forms are generally chemically less stable. They also tend to have very low bulk densities, making the materials difficult to isolate and handle. The irregular shape of powder of amorphous forms creates high surface area, which attracts water molecules making them inherently more hygroscopic.

Whereas all of these problems can be resolved, generally, the amorphous forms are to be avoided unless the differences in solubility make a significant impact on the bioavailability.

Solvates

In additions to polymorphs, solvates (inclusion of the solvent of crystallization) are also often formed during the crystallization process. These forms are also called psudopolymorphs. The solvent molecules fill spaces in the crystal lattice and generally reduce the solubility and dissolution rates. This phenomenon is thermodynamically driven. If the solvate contains an organic solvent, this would not be admitted by regulatory authorities. According to the

TABLE 10 Effects of Salvation on Drug Activity

Example	Explanation
Ampicillin	Anhydrate shows higher bioavailability compared to trihydrate (?)
Hydrocortisone	Hemiacetone solvates show higher absorption compared to asolvates
Caffeine, theophylline	Increased dissolution of anhydrous forms
Glutethimide	Compared to hydrates
Mercaptopurine, Prednisolone	Higher dissolution and activity of asolvate from pellets implanted
Griseofulvin	Chloroformate solvate gives higher bioavailibility compared to asolvate, also solvation–desolvation results in increased surface area
Citric acid	Hydrate used to provide mole of water as granulating agent in effervescent preparations

International Conference on Harmonization guidelines, the Class I solvents must be avoided as these are carcinogenic such as benzene, carbon tetrachloride, and 1,2-dichloromethane; the Class II solvents should be limited and include non-genotoxic animal carcinogens such as cyclohexane, acetonitrile; the Class III solvents have low-toxicity potential including acetic acid, alcohol, and acetone, which are allowed as long as the daily permissible dose does not exceed 50 mg. Generally, an allowed solvate would likely be removed during the manufacturing process but in some instances, the presence of solvate is desired like in beclomethasone dipropionate product of Glaxo that includes trichlorofluoromethane solvate; this solvate prevents crystal growth in sprays containing trichlorofluoromethane as propellant.

During seeding, crystals may incorporate one or more of the molecules of the solvent into their structure and the resultant forms are referred to as SOLVATES. The solvates themselves may exist in various *polymorphic* forms and are referred to as *pseudopolymorphs*. Some examples of pseudopolymorphs include mercaptopurine, fluprednisolone, and succinylsulfathiazole. The number of drug solvates is well over 100 and some of the most common examples include steroids, antibiotics, sulfonamides, barbiturates, xanthines, and cardiac glycosides.

The use of a solvates in increasing bioavailability is on the premise that some anhydrates dissolve faster than their corresponding hydrates in aqueous media. However, the relationship becomes much more complex when alcohlates or other nonaqueous solvate are dissolved in water. Table 10 lists several examples of effect of solvation on drug and dosage form.

Whereas the selection of an appropriate solvate or asolvate form can be advantageous in increasing the dissolution rates and bioavailability in some instances, their use requires careful monitoring of manufacturing and storage conditions because of the possibility of inadvertent solvation or desolvation.

Hydrates

When solvate happens to be water, these are called hydrates wherein water is entrapped through hydrogen bonding inside the crystal and strengthens crystal structure and thereby invariably reduces the dissolution rate (Table 11). The water molecules can reside in the crystal either as isolate lattice where they are not in contact with each other, or lattice channel water where they fill space and metal coordinated water in salts of weak acids where meta ion coordinates with water molecule. Metal-ion coordinates may also fill channels such as in the case of nedocromil sodium trihydrate. Crystalline hydrates have been classified by structural aspects into three classes: isolated lattice sites, lattice channels, and metal-ion coordinated water. There are three classes discernible by the commonly available analytical techniques.

1. Class I includes isolated lattice sites, represent the structures with water molecules that are isolated and kept from contacting other water molecules directly in the lattice structure. Therefore, water molecules exposed to the surface of crystals may be easily lost. However, the creation of holes that were occupied by the water molecules on the surface of crystals does not provide access for water molecules inside the crystal lattice. The analyses by thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) for the hydrates in this class show sharp endotherms. Cephradine dihydrate is an example of this class of hydrates.

TABLE 11 Drug Substance Hydrate Forms as Reported in the Pharmacopoeia

Compound	Water of hydration
Aminophylline	2
Ampicillin	3
Beclomethasone dipropionate	0 or 1
Caffeine	1
Calcium citrate	4
Calcium gluceptate	0 or variable; effloresces
Calcium gluconate	0 or 1
Dextrose	1
Diatrizoic acid	2
Dibasic sodium phosphate	0, 1, or 2
Ephedrine	1/2
Fluocinolone acetonide	2
Hydrocortisone hemisuccinate	1
Magnesium citrate oral solution	1
Magnesium gluconate	2
Magnesium sulfate	0, loses gradually
Monosodium sodium phosphate	0, 1, or 2
Naloxone hydrochloride	2
Nitrofurantoin	0 or 1
Potassium gluconate	0 or 1
Prednisolone	0 or 1
Saccharin sodium	1/3; effloresces
Sodium acetate	3
Sodium citrate	0 or 2
Sodium sulfate	0 or 1; effloresces
Succinyl chloride	2
Theophylline	0 or 1
Thioguanine	0 or 1/2
Thiothixene hydrochloride	0 or 2
Zinc sulfate	1 or 7

Source: From United States Pharmacopoeia 24.

2. Class II includes hydrates that have water molecules in channels. The water molecules in this class lie continuously next to the other water molecules, forming channels through the crystal. The TGA and DSC data show interesting characteristics of channel hydrate dehydration. Early onset temperature of dehydration is expected and broad dehydration is also characteristic for the channel hydrates. This is because the dehydration begins from the ends of channels that are open to the surface of crystals. Then, dehydration keeps on happening until all water molecules are removed through the channels. Ampicillin trihydrate belongs to this class. Some hydrates have water molecules in two-dimensional space and they are called planar hydrates.
3. Class III includes ion-associated hydrates. Hydrates contain metal-ion coordinated water and the interaction between the metal ions and water molecules is the major force in the structure of crystalline hydrates. The metal–water interactions may be quite strong relative to the other nonbonded interactions and, therefore, dehydration occurs at very high temperatures. In TGA and DSC thermograms, very sharp peaks corresponding to dehydration of water bonded with metal ions are expected at high temperatures.

Hydrates can also exist in various polymorphs such as in the case of amiloride hydrochloride. A myriad of methods are available to study hydrates and their polymorphs including differential thermal analysis (DTA), DSC, XRPD, and moisture uptake studies.

Complexation

A molecular complex consists of constituents held together by weak forces such as hydrogen bonds. The physical properties of drug complexes, such as solubility, molecular size, diffusivity,

TABLE 12 Effect of Complexation on Release and Bioavailability

Drug	Complexing agent	Effect
Amphetamine	Na-CMC	Decreased bioavailability
Phenobarbital	PEG 4000	Decreased bioavailability
Tetracycline	Heavy	Decreased bioavailability
Salicylic acid	Caffeine	Increased lipid solubility, no effect on bioavailability due to rapid dissociation
Diphenhydramine	Methyl orange	
Acidic dyes	Basic nitrogen	
Atropine	Eosin B	
Prednisolone/predisone	Propylamide/propionamides	Increased bioavailability
Digoxin	Hydroquinone	Increased bioavailability
Benzocaine, erogtamine	Caffeine	Increased dissolution
M-Benzoic acid	Tartaric acid	Increased dissolution
Maleic acid	Creatinine	Increased dissolution
Several antihistamines	Beta cyclordextrin	Increased dissolution
Caffeine	Citric acid	Increased dissolution

Abbreviations: NA-CMC, sodium carboxymethylcellulose; PEG, polyethylene glycol.

and lipid–water partition coefficient can differ significantly from those of the drug itself, resulting in possible bioavailability variations. Complexation will generally increase the total solubility of a poorly water-soluble drug if the complex itself is soluble in aqueous media. If the complexation process is reversible (Drug + Complexing agent complex) then the absorption rates and the extent of absorption will be increased for poorly soluble drugs. For drugs, which are generally adequately absorbed, the complex formation may result in a slowing down of absorption, but the overall quantity of drug absorbed may not change.

The most frequently observed complex formation is between various drugs and macromolecules, such as gums, cellulose derivatives, high-molecular weight polyols and nonionic surfactants. Mostly, however, these complexations are reversible with little effect on the bioavailability of drugs. But in those instances where the complex is insoluble in aqueous media, these interactions are clearly contraindicated. Table 12 lists several examples of complexation where bioavailability has been altered.

Surface Activity

Surfactants have variable effects on the dissolution and absorption processes. The lowering of surface tension increases the dissolution rates by increasing the solubility of drugs if the concentration of the surfactant is above the critical micelle concentration. The lowering of surface tension also increases the diffusion of free molecules in the medium, increasing the contact between free drug and the absorption surface. The surfactants can also increase the membrane permeability, allowing greater absorption of most chemical structures.

The overall effect of surfactants on the bioavailability of drugs is complex, since the molecules contained in the micelle are not available for absorption unless a quick equilibration between the free drug molecules and those inside the micelles can be established. A number of drugs have surface active properties themselves and form their own micelles, thus facilitating the absorption. Examples of these drugs include potassium benzyl penicillin, mixtures of penicillin and streptomycin salts, amphetamine sulfate, cyclopentamine hydrochloride, ephedrine sulfate, propoxyphene hydrochloride, ionic derivatives of phenothiazines, dyes, quarternary ammonium salts of drugs, and liquoris.

There are numerous examples in the literature where the use of surfactants in a formulation has resulted in increased absorption. Some of these examples are listed in Table 13.

TABLE 13 Examples Where Bioavailability Has Been Increased Due to the Addition of Surfactants

Drug	Surfactant
Vitamin A	Sodium laurylsulfate
Vitamin B12	Polysorbate 80/85 G-1096
O-benzoylthiamine disulfide	Sodium laurylsulfate
Cephaloridine	Various
G-Strophanthin	Sodium laurylsulfate
Heparin	Sodium laurylsulfate, dioctylsodium sulfosuccinate
Iodoform	Polysorbate 80
Phenosulfonphthalein	Dioctyl sodium sulfosuccinate
Riboflavin	Sodium deoxycholate
Salicylamide	Polysorbate 80
Salicylic acid	Various
Spironolactone	Polysorbate 80
Sulfasoxazole	Polysorbate 80
Thiourea	Alkylbenzone sulfonate dodecyl trimethyl ammonium chloride

The surface-active properties of gastrointestinal contents primarily involve the various fluids, e.g., gastric juice and intestinal juice, and can be a major factor in the dissolution of several hydrophobic drugs. The most important component of gastrointestinal fluids is the high concentration of bile salts (100–200 mM) present, well above their critical micelle concentration (2–3 mM). The bile salt micelles solubilize polycyclic aromatic hydrocarbons, progesterone, cholesterol, esterone, griseofulvin, and reserpine and thus increase their absorption. Enhancement of drug absorption after meals is often related to the increased flow of bile, which can solubilize the drug molecules, as demonstrated by griseofulvin. However, if micellization results in removing the otherwise available molecule for absorption, the bioavailability may decrease.

Hygroscopicity

Water molecules have polar ends and readily form hydrogen bonding. As a result, several compounds interact with water molecules by surface adsorption, condensation in capillaries, bulk retention, and chemical interaction and are called hygroscopic. At times the interaction between compounds and water is so strong that interacting water vapors result in dissolving the compound, this process is called deliquescence, wherein there is formed a saturated layer of solution around particles. Most of these interactions are dependent on critical water vapor pressure or relative humidity. Moisture also induces hydrolysis and other degradation reactions; presence of moisture also affects physical properties such as powder flow, dissolution and even crystal structure. The impact of moisture on physical or chemical properties of compounds depends on the strength of bonding between water molecule and the surrounding space where water molecules are contained. In tightly bound state, the water molecules are generally not available to induce chemical reactions. Free water molecules can participate in the creation of a liquid environment around crystal lattice where the pH may be altered due to dissolution process. Similarly water molecules held as crystal hydrates or trapped in an amorphous form are not available to modify the milieu interior of solid powders.

The classification of compounds into different hygroscopic categories is based on two types of models; one where the relative humidity and temperature are kept constant and gain in the weight of compound is recorded such as prescribed in the European Pharmacopoeia. In one test, the compound is stored at 25°C for 24 hours at 80% relative humidity and if it shows less than 2% mass gain, it is called slightly hygroscopic; hygroscopic compounds show less than 15% and very hygroscopic compounds show more than 15% mass gain; the deliquescent compounds simply liquefy at this test condition. The dynamic model tests hygroscopic nature at various humidity; compounds showing no mass gain at 90% are called nonhygroscopic, those not gaining at 90% are slightly hygroscopic but those gaining 5% over a week period are

called moderately hygroscopic. Where mass increases at 40% to 50% humidity these compounds are called very hygroscopic.

Generally, a compound that is very hygroscopic will be less desirable but if studies show that despite moisture uptake the compound stays stable and workable in the formulation studies, this is an important consideration.

Particle Size

Of all the possible manipulations of the physical properties of drugs to yield better absorption, the reduction of particle size is most widely exploited. Increased absorption due to reduction of particle size is a result of increased dissolution, which is in turn the result of a larger specific surface area being exposed to the fluids in the gastrointestinal tract or other sites of administration. For example, the breakdown of a 3 mm Cu particle into 1 mm Cu particles results in a 300% increase in the exposed surface area.

Reduction in particle size can be achieved by several methods, including milling, grinding, precipitating the drug on an absorbent, and dispersing the drug in an inert water-soluble carrier (referred to as a solid dispersion). The solid dispersion formulation techniques have received great attention in the recent past and provide an innovative method of particle size reduction. If a hydrophobic drug is dispersed in a hydrophilic medium in a solid state, a faster release of the drug can be expected from this system since the rate limiting steps in the dissolution of the drug will be fewer. The state of drug dispersion can vary from microcrystalline to molecular and thus a wide range of dissolution rates is possible. For example, dispersion of sulfathiazole in urea results in a monomolecular dispersion (solid solution) and the dissolution rates are increased by almost 700 times. The dispersion of griseofulvin in polyethylene glycol 6000 results in an almost 100% increase in its bioavailability as compared to the micronized form of griseofulvin. The solid dispersions are generally prepared by either fusing or dissolving the drug and the water-soluble carrier and then solidfying the melt or solution by cooling or evaporation. The drugs also often coprecipitate, as with the solid dispersion of reserpine and deoxycholic acid. Examples of drugs whose dissolution rates have been increased as a result of solid dispersion formulation include salicylic acid, reserpine, chloramphenicol, prednisone, salicylamide, pentaerythritol, and others.

Quite often solid dispersions can also be used to decrease the release of drugs, so as to provide sustained release as in the dispersion of chlorpheniramine in maleic anhydride copolymers.

The conventional methods of particle size reduction have long been employed to improve the bioavailability of drugs, some of these examples include: vitamin A, medroxyprogesterone acetate, 4-Acetamidophenyl 2,2,2-trichlorethyl carbonate, nitrofurantoin, aspirin, phenobarbital, bishydroxycoumarin, phenacetin, chloramphenicol, procaine penicillin, cyheptamide, reserpine, digoxin, spironolactone, fluocinolone acetonide, sulfadiazine, griseofulvin, sulfasoxazole, *p*-hydroxypropiophenone, sulfur, and tolbutamide.

The reduction in the particle size is, however, not always desirable. For example, nitrofurantoin, when administered in its fine particle size, causes more gastrointestinal irritation than when administered in its coarser size. This is due to the higher plasma and gastrointestinal concentrations resulting from use of a fine particle size. The use of the coarser size is therefore preferred even though this results in retarded absorption. When chemical instability is a problem the reduction of particle size is also contraindicated, as with penicillin G and erythromycin, which decompose in the gastrointestinal tract quickly. Even in the solid state a small particle size means a greater surface area available for the absorption of moisture, which can result in an increased rate of decomposition. The reduction of the particle size of hydrophobic drugs also leads to increased surface charges (static) resulting in the agglomeration of the particles, especially in an aqueous media because of thermodynamic repulsion. This results in a significant decrease in the effective or exposed surface area available for dissolution. This problem can usually be resolved by adding appropriate surfactants, which will reduce the interfacial tension and allow penetration of water molecules through the pocket of hydrophobic air surrounding these particles. As an example, the dissolution of phenacetin is highly

dependent on its particle size where decreasing the particle size decreases dissolution due to absorption of a large quantity of air around the particles. Addition of surfactants or other adjuvants, which reduce this hydrophobic layer of air will increase the effective surface area and thus the dissolution rate. Gastric fluids have relatively lower surface tension, 43 dynes/cm compared to water and may improve wetting effect of hydrophobic particles. The surface activity of gastric fluid is mainly due to the regurgitation of the intestinal fluids into the stomach. For example, phenacetin granules dissolve faster than the phenacetin powder in diluted gastric fluid.

The first step in the commencement of dissolution is the wettability of solid particles—there is a direct correlation between wettability and bioavailability. Since the milieu of drug administration sites is mostly aqueous in nature, low wettability makes particles less hygroscopic.

Dissolution of salts leads to a change in the pH of the dissolution media because of the buffering effect; a base dissolved in acidic media increases the pH since the acidic counterions are trapped into salt forms. Similarly as salts dissolve the pH shift depends on whether it is acid or basic component, which is weaker. The final balance is always dependent on the relative pK_a of the acidic and alkaline components. This is an important consideration as it explains the difference in the results obtained if the studies are conducted in water or buffer. When enteric protection is desired the dissolution rates should be determined in 0.1 N HCl wherein many differences in the dissolution rates between water and buffer are obviated.

Dissolution of a solid usually takes place in two stages: salvation of the solute molecules by the solvent molecules followed by transport of these molecules from the interface into the bulk medium by convection or diffusion. The major factor that determines the dissolution rate is the aqueous solubility of the drug, however, other factors such as particle size, crystalline state (polymorphs, hydrates), pH and buffer concentration can affect the rate. Moreover, physical properties such as viscosity and wettability can also influence the dissolution process.

SOLUBILITY

The discussions of pK_a, log *P*, log *D* above is relevant to understanding the factors that affect the solubility of a drug at the site of administration and thus determining the activity, toxicity, stability, and dosage form and route of administration. The USP classifies drugs based on their solubility (Table 14).

High solubility is defined as the highest dose strength that is soluble in 250 mL or less of aqueous media across the physiologic pH range. Poorly soluble drugs can be defined as those with an aqueous our solubility of less than 100 μg/mL. If a drug is poorly soluble, then it will only slowly dissolve, perhaps leading to incomplete absorption. Some general observations about the behavior of solutes in solution systems include:

- Electrolytes dissolve in conducting solvents.
- Solutes containing hydrogen capable of forming hydrogen bonds dissolve in solvents capable of accepting hydrogen bonds and vice versa.

TABLE 14 The United States Pharmacopoeia Solubility Classification

Descriptive term	Parts of solvent required for one part of solute
Very soluble	<1
Freely soluble	1–10
Soluble	10–30
Sparingly soluble	30–100
Slightly soluble	100–1000
Very slightly soluble	1000–10,000
Practically insoluble or insoluble	10,000 and over

- Solutes having significant dipole moments dissolve in solvents having significant dipole moments.
- Solutes with low or zero dipole moments dissolve in solvents with low or zero dipole moments.

There are always exceptions to these rules, but a good rule of thumb "like dissolves like" mostly applies. Therefore, solvents fall into three classes,

1. Protic solvents such as methanol and formamide which are hydrogen bond donors,
2. Dipolar aprotic solvents (e.g., acetonitrile nitrobenzene) with dielectric constants greater than 15 but which cannot form hydrogen bonds with the solute, and
3. Aprotic solvents in which the dielectric constant is weak and the solvent is nonpolar, e.g., pentane or benzene.

The solubility of ionizable compounds is pH dependent. For weak acids, as pH decreases, the solubility decreases. At equilibrium:

$$[\mathrm{HA}]\ \text{solid} <-> [\mathrm{HA}]\ \text{solution} \tag{12}$$

Where, S_0 is the molar solubility assumed to be pH independent. The equilibrium dissociation constant is:

$$K_a = [H_3O^+][A^-]/[HA] \tag{13}$$

or

$$[A^-] = K_a[HA]/[H_3O^+] = K_aS_0/[H_3O^+] \tag{14}$$

The total solubility (S) is expressed as:

$$S = [A^-] + [HA] \tag{15}$$

or

$$S = K_aS_0/[H_3O^+] + S_0 \tag{16}$$

or

$$S - S_0 = K_aS_0/[H_3O^+] \tag{17}$$

$$\log(S - S_0) = \log K_a + \log S_0 - \log[H_3O^+] \tag{18}$$

$$\log\left[\frac{(S - S_0)}{S_0}\right] = -\mathrm{p}K_a + \mathrm{pH} \tag{19}$$

The solubility of a drug is the maximum amount of drug that dissolves at a specified temperature and solvent. It is a thermodynamic parameter given by:

$$\ln N(\mathrm{sat}) = -\Delta H/R(1/T - 1/T_{mp}) \tag{20}$$

where N is the mole of solute dissolved in the solvent, H is the enthalpy of dissolution and T_{mp} is the melting point.

Thus, the two characteristics that determine the solubility are the melting point and enthalpy of dissolution, both of which are dependent on factors such as chemical structure, physical state (polymorphism, etc.), etc. These categories of factors will be discussed in detail.

Since the majority of drugs used are either weak acids or bases, their total solubility in any given media will depend on their ionization constant in the solution phase. The total solubility, S_t, of a drug is dependent on the relative contribution of the solubility of its unionized form, S_u, and the ionized form, S_i:

$$S_t = S_u + S_i \tag{21}$$

TABLE 15 Ratio of Total and Unionized Drug as a Function of Difference in pH and pK_a

PH–pK_a	S_t/S_u (acid)	S_t/S_u (base)
−2.0	1.01	101
−1.5	1.03	32.6
−1.0	1.10	11.0
−0.5	1.32	4.16
0.0	2.0	2.0
+0.5	4.6	1.32
+1.0	11.0	1.10
+1.5	32.6	1.03
+2.0	101	1.01

Whereas the solubility of the unionized form is a thermodynamic parameter which is constant under given temperature, solvent, and pressure conditions, the fraction of the unionized form changes as a function of solution pH (Table 15).

In applying the equations given above to in vivo situations in man, one should remember that the stomach contents are usually in the pH range of l to 3 and the contents of the upper small intestine, where most drug absorption occurs, have a pH range of 5.5–7.0, and are not "alkaline," only less acidic. Weakly basic compounds will therefore generally dissolve faster in the gastric fluids, and weakly acidic compounds will dissolve faster in the intestinal fluids. For example, salicylic acid with a pK_a of 3.0 shows an approximately 16-fold increase in its dissolution rate when the surrounding pH is changed from 1.5 to 6.8. However for weak acids a linear relationship between $1/H^+$ and its dissolution rate or for weak bases a linear relationship between the dissolution rate and H^+ may not always be possible, especially around neutral pH ranges. This is due to the mechanism of dissolution, which involves formation of a diffusion layer saturated with the dissolving compound, weak acid or base, which results in pH values which may not correspond to the pH values of the bulk medium. For example, dissolution of salicylic acid will result in a pH around the dissolving particles lower than the bulk pH of intestinal fluids. It is this pH of the diffusion layer that determines the actual rate of dissolution. As discussed earlier, inclusion of agents such as sodium bicarbonate in aspirin formulations results in a higher pH in the diffusion layer, increasing the dissolution rates.

In describing the dissolution rates by using the Noyes–Whitney equation, the term saturation solubility is replaced by the solubility in the boundary layer around the dissolving particle and thus for weak acids the equation is transformed as follows:

$$\frac{dC}{dt} = K_s(C_s(1 + K_a/H^+) - C_t) \tag{22}$$

and for weak bases:

$$\frac{dC}{dt} = K_s(C_s(1 + H^+/K_a) - C_t) \tag{23}$$

Thus, for weak acids, dissolution rate increases at basic pH such as shown for tolbutamides and for weak bases, the dissolution rate increases at acid pH, such as shown for tetracycline. Tolbutamide dissolution in gastric fluid is 15 times lower than in simulated intestinal fluid, whereas its salt has slightly higher dissolution in acid media. For tetracycline, the dissolution rate is decreased 2600 times in intestinal fluid whereas its hydrochloride salt shows almost 100% increase in dissolution in the intestinal fluid compared to gastric fluid.

It should be noted that the dissolution of some dosage forms is dependent on pH such as used for enteric-coated forms or other designs where the dissolution and disintegration of the dosage form may be highly pH dependent.

It is generally agreed that the unionized form of a drug is most suitable for gastrointestinal absorption. Thus the efficiency of absorption of a weakly acid or weakly basic compound will change as the dosage form passes through various pH conditions in the gastrointestinal tract. This theory is also referred to as the pH-partition theory and holds true for a variety of

drugs. However, if one takes into account the large differences in the absorption surface areas of the stomach, the small intestine, and the colon, it seems logical to assume that most of the drugs will show sufficient absorption from the upper part of the small intestine if equilibrium is always established between the unionized absorbable species and the ionized form of the drug. For example, in situ studies show that at pH 6.8, where salicylic acid is almost 100% ionized, the absorption is very fast from the rat intestine (50% absorbed in 7 min).

Molecular Size

Large organic molecules have a smaller aqueous solubility than smaller molecules, this being due to interactions between the nonpolar groups and water, i.e., solubility is dependent on the number of solvent molecules that can pack around the solute molecule.

Poorly soluble compounds represent an estimated 60% of compounds in development and many major marketed drugs. It is important to measure and predict solubility and permeability accurately at an early stage, and interpret these data to help assess the potential for development of candidates. This requires developing an effective strategy to select the most appropriate tools to examine and improve solubility in each phase of development, optimization of solid-state approaches to enhance solubility including the use of polymorphs, co-crystals and amorphous solids. All of these would affect the dissolution rates and bioavailability that can be studied with nanocrystal technology.

With this trend of increasingly insoluble drugs stretching resources, many companies are now re-evaluating their strategy. They know that there are many available technologies to measure and predict and finally improve solubility, and several new techniques emerging. Studies that encompass this scope would include how membrane permeation of drugs can be enhanced by means of solubilizing agents, how the solid state is characterized and modified to improve solubility and drug performance, how salt screening and selection can impact on dissolution rate and oral absorption, apply nanocrystal technology to increase dissolution rate, analyze the use of pharmaceutical co-crystals in enhancing drug properties.

Many different approaches have been developed to overcome the solubility problem of poorly soluble drugs, e.g., solubilization, inclusion compounds, and complexation. A basic disadvantage in these formulation approaches is that these can only be applied to a certain number of drugs exhibiting special features required for implementing the formulation principle (e.g., molecule fits into the cavity of the cyclodextrin ring). The use of solvent mixtures is also very limited due to toxicologic considerations. In addition, more and more newly developed drugs are poorly soluble in aqueous media and simultaneously in organic media, thus excluding the use of solvent mixtures. Ideally the formulation principle should be able to be applied to all or at least most of the poorly soluble drugs.

Solubilizers (e.g., organic solvents, detergents, and Pluronics) are often used to solubilize drugs in aqueous solution without considering their effects on biologic systems such as lipid membranes and multidrug resistance efflux transporters (e.g., P-glycoprotein or multidrug resistance gene, MDR1). Liposomal solubilization is an effective approach for the delivery of potent, insoluble drug candidates.

An alternative to other methods developed is the production of drug nanoparticles by high-pressure homogenization either as pearl milling or the continuous high-pressure homogenization. Of importance is the consideration of metallic contamination during fast speed milling processes to keep it less than 1 ppm. Drug nanoparticles are produced by dispersing the drug powder in an aqueous surfactant solution, the obtained pre-suspension is passed through a high-pressure piston-gap homogenizer, e.g., 5 to 20 homogenization cycles at typically 1000 to 1500 bars and works on the principle that cavitation occurs in the aqueous phase. The particle suspension has a very high flow velocity when passing the tiny gap of the homogenizer, the static pressure on the water decreases below the vapor pressure of water, the water starts boiling at room temperature leading to the formation of gas bubbles, at the exit of the gap the gas bubbles implode. The implosion shock waves disintegrate the drug particles to drug nanoparticles. Further improvement on nanoparticle production includes homogenization in nonaqueous phases or with reduced water content to produce more pronounced cavitation at higher temperatures. The chemical stability of drugs is less impaired when

homogenizing in nonaqueous or water-reduced media at low temperatures. The drug powder is dispersed in a nonaqueous medium (e.g., polyethylene glycol, PEG 600, Miglyol 812) or a water-reduced mixture (e.g., water–ethanol) and the pre-suspension homogenized in a piston-gap homogenizer. A suitable machine for lab scale is the Micron Lab 40 (APV Deutschland GmbH, Lübeck, Germany). Ostwald ripening occurs due to different saturation solubilities in the vicinity of very small and of larger particles. The particles produced are relatively homogeneous. The differences in the size in combination with the generally poor solubility of the drug nanoparticles are sufficiently low to avoid Ostwald ripening. Aqueous drug nanoparticle suspensions generally prove to be physically stable for several years.

The application of micronization and nanonization is increasing surface area leading to an increased dissolution rate according to the Noyes–Whitney equation. However, this is only one aspect. The dissolution pressure is a function of the curvature of the surface that is much stronger for a curved surface of nanoparticles. Below a size of approximately 1–2 μm, the dissolution pressure increases distinctly leading to an increase in saturation solubility. In addition the diffusional distance h on the surface of drug nanoparticles is decreased, thus leading to an increased concentration gradient $(C_s–C_x)/h$. The increase in surface area and increase in concentration gradient lead to a greater increase in the dissolution velocity compared to a micronized product. In addition, the saturation solubility is increased as well, even though it is a thermodynamic parameter; the increase in solubility occurs as the supersaturation stage is reached. Saturation solubility and dissolution velocity are important parameters affecting the bioavailability of orally administered drugs. From this, nanoparticles have the potential to overcome these limiting steps.

Nanoparticle-based products are likely to have some unique characteristics: general adhesiveness of nanoparticles to the gut wall, adhesion to the gut wall being a reproducible process thus minimizing variation in drug absorption, increase in dissolution velocity overcoming this rate-limiting step and additionally increase in saturation solubility leading to an increased concentration gradient between gut and blood. Orally administered drug nanoparticles can increase the bioavailability and can be the only tool available to achieve a sufficient bioavailability with poorly soluble drugs. However, the possibility of faster absorption may have its own drawbacks, both from pharmacology as well as stability in the gut. For intravenous administration, the drug nanoparticles should possess a bulk population in the nanometer range by simultaneously having a low microparticle content, i.e., especially particles larger than 5 μm which can cause capillary blockade. The homogenization process yields a product with minimized content of particles larger 1 μm. Intravenous administration of drug nanoparticles allows achievement of sufficient blood levels and finds good application in the evaluation of new compounds. In addition, toxicologically critical excipients such as Cremophor EL used in Taxol formulations can be avoided when stabilizing the drug nanoparticles with accepted emulsifiers, e.g., lecithin or Tween 80. It is interested to note that when taxol is administered with a cremophor EL, the pharmacokinetics of drug turns out to be nonlinear. For intravenous administration, a small particle size below 150 nm is only desirable in case one wants to pass fenestrated endothelia (e.g., treatment of tumors), however, this is a very limited case. More realistic and short-term achievable goals are passive targeting of drugs to treat mononuclear phagocytes (MPS) infections (i.e., targeting to the macrophages, e.g., treatment of *Mycobacterium tuberculosis* and *Mycobacterium avium* infections, especially in HIV patients). Here it is more desirable to have larger particles to ensure fast and efficient removal from the blood streams by the macrophages. Another therapeutic goal is the creation of stealth drug nanoparticles circulating in the blood, minimizing free drug concentration but simultaneously prolonging the drug release by slow dissolution. For this purpose, very small particles are not suitable because they will dissolve too fast. Another therapeutic goal is targeting to non-MPS targets, e.g., the brain and the bone marrow.

The particle size should be customized depending on the therapeutic requirements and purpose. The nanoparticle suspensions are physically stable on long term in case they are stabilized by emulsifiers/polymers in optimized composition. However, aqueous suspensions might not be the most convenient dosage form for the patient. The nanoparticle suspension can be used as granulation fluid to produce tablets or as wetting liquid for pellet production. The dispersions can also be spray-dried to be filled into hard gelatin capsules or sachets. Drug

nanoparticles produced in PEG 600 or Miglyol can directly be filled into soft gelatin capsules. Lyophilization of drug nanoparticles produced in water-reduced media can be used to produce fast dissolving delivery systems. For parenteral application, nanoparticles can be lyophilized and reconstituted prior to injection with isotonic media (e.g., water with glycerol). There are also other areas of application, e.g., ocular delivery (prolonged retention time) or topical application (increased saturation solubility leading to increased diffusion pressure into skin).

DISSOLUTION

Dissolution is the conversion of solid state (highly aggregated state) to a solution state (highly dispersed state). Some of the key factors, which affect this transition are the solubility of the drug, the diffusion process, hydrodynamic processes and possible reactivity of the solute to solvents. The dissolution rates of dosage forms are affected by additional factors that almost invariably affect bioequivalence of the drug products. In order to understand the basic factors that affect dissolution rates, it is necessary to examine various mathematical models, which describe the kinetic phenomenon of dissolution. The dissolution models are derived from the known principles of physics and chemistry such as Fick's laws of diffusion, concentration, or chemical potential gradients and the hydrodynamic principles. Since all models require simplification, sometimes oversimplification of the actual mechanism of dissolution, more often than not deviations are observed between theoretical and actual rates of dissolution, which are often corrected by introducing a variety of empirical constants.

Diffusion Model

This is the simplest model (Fig. 12) where the solvent phase in contact with the solid surface becomes saturated with the solute and if there is no turbulence in the system, the liquid at the surface remains motionless and the dissolution across the liquid is primarily the function of the diffusion of molecules across and is given by:

$$M = 2C_s ADt \tag{24}$$

where M is the amount dissolved at time t, C_s is the solubility in the medium, A is the surface area, and D is the diffusion coefficient in the medium. This is entirely a diffusion-dependent model, which does not take into consideration build up of drug concentration in the solution (sink condition) and movement of liquid past the dissolving surface. Since the concentration build-up in the bulk solvent decreases the concentration gradient, dissolution process slows down with time.

Convection Model

If the liquid is moving past the dissolving surface, convection process is set up along with the diffusion process. It should be noted that the diffusion process is taking place normal to the dissolving surface whereas the fluid flow is parallel to it as shown in Figure 12. The following equation describes the dissolution process in convection:

$$M = 0.81D^{2/3}C_s x^{1/3} bL^{2/3} t \tag{25}$$

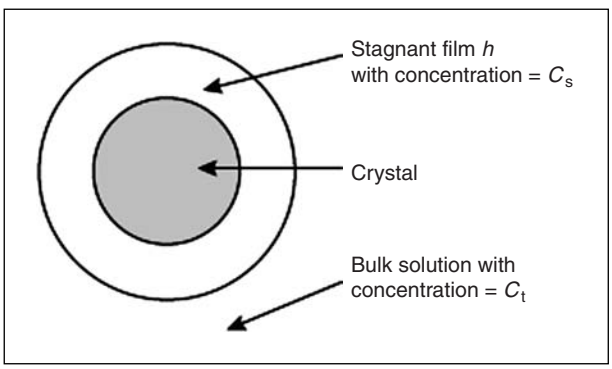

FIGURE 12 Diffusion layer model of dissolution.

where D is the rate of shear and b is the width of the dissolving surface and L is the length in the direction of flow. Unlike the previous model where a build up of concentration in the medium slows down the dissolution, in this model, a sink condition exists and no change in the dissolution is noted. The sink condition is defined as the state where C is less than 10% of the C_s. However, as the fluid flows past the surface, the dissolution rate due to diffusion slows down towards the ends of the surface due to concentration build up in the diffusion layer as shown in Figure 12.

Surface Reaction Model

The models described above assume that a saturation concentration of the drug is maintained in the diffusion layer. However, in some instances the surface reaction leading to that state may be rate limiting step and thus the dissolution rate is given by the following equation:

$$M = AC_s t/(1/K_s + h/D) \tag{26}$$

where K is the surface rate constant and h is the thickness of the hypothetical diffusion layer. The concept of having an unstirred diffusion layer in this model is often questioned on the basis of hydrodynamic theory.

Cube-Root Model

In most instances, dissolution occurs from a suspended particle wherein the total surface area changes with time; the dissolution rate for such a model is given by:

$$M_0^{1/3} - (M_0 - M)^{1/3} = Kt \tag{27}$$

where M is the mass of the powder dissolved at time t and M is the initial mass. The constant K is directly proportional to the diffusion coefficient, the solubility and the cube root of the number of particles, the particle size, and the diffusion layer thickness and is thus referred to as model-dependent constant. This model provides excellent fit for dissolution of single particles. In an actual system, a large number of particles exist with different (often log normal/ distribution) diameters, which decrease with time. These situations are also well characterized by the above model.

Tablet Dissolution Model

The dissolution of dosage forms, e.g., a tablet is preceded by its disintegration, which is given by:

$$q = \mathrm{d}m/K\mathrm{d}t + m \tag{28}$$

where q is the fraction of the tablet disintegrated and m is the fraction that has dissolved. As the disintegration rate becomes faster, the shape of the dissolution curve becomes more exponential and less sigmoidal. The fitting of dissolution data to equations is an empirical process such as used in "sigma-minus" plots where log of the amount of drug remaining in the dosage form versus time is plotted:

$$\ln(M_0 - M) = -Kt \tag{29}$$

where M_0 is the dose (either actual or the amount dissolved at infinity time … a very important factor) and K is the first-order rate constant.

Since there is often a lag time before the dissolution becomes appreciable, this function can also be introduced into the dissolution equation:

$$\ln(1 - M/M_0) = -K(t - T) \tag{30}$$

where T is the lag time. This lag time may also be associated with dosage form characteristics such as breaking down of a coating, etc. This lag function can also be introduced into the cube-root model described above [equation (27)].

Another common equation used to fit dissolution data is called Rosin-Rammler-Sperling-Weibull equation where T is lag time, and a and b are adjustable parameters, wherein a is the scale parameter and b is the shape parameter:

$$-\ln(1-M) = (t-T)b/a \tag{31}$$

The value of b determines whether the curve has sigmoidal or exponential shape. This flexibility in the use of equation allows its use in most common types of dissolution curves. The use of log-normal probability graph paper has also been made to linearize dissolution rate data.

Noyes–Whitney Model

The classic model for describing dissolution rates is given by Noyes–Whitney equation:

$$\frac{dC}{dt} = KS(C_s - C) \tag{32}$$

where dC/dt is the rate of dissolution, C_s is the saturation concentration of drug in the diffusion layer, C_t is the concentration of drug in dissolution media (or the bulk), S is the surface area of the dissolving solid, and K is the dissolution rate constant and is given by:

$$K = \frac{D}{h} \tag{33}$$

where D is the diffusion coefficient and h is the thickness of diffusion layer.

This equation is of great value in the formulation studies wherein increase in the surface area of aggregates is the most power tool to optimize dissolution. In dissolution theory, it is assumed that an aqueous diffusion layer or stagnant liquid film of thickness h exists at the surface of a solid undergoing dissolution, as observed in the following figure. This thickness h represents a stationary layer of solvent in which the solute molecules exist in concentrations from C_s to C. Beyond the static diffusion layer, at x greater than h, mixing occurs in the solution, and the drug is found at a uniform concentration, C, throughout the bulk phase (Fig. 12).

The diffusion layer model of dissolution assumes that the dissolution of drug at the solid–liquid interface into a concentrated layer surrounding the solid particle is more rapid than the diffusion of dissolved drug from that layer into the bulk solution. This diffusion is therefore rate limiting in observed dissolution. Since diffusion involves kinetic energy, it is highly dependent on the temperature. For an ideal solution, no heat is absorbed or given off upon dissolution; however, for a real solution, the heat of solution (ΔH) can be either negative (heat is given off) or positive (heat is absorbed). The mathematical relationship of solubility (C_s) to temperature is:

$$\log C_s = \left(\frac{-\Delta H}{2.303\,RT}\right) + \text{constant} \tag{34}$$

where R is the gas constant and T is the absolute temperature. A plot of log C_s versus $1/T$ gives the value of the constant. A heat effect depends on whether the material absorbs heat an endothermic process or gives off heat (an exothermic process) when it dissolves. Most materials absorb heat as they dissolve. According to the La Chatelier's principle, a system at equilibrium will adjust in such a manner so as to reduce external stress. Therefore, if a substance absorbs heat when it dissolves and heat is added to the system, equilibrium can be restored, i.e., the external stress can be reduced, by the absorption of heat. This can only be done in such a system by the dissolution of more of the substance, i.e., an increase in solubility at the higher temperature until the equilibrium is restored.

The thermodynamic driving force for dissolution is therefore the heat of solution of the substance. For a crystalline solid, this represents the difference between the heat of sublimation of the compound and the heat of hydration of the ions. The heat of sublimation is the heat required to bring ions from the solid state to the gaseous state and is a measure of the energy required to pull apart the crystalline lattice. The heat of hydration is the heat given off by the hydration of those ions. For dissolution to be an endothermic process the heat of sublimation is greater than the heat of hydration and ΔH is positive, i.e., the heat is absorbed upon dissolution;

therefore, solubility increases with an increase in temperature. If heat of sublimation is equal to the heat of hydration, solubility is independent of temperature.

Dissolution Factors

Regardless of the dissolution model chosen, fundamental considerations of the physicochemical nature of the drug significantly affect dissolution.

The Concentration Gradient

The saturation solubility of the drug in the diffusion layer determines the dissolution rate by providing the driving force for dissolution, the difference between C_s, the saturation C, the concentration of dissolved drug in the bulk fluids, e.g., in the gastrointestinal tract. The C is generally much smaller, which means dissolution occurs under sink conditions (defined accurately as condition where C_s is 0.1°C). However, if a drug is absorbed slowly the concentration in the gastrointestinal fluids may increase and thus decreasing the concentration gradient and the dissolution rate.

Additionally, the bulk fluids may not be identical to the dissolving fluids of the diffusion layer. A drug may be more soluble in the diffusion layer and then precipitate in the bulk fluids, especially if the pH differs. However, these precipitated particles are generally quite small, and redissolve rapidly. If the drug is more soluble in the bulk fluids than in the diffusion layer because of a difference in pH or due to the complexation with other components in the bulk fluid, the concentration may increase and dissolution may increase but if the solubility of the drug is lower in the bulk medium the dissolution rate will slow down or even stop. Furthermore, the volume of the bulk fluids is much larger than the volume of the diffusion layer resulting in smaller C despite dissolution of large amounts. In most instances, an increase in C that would affect dissolution rate would only occur when other processes such as membrane transport or stomach emptying becomes the rate-limiting step in drug absorption. However, in general it is advised that patients take their oral medications with a full glass of water in order to insure that the drug dissolves adequately. Another advantage in taking drugs with large volumes of fluid is that it results in greater contact with the absorption surface resulting in faster and higher absorption.

The buffering agent provides higher pH in the diffusion layer increasing the dissolution of weakly acidic drug which precipitates and redissolves in the gastrointestinal tract. In the absence of buffering agent the pH of the diffusion layer will be acidic as a result of the dissolution of weak acid.

Dissolution Constant

Although it is possible to control the dissolution rate of a drug by controlling its particle size and solubility, there is very little, if any, control over the D/h term. In this equation it is assumed that h, the thickness of the stationary diffusion layer, is independent of particle size. In fact this may not be true. The diffusion layer thickness generally increases as the particle size increases. Furthermore, h decreases as the "stirring rate" increases. Under in vivo, as gastrointestinal motility increases or decreases, h would be expected to decrease or increase correspondingly. Another assumption made here is that all the particles are spherical and of the same size, whereas in reality, the particles are polydisperse and of multiparticulate nature whose size distribution in terms of the number of particles tends to be skewed toward the smaller particles. Furthermore, as dissolution proceeds, the particles become smaller and hence, h is more a variable than a constant.

Another uncontrollable term is D, the diffusion coefficient of the drug. For a spherical molecule in solution, it is given by the following:

$$D = \frac{kT}{6nr} \tag{35}$$

where k is the Boltzmann's constant, T the absolute temperature, r the radius of molecule in solution, and n the viscosity of the solution.

The two variables in this equation are the viscosity and temperature. Increasing the viscosity of the gastrointestinal fluid will decrease dissolution and will slow gastric emptying, thus delaying delivery of the drug to the absorption site. Increasing the temperature of the gastrointestinal fluids increases diffusion and thus taking oral dosage forms with warm liquids may be advised. However, extremely hot liquids generally delay stomach emptying. Whereas D and h are regarded as constants, these may be variable under in vivo conditions.

Dissolution Testing

Ideally, dissolution should simulate in vivo conditions. To do this, it should be carried out in a large volume of dissolution medium, or there must be some mechanism whereby the dissolution medium is constantly replenished by fresh solvent. Provided this condition if met, the dissolution testing is defined as taking place under sink conditions. Conversely, if there is a concentration increase during dissolution testing, such that the dissolution is retarded by a concentration gradient, the dissolution is said to be nonsink. Whilst the use of the USP paddle dissolution apparatus is mandatory when developing a tablet, the rotating disc method has great utility with regard to preformulation studies. The intrinsic dissolution rate is the dissolution rate of the compound under the condition of constant surface area. The rationale for the use of a compressed disc of pure material is that the intrinsic tendency of the test material to dissolve can be evaluated without formulation of excipients.

The dissolution testing is performed not only on the finished products but also on the pure drug and in combination with various excipients to ascertain individual contributions of the components to overall dissolution. Basically, the dissolution test systems are of two types: the stirred-vessel type and the flow-through column. In the stirred type, agitation is provided by some kind of paddle whereas in the column type the solvent flows over the drug. A large number of variations of these systems are currently used. However, the USP apparatus is used for official certification of batches. The monographs describe the specific temperature, the dissolution medium (distilled water, simulated gastric fluid, or simulated intestinal fluid), the rotation speed of the basket (60–150 rpm), and the percentage of drug to be dissolved as an endpoint. These conditions are determined by the intrinsic properties of the drug and its dissolution behavior. The list of drugs included in official compendium where dissolution test must be conducted as requirement of dosage form release is extensive and likely to grow; some examples include: acetohexamide, nitrofurantoin, digoxin, phenylbutazone, ergotamine tartarate and caffeine tablets, prednisolone, hydrochlorothiazide, prednisone, lithium carbonate, sulfamethoxazole, meprobamate, sulfisoxazole, methaqualone, theophyllin, ephedrine, methylprednisolone, hydrochloride and phenobarbital tablets, and tolbutamide.

In the official dissolution tests, 6 or 12 tablets or capsules are tested individually for their dissolution properties. In the first stage six units are tested and each unit must fall within less than 5% of the specified limit (e.g., 60% dissolved in 30 min). If one or more units fail then another six units are tested and the average of 12 units (six from first test) should be equal to or greater than the specified percentage and no unit should be less than 15% of the specified limit. If this stage also fails then additional 12 units are tested and the average of all (now 24) should be equal to or greater than the specified limit and not more than two units can be less than 15% off the limit.

An inherent problem in this type of testing is that it requires the use of labeled amount of drug for calculation purposes and any content variability is not considered. Conceivably, large variations in the dissolution rates are possible due to these differences, e.g., tablets containing 80% to 120% of the labelled amount will require 75% to 50% dissolution if the requirement is 60%. In addition, the statistical design of the dissolution testing allows a batch with 20% defective tablets to pass 58% of the time. There are also serious problems in the reproducibility of dissolution data since the dissolution is dependent on human errors and subtle factors such as the vibrations in the room. Despite these drawbacks, FDA considers dissolution testing to be the most discriminating in vitro test with which to establish in vivo correlations.

The dissolution media comprises the fluids at the site of administration. Composition of these fluids may increase or decrease the solubility of a drug. In the case of salt, those that increase the solubility are said to "salt in" the solute, and those that decrease the solubility to

"salt out" the solute. The effect of the additive depends very much on the influence it has on the structure of the water or its ability to compete with solvent water molecules. Both effects are described by the empirically derived Setschenow equation:

$$\log \frac{S_0}{S} = kM \tag{36}$$

The above equation describes the relationship between the aqueous solubility of sparingly soluble salts (S_0) and the empirical Setschenow salting-out constant k is equal to $0.217/S_0$. This relationship and the Setschenow equation are valid only at low concentrations of added salt. As the concentration of added salt increases, the apparent k value is not constant but is dependent on solubility and the rate of change of solubility with added salt concentration. It was concluded that the Setschenow treatment is generally inappropriate for description and analysis of common ion equilibrium.

Therefore, since S_0 is assumed to remain constant and pK_a is a constant, if pH decreases, the value of S must also decrease. In a similar manner, the solubility of a weak base decreases as pH increases.

Another aspect of the effect of electrolytes on the solubility of a salt is the concept of the solubility product for poorly soluble substances. The experimental consequences of this phenomenon are that if the concentration of a common ion is high, then the other ion must become low in a saturated solution of the substance, i.e., precipitation will occur. Conversely, the effect of foreign ions on the solubility of sparingly soluble salts is just the opposite, and the solubility increases. This is called salt effect.

Since dissolution is usually an endothermic process, increasing solubility of solids with a rise in temperature is the general rule. Therefore, most graphs of solubility plotted against temperature show a continuous rise, but there are exceptions, e.g., the solubility of sodium chloride is almost invariant, whereas that for calcium hydroxide falls slightly from a solubility of 0.185 g/mL at 0°C to 0.077 g/mL at 100°C.

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8 Drug Delivery Factors

BACKGROUND

Drugs must be present in a solution form to cross biologic barriers such as the gastrointestinal mucosa. Thus the process of dissolution becomes an integral part of the various rate-limiting steps leading to a clinical response. However, dissolution alone is not sufficient to provide the absorption of drugs. The drug molecules must have the characteristics required for crossing the various lipoid layers or membranes in order to reach the general circulation. Lack of sufficient aqueous solubility is usually the rate-limiting step in the dissolution process, and lack of sufficient lipophilic properties is the usual rate-limiting step in the penetration of the lipoid barriers. Attempts to rectify dissolution problems can therefore lead to problems in membrane transport, and vice versa. A fine balance between the hydrophilic and lipophilic properties is needed to provide optimum delivery of drugs to the site of action.

The variations in bioavailability extend to almost all classes of drugs and as a result the chemical modifications required for optimum bioavailability and difficult to summarize. For example, the bioavailability of many antibiotics in the same class varies widely. Besides chemical modifications, formulation manipulations also significantly affect bioavailability. For example, almost 60-fold differences have been reported in the rates of absorption of different formulations of spironolactone. The regulatory agencies have well recognized the drugs with potential bioequivalency problems. Most of these drugs are highly potent, with log–dose response curves and some exhibiting "all or none" effects. It is important to recognize that bioavailability variations can cause significant pharmacologic response variability. For those drugs where a minimum therapeutic level (e.g., minimum inhibitor concentration of antibiotics) must be achieved, lower bioavailability may mean totally ineffective dose. It should be noted that similar variations in bioavailability result in different changes in pharmacologic response depending on the potency of drugs and the segment of log–dose response curve at which the dose is administered. For instance, doses at less steep ranges of very low or very high doses result in proportionally smaller changes in the pharmacologic responses as a result of bioavailability variations.

An identical variation in the bioavailability results in a significantly higher variation in the pharmacologic response for high potency drugs when compared with low potency drugs.

The purpose of dosage formulation is to design a dosage form with a suitable combination of the following attributes:

- Contains the labeled amount of drug in an active form.
- Is free from extraneous materials.
- Consistently delivers the drug to the general circulation at an optimum rate and to an optimum extent.
- Suitable for administration through an appropriate route.
- Acceptable to patients.

The dosage form characteristics, such as particle size, salt form, solvent type, and dissolution rate, as well as the various additives, all contribute to the dosage form design. The additives may be pharmacologically inert, as with tablet binders and lubricants, or they

may have the function of modifying the absorption, the biotransformation, or the excretion of the primary therapeutic agents.

A large number of formulation factors are common to many dosage forms and some of these are summarized here to make the reader aware of their complexity:

- The vehicle must be either miscible or spreadable throughout the biologic tissue before partitioning and absorption can take place.
- Sugars in the formulation increase viscosity delayed gastric emptying and also alter passive drug diffusion by fluid uptake and other mechanisms.
- Various buffer systems affect surface tension, pH and fluid uptake and this causes altered drug absorption.
- Surfactants affect solubility, dissolution, diffusion across lumen and gastrointestinal membrane permeability.
- Complexing agents affect solubility, partition coefficient can form nonabsorbable complexes.
- Chelating agents added to retard oxidation affect intestinal membrane permeability.
- Dyes adsorb on crystal surfaces and often retard dissolution.
- Absorbents such as kaolin, attapulgite, talc, activated charcoal, etc., reduce the rate and the extent of drug absorption.

In many instances, several different dosage forms are available for a given drug, and an appropriate selection must be made based on the attributes listed above. In general, the dissolution and hence absorption of drugs from the dosage form depends on the degree of dispersion. The following discussion attempts to characterize various dosage forms and provides a rational basis for their selection.

SOLID DOSAGE FORMS CONSIDERATIONS

Most pharmaceutical companies would rather have their new molecule enter the market as a tablet or capsule for a variety of safety, cost, and marketing considerations. As a result, almost 70% of all drugs administered today are in solid dosage forms. When so intended and the default form should be solid dosage form (unless it is predetermined in the case of therapeutic proteins or other drugs that must be administered by parenteral route or other specific routes for specificity of activity desired. The typical parameters studies for solid dosage forms relate to the ability of a powder mix, to flow well in manufacturing machines, as well as to the intrinsic characteristics that make it compressible. Some examples of properties studied include: crystal structures (polymorphs), external shapes (habits), compression properties, cohesion, powder flow, micromeritics, crystallization, yield strengths and effects of moisture and hygroscopicity, particle size, true, bulk and tapped density, surface area.

Particle Size

The particle size of new drug substance is a critical parameter as it affects every phase of formulation and its effectiveness. Appropriate particle size is required to achieve optimal dissolution rate in solid dosage forms, control sedimentation, and flocculation in suspensions, small particle size (2–5 μm) is required for inhalation therapy, content uniformity and compressibility is governed by particle size. As a result, the preformulation studies must develop a specification of particle size as early as possible in the course of studies and develop specifications that need to be adhered to throughout the studies.

Conventional methods of grinding in mortar or ball milling (where sample quantity is sufficient; generally it is not and limited to about 25–100 mg) or micronization techniques are used to reduce the particle size. The method used can have significant effect on the crystallinity, polymorphic structures (often to amorphous forms) and drug substance stability that can range from discoloration to significant chemical degradation. Changes in polymorphic forms can be determined by performing X-ray powder diffraction (XRPD) before and after milling.

Micronization where possible allows increase in the surface area to the maximum which can impact on the solubility, dissolution and as a result, bioavailability. Since the aim of most preformulation studies is to determine if a solid dosage form can be administered, knowing that reduction of particle size where it changes dissolution rates can be pivotal in decision making for the selection of dosage forms. In the process of micronization, the drug substance is fed into a confined circular chamber where it is suspended in a high-velocity stream of air. Interparticulate collisions result in a size reduction. Smaller particles are removed from the chamber by the escaping air stream toward the center of the mill where they are discharged and collected. Larger particles recirculate until their particle size is reduced. Micronized particles are typically less than 10 μm in diameter. In some instances micronization can prove counter-productive, where it results in increased aggregation (leading to reduced surface area) or alteration of crystallinity, which must be studied using such methods as microcalorimetry, dynamic vapor sorption (DVS) or inverse gas chromatography.

The introduction of DVS in 1994 revolutionized the world of gravimetric moisture sorption measurement, bringing outdated, time and labor intensive desiccator use into the modern world of cutting-edge instrumentation and overnight vapor sorption isotherms. With a resolution down to 0.1 μg, 1% change in mass of a 10 mg sample on exposure to the humidity controlled gas flow is both easily discernable and reproducible. DVS is a valued tool for studies related to polymorphism, compound stability, bulk and surface adsorption effects of water and organic vapors. The DVS studies would typically show percent mass increases but often a hysteris loop relationship is observed where there is crystallization of compound that results in the expelling of excess moisture. This effect can be important in some formulations, such as dry powder inhaler devices since it can cause agglomeration of the powders and variable flow properties. The DVS is useful study when amorphous forms are involved upon size reduction; in many cases, a low level of amorphous character cannot be detected by techniques such as XRPD; microcalorimetry can detect less than 10% amorphous content (the limit of detection is 1% or less). The amorphous content of a micronized drug can be determined by measuring the heat output caused by the water vapor inducing crystallization of the amorphous regions.

Surface Area

Since the surface area exposed to the site of administration determines how fast a particle dissolves in accordance with the Noyes–Whitney equation, these determinations are important. In addition, in those instances where the particle size is difficult to measure, a gross estimation of surface area is the second best parameter to have to characterize the drug. The most common methods of surface area measurement including gas adsorption (nitrogen or krypton) based on what is most commonly described as the Braunauer, Emmet, and Teller, or BET, method applied either as a multipoint or single-point determination.

Porosity

Most solid powders contain a certain void volume of empty space. This is distributed within the solid mass in the form of pores, cavities, and cracks of various shapes and sizes. The total sum of the void volume is called the porosity. Porosity strongly determines important physical properties of materials such as durability, mechanical strength, permeability, adsorption properties, etc. The knowledge of pore structure is an important step in characterizing materials, predicting their behavior.

There are two main and important typologies of pores: closed and open pores. Closed pores are completely isolated from the external surface, not allowing the access of external fluids in neither liquid nor gaseous phase. Closed pores influence parameters like density, mechanical, and thermal properties. Open pores are connected to the external surface and are therefore accessible to fluids, depending on the pore nature/size and the nature of fluid. Open pores can be further divided in dead-end or interconnected pores. Further classification is related to the pore shape, whenever is possible to determine it. The characterization of solids in terms of porosity consists in determining the following parameters.

Pore Size

Pore dimensions cover a very wide range. Pores are classified according to three main groups depending on the access size:

- Micropores: less than 2 nm diameter
- Mesopores: between 2 and 50 nm diameter
- Macropores: larger than 50 nm diameter.

Specific Pore Volume and Porosity

The internal void space in a porous material can be measured. It is generally expressed as a void volume (in cc or mL) divided by a mass unit (g).

Pore Size Distribution

It is generally represented as the relative abundance of the pore volume (as a percentage or a derivative) as a function of the pore size.

Bulk Density

Bulk density (or envelope density) is calculated by the ratio between the dry sample mass and the external sample volume.

Percentage Porosity

The percentage porosity is represented by ratio between the total pore volume and the external (envelope) sample volume multiplied by 100.

Surface Area

See above for discussion.

True Density

Density is the ratio of the mass of an object to its volume and for solids this term describes the arrangement of molecules. The study of compaction of powders is described by the Heckel equation:

$$\ln\left[\frac{1}{1-D}\right] = KP + A \tag{1}$$

where D is the relative density, which is the ratio of the apparent density to the true density, K is determined from the linear portion of the Heckel plot and P is the pressure. The densities of molecular crystals can be increased by compression. Information about the true density of a powder can be used to predict whether a compound will cream or sediment in a suspension such as metered dose inhaler (MDI) formulation. Therefore, suspensions of compounds that have a true density less than these figures will cream (rise to the surface), and those that are denser will sediment. It should be noted, however, that the physical stability of a suspension is not merely a function of the true density of the material. The true density is thus a property of the material and is independent of the method of determination. In this respect, the determination of the true density can be determined using three methods: displacement of a liquid, displacement of a gas (pycnometry) or floatation in a liquid. The liquid displacement is tedious and tends to underestimate the true density, displacement of a gas is more accurate but needs relatively expensive instrumentation. As an alternative, the floatation method is simple to use and inexpensive.

FLOW AND COMPACTION OF POWDERS

The flow properties of a powder will determine the nature and quantity of excipients needed to prepare a compressed or powder dosage form. This refers mainly to factors such as ability

to process the powder through machines. To make a quick evaluation, the compound is compressed using an infrared (IR) press and die under 10 tons of pressure with variable dwell times, and the resulting tablets are tested with regard to their crushing strength after storing the tablets for about 24 hours. If longer dwell times result in higher crushing strength then the material is likely plastic; elastic material will show capping at low dwell times; the brittle material will not show any effect of dwell times. It is recommended that the compressed tablets be subject to XRPD to record any changes in the polymorphic forms.

Electrostaticity

When subjected to attrition, powders can acquire an electrostatic charge, the intensity of which is often proportional to physical force applied as static electrification of two dissimilar materials occurs by the making and breaking of surface contacts (triboelectrification or friction electrification). Electrostatic charges are often used to induce adhesive character to bind drugs to carrier systems, e.g., glass beads coated with hydroxypropylmethyl cellulose containing drugs. The net charge on a powder may be either electropositive or electronegative depending on the direction of electron transfer. The mass charge density can vary from 10^{-5} to 100 μC/kg depending on the stress, ranging from gentle sieving to micronization process. This can be determined using electric detectors to determine polarity as well as the electrostatic field. The electrostaticity results in significant changes in the powder flow properties.

Caking

Powders cake due to agglomeration as a result of such factors as: static electricity, hygroscopicity, particle size, impurities of the powder and, storage conditions, stress temperature, relative humidity (RH) and storage time, etc. The mechanisms involved in caking are based on the formation of five types of interparticle bonds such as bonding resulting from mechanical tangling, bonding resulting from steric effects, bonds via static electricity, bonds due to free liquid, and bonds due to solid bridges. During the process of micronization, the formation of localized amorphous zones can lead to caking as these zones are more reactive to factors described above specially when exposed to moisture; the mechanisms involve moisture sorption due to surface sintering and recrystallization at well below the critical RH. In most instances increase in RH begin to show some impact at values above 20% resulting in most dramatic effects above 75% to 80% RH for powders that are subject to humidity effects.

Polymorphism

Because polymorphism can have an effect on so many aspects of drug development, it is important to fix the polymorph (usually the stable form) as early as possible in the development cycle. Whereas it is not necessary to create additional solid state forms by techniques or conditions unrelated to the synthetic process for the purpose of clinical trials, regulatory submission of a thorough study of the effects of solvent, temperature and possibly pressure on the stability of the solid state forms is advised. A conclusion that polymorphism does not occur with a compound must be substantiated by crystallization experiments from a range of solvents. This should also include solvents that may be involved in the manufacture of the drug product, e.g., during granulation.

Powders

The formulation and bioavailability problems associated with suspensions are also characteristic of powders, whereby the active ingredient is mixed with inert diluents and administered either directly or in a capsulated form. An additional problem therefore arises due to possible adsorption of drugs onto diluents, from which the drug may not be released quickly enough for adequate absorption. For example, only 40% of thiamine and 79% of riboflavin are available for absorption from capsules containing Fuller's earth, which adsorbs these drugs. Similarly, calcium phosphate used as a diluent in tetracycline capsules reduces absorption by the formation of insoluble complexes.

The particle size of powders is significant in their dissolution and bioavailability, as demonstrated by spironolactone and griseofulvin, the micronization of which leads to significantly higher absorption in humans. However, smaller particle powders have a greater tendency to adsorb moisture from the atmosphere, which results in possibly unstable preparations. Smaller particle size also means increased electrostatic charges on the particle surface, especially with hydrophobic drugs. This might result in aggregation and the consequent loss of an effective or exposed surface area for dissolution.

An example in which smaller particle size is not always desirable, even though it does increase bioavailability, is in the use of nitrofurantoin. The use of a larger particle size is recommended to avoid the gastrointestinal irritation and accompanying nausea which occurs very frequently with the use of fine particles in oral dosage forms.

When powders are administered in a gelatin capsule, the capsule shell itself may affect the absorption process. Hard gelatin capsules dissolve more readily in the gastrointestinal fluids than soft gelatin capsules. For example, the slow absorption of vitamin B from soft capsules may be attributed to the slow dissolution of capsules themselves. However, a recent study showed that soft capsules might produce an unexpected increase in the absorption of digoxin, with which absorption rates even higher than the solution dosage forms were obtained. This finding was attributed to the possible interaction of digoxin with the soft elastic capsule walls and also to the protection of digoxin against possible chemical decomposition from gastrointestinal fluids.

A large number of drugs are administered in powder form, such as iodochlorhydroxyquin and methylbenzethonium chloride, or contained in a capsule, as are phenytoin, chloramphenicol, erythromycin, tetracycline, lithium carbonate, quinine sulfate, chlordiazepoxide hydrochloride, cephalexin, and propoxyphene.

Tablets

Whereas solutions represent a state of maximum dispersion, compressed tablets have the closest proximity of particles. Complexities in dissolution and bioavailability are generally inversely proportional to the degree of dispersion-compressed tablets are thus most prone to bioavailability problems. This is primarily due to the smaller surface area exposed for dissolution until the tablets break down into smaller particles. Factors responsible for the primary break down of tablets into granules and their subsequent breakdown into finer particles include such parameters as the concentrations of binder, disintegrant, and lubricant; the hydrophobicity of the drug and the adjuvants; therefore, it can be expected that a significant difference is always possible in the dissolution and bioavailability of various tablets.

The problem of tablet disintegration is well demonstrated by such drugs as dipyridamole, thioridazine, and digoxin, which exhibit higher blood levels if the tablets are crushed before administration.

The disintegration test for tablets has long been used to detect ineffective products, as determined by a lack of disintegration into large particles within a given period of time. This test allows monitoring of batch-to-batch variations in the manufacturing process. However, adequate disintegration alone does not assure ultimate dissolution, which may be retarded by the absorption of drug on hydrophobic lubricants in the formulation, the recrystallization of drugs, the presence of large primary granules, and the failure of these granules to break down further into finer particles. The importance of using smaller particles in tablet formulations is well demonstrated in the use of griseofulvin, with which the reduction of particle size has been consistently related to bioavailability. Recently, a solid dispersion of griseofulvin was formulated which contained ultramicrosize particles of the drug, resulting in an almost 100% improvement in its bioavailability compared to the micronized forms.

The coatings of tablets, which are applied for a variety of reasons, add another rate-limiting factor, since a coating must dissolve or disrupt before the tablet can disintegrate and the dissolution process begins. The sugar coating used to mask unpleasant taste, appearance, and odor, or to protect a tablet ingredient from decomposition during storage, consists of an application of poorly soluble polymers which can interfere with the disintegration of tablets.

Film coatings are generally less problematic, but enteric coatings used to protect both the gastric mucosa from the drugs and the drugs from the gastric fluids give the most variable bioavailability, since their disintegration is often dependent on gastrointestinal pH and other highly variable physiologic and physicochemical factors.

Solutions

Solutions are thermodynamically stable monomolecular dispersions of drug molecules in a liquid or solid phase. Absorption from aqueous solutions is generally very fast and complete from all sites of administration, provided that penetration through the absorption barrier (such as the gastrointestinal membrane) is not a rate-limiting factor. The rate-limiting steps as disintegration and dissolution are minimal in the use of solutions. For example, potassium penicillin V gives higher blood levels than benzathine penicillin V when both are administered orally in tablet form, but solutions of the two drugs yield essentially equal blood levels of penicillin.

Besides providing the highest bioavailability, solutions are also convenient for administration to pediatric and geriatric patients. In some instances the use of solutions is a crucial part of the drug delivery. For example, calcium must be administered as a solution in its citrate form to achlorhydric patients, since the solid carbonate form will not dissolve sufficiently in the gastrointestinal tract without the presence of hydrochloric acid. An analogous problem exists in the administration of sodium salts of weakly acidic drugs, which precipitate in the stomach in crystalline form. These crystals are usually very fine and redissolve quickly, but there is always a possibility of retarded absorption due either to precipitation as large particles or to coating of the particles with hydrophobic acid, as demonstrated with such poorly water soluble drugs as warfarin and phenytoin. These drugs can therefore be absorbed better from a suspension dosage form than from a solution of their sodium salts.

Quite often, solutions of poorly water-soluble drugs are affected by adding cosolvents, such as alcohol or propylene glycol, and by adding complexing agents, which form a water-soluble complex with the drug or with surfactants that solubilize the drugs. In all of these instances the drugs will precipitate because of the dilution effect and are subject to possible difficulty in redissolution.

Sometimes nonaqueous solutions provide better absorption than aqueous solutions, as demonstrated by indoxole. A solution of indoxole in oil, administered as an oil-in-water emulsion shows three times better absorption than the aqueous solution.

The use of solid solutions is a novel application of dispersion techniques, whereby the drug is dispersed in a solid water-soluble vehicle, such as urea, succinic acid, or polyethylene glycols, which dissolves rapidly in water, releasing the macrocrystalline or monomoleclar form of the drug. Although there is a large volume of data about the applications of the principles of solid dispersions and solutions, only one product is currently available which utilizes this concept, i.e., Gris-PEG, a dispersion of griseofulvin in polyethylene glycol.

Solution dosage forms offer several advantages particularly the resolution of bioavailability problems, instant administration as injectable forms (though nonsolution forms are also given parenterally). At the preformulation stage more important factors are the solubility (and any pH dependence) and stability or the new compound.

Solubility

Where a solution form is desired and the compound has low solubility, there are several techniques, some very simple to some very complex, to achieve the desirable property of the lead drug including pH manipulation, use of cosolvents, surfactants, emulsion formation and adding complexing agents. On a more complex stage, the liposomes or similar drug delivery systems can be used.

Since many compounds are weak acids or bases, their solubility will then be a function of pH. However, ionic strength of medium plays a significant role and as a result most paretneral formulations are buffered to prevent crystallization of drugs.

The use of cosolvents improves solubility as a result of the polarity of the cosolvent mixture being closer to the drug than it is in water:

$$\log S_m = f \log S_c + (1-f) \log S_w \quad (2)$$

where S_m, the solubility of the compound in the solvent mix; S_w, solubility in water; S_c is the solubility of the compound in pure cosolvent; f, the volume fraction of cosolvent; and σ, the slope of the plot of log (S_m/S_w) versus f. There is a definite correlation between the S value to indices of cosolvent polarity such as the dielectric constant, solubility parameter, surface tension, interfacial tension and octanol–water partition coefficient. The aprotic cosolvents give a much higher degree of solubility than the amphiprotic cosolvents. This means that if a cosolvent can donate a hydrogen bond, it may be an important factor in determining whether it is a good cosolvent. Use of cosolvents with polar drugs can reduce the solubility.

Emulsion Formulations

For drugs with poor water solubility, and emulsion formulation such as oil-in-water (o/w) where the drug has good partitioning in the oil phase chosen, offers often an excellent choice. The particle size of the emulsion and its stability (physical and chemical) then become significant factors since larger globule sizes may lead to phlebitis. To achieve smaller particle size the technique of microfluidization is often used among other such homogenization available methods. Phospholipids added stabilize emulsions through surface charge changes as well as providing a good mechanical barrier.

Many drugs show surface active behavior because they have the correct mix of chemical groups that are typical of surfactants. The surface activity of drugs can be important if they show a tendency to, e.g., adhere a surfaces, or if solutions foam. Not all surface active drugs form micelle because of steric hindrances.

Suspensions

Where the drug has limitations in its solubility and efforts to enhance fail, where there is a tendency for fast crystallization from solutions or even where chemical stability is a problem, often formulating suspension dosage forms obviates some of these drawbacks. However, suspensions, by nature, must have higher viscosity to prevent settling of particles and thus create problems in pourability, syringability, etc. Appropriate selection of a vehicle that provides an ideal compromise among all characteristics thus becomes a critical factor because the intent is to have as little solubility in the vehicle as possible to prevent crystallization from the solution that surrounds the suspended particles; as a result, weak acids and bases appear as poor choice for suspension formulation. In some instances it may be possible to prepare a derivative with larger hydrophobic groups or salt formation that would have lower solubility if preparing a suspension dosage form is particularly desired. Compounds that can form hydrates while in suspension state can create stability problem. A significant thermodynamic problem in suspension formulation comes from Ostwald ripening, crystal growth, not due to phase change but as a result of differences in the solubility as a function of crystal size:

$$\frac{RT}{M} \ln\left(\frac{S_2}{S_1}\right) = \frac{2\sigma}{\rho}\left(\frac{1}{r1} - \frac{1}{r2}\right) \quad (3)$$

where R is the gas constant, T is the absolute temperature, S_1 and S_2 are the solubilities of crystals of radii $r1$ and $r2$ respectively, σ is the specific surface energy, ρ is the density and M is the molecular weight of the solute molecules. Temperature fluctuations are obviously one factor that promotes Ostwald ripening. Whereas phase changes can be studied using such standard techniques as DSC, hot stage microscopy or XRPD, Ostwald ripening is best studied using microscopic methods. The art of suspension formulation is complex as a large number of factors including additives can have a significant influence on crystal growth; for example, dyes molecules often attach to high-energy points on crystals affecting their growth; similarly it is reported that PVP, a common ingredient of many suspension formulations inhibits crystal growth. Albumin is also known to have similar impact. The choice of additives is also governed

by the final form of suspension; if it has to be sterilized, the additives must be able to sustain autoclave temperatures; besides, autoclaving itself can affect both physical and chemical stability of the drug. Zeta potential measurements of suspensions often prove useful.

Suspensions require the dissolution of particles before they can be absorbed. The dissolution process can be rate limiting, depending on the aqueous solubility of the drug and the formulation additives involved. Thus there are many more factors that can affect drug absorption in the use of suspensions than are possible in the use of solutions. Generally, however, suspensions will provide better absorption than such other dosage forms as capsules and tablets such as shown by trimethoprim and sulfamethoxazole combinations and sulfadimethoxine. Suspensions are also used when a slow release of the drug is desired, as with intramuscular administration of triaminocolone acetonide or with tetracycline ophthalmic suspensions. Since suspensions provide a large surface area, various antacid products are most effectively administered as suspensions, since the mode of action involves both the chemical neutralization of hydrochloric acid and its physical adsorption onto the suspended particles. It is interesting to note that a majority of official oral suspensions in current use involve antiinfective agents, e.g., pyrantel pamoate, pyrvinium pamoate, thiabenzadole, chloramphenicol palmitate, democlocycline, methacycline, oxytetracycline, penicillin, tetracycline, methenamine mandelate, nitrofurantoin, sulfonamides, trisulfapyrimidines, nystatin, etc. Most of the anti-infective agents are chemically unstable, can cause gastrointestinal irritation, and are often erratically absorbed from such solid dosage forms as tablets and capsules. The use of suspensions for these drugs provides an ideal mechanism for solving formulation problems related to these attributes. Consider a drug with a decomposition constant of 0.21 hours and aqueous solubility of 4 g/L. A 500 mg dose in aqueous solution will have a shelf life (10% decomposition) of 0.53 hours, whereas 500 mg suspended in 10 mL of saturated solution will have a shelf life of 6.25 hours, indicating a more than 1000% increase in drug stability.

The use of suspensions is also advantageous in pediatric or geriatric practice, where they can be accurately and conveniently administered using droppers, or oral syringes. Suspension dosage forms are utilized for all routes of administration except intravascular.

Controlled-Release Dosage Forms

Unless specific formulation efforts are made to control the release of drugs, the rates of drug absorption are generally proportional to the amount of drug at the site of absorption. In many instances it is necessary to prolong the action of drugs by sustaining their absorption over a longer period of time.

The design of oral prolonged-action dosage forms includes such modifications as:

- Barrier coating, whereby the drug diffuses out through a membrane within which it may be dissolved by the penetrating gastrointestinal fluids.
- Fat embedment, which involves suspending the drug in a fatty medium in a solid dosage form from which the drug is released by erosion, hydrolysis of fat, and direct dissolution.
- Plastic matrices, which allow leaching and diffusion of drugs from a solid plastic matrix which is left intact after the drug has been released.
- Repeat action tablets, utilizing a double coating which releases an initial dose followed by another dose released either instantaneously or by slow diffusion.
- Ion-exchange resins, which provide prolonged dissolution by the formation of drug salts with resins, which then react with either hydrochloric acid in the stomach or sodium chloride in the intestine to exchange the drug.
- Hydrophilic matrices, utilizing hydrophilic gums for compression into tablets which undergo gelatin and release the drug by diffusion.
- Polymer resin beads, in which the drug is first dissolved or suspended in plastic monomers and then polymerized. The beads are then either filled into a capsule or compressed into tablets. Drug release is controlled by the dissolution and swelling of the resin and the diffusion of drug from the beads.

- Soft gelatin depot capsules involve the dissolution or suspension of drugs in sponge-forming solutions and consequent filling into capsules which leave a solid skeleton upon diffusion of the drugs.
- Drug complexes utilizing macromolecules provide prolonged release upon the hydrolysis of the complex.

The release of drugs administered parenterally can also be controlled by the following methods:

- Pharmacologic methods: Intramuscular or subcutaneous administration instead of intravenous. Simultaneous administration of vasoconstrictors (adrenalin in local anesthetics, ephedrine in heparin solution), blocking elimination of drugs through the kidney by simultaneous administration of a blocking agent such as probenecid with penicillin or *p*-aminosalicylic acid.
- Chemical methods: Use of salts, esters, ethers, and complexes of the active ingredient with low solubility.
- Physical methods: Selection of a proper vehicle giving prolonged release, as with the use of oleaginous solutions instead of aqueous solution; the addition of macromolecules which increase the viscosity, such as carboxymethylcellulose, tragacanth, etc.; the use of swelling material to increase the viscosity of oleaginous solutions, as with aluminum monstearate; the addition of absorbents; the use of a solution from which the drug is precipitated upon contact with body fluids; the use of aqueous and oleaginous suspensions; and the use of implants.

Therapeutic Systems

Several dosage forms, termed Therapeutic Systems, have recently been marketed in this county. The Therapeutic System is a dosage form that provides preprogrammed, unattended delivery of drugs at a rate, and for a given time period, designed to meet a specific therapeutic need. These systems have been developed for introducing drug substances both via the systemic circulation and directly to specific target organs. Many new drug delivery techniques have been developed, including:

- Diffusion of drugs through rate controlling membranes.
- Osmotic pumping.
- Biodegradable polymer matrices.
- Polymer-bound active species.
- Nanosystems.

The Therapeutic Systems are composed of an active drug in a delivery module, which consists of a drug reservoir, which may be a single or multicompartment element; a rate controller; and an energy source to effect the release of the drug molecules through a delivery portal. The drug delivery module is housed in a "platform" which is compatible with the tissues and couples the system to the body site in which it is deployed. The platform may be either fixed or mobile within a defined area. Some examples include the ocular platform, which is designed so that it can float comfortably and inconspicuously in the tear film on the eye beneath the eyelid for controlled delivery of (Occusert); and the T-shaped progesterone impregnated polymer unit for intrauterine deployment for fertility control (Progestasert).

The osmotic drug delivery system resembles an ordinary tablet in appearance and is comprised a solid core of drug surrounded by a semipermeable membrane with a single minute orifice. The membrane allows steady entry of water at a predetermined rate to dissolve the drug. Drug solution is then continuously pumped through the orifice, providing a constant rate of release.

Other novel ideas include a transdermal therapeutic system consisting of a disc 0.2 mm thick and 2 cm in diameter, which is worn behind the ear like a tiny adhesive bandage and

releases scopolamine for its antiemetic properties and the use of nitroglycerin patches for angina pectoris. The use of biodegradable polymers has also been suggested for implant systems for the controlled release of drugs.

The foregoing innovations are cited here to make the reader aware of the possibilities of bioavailability variation as a result of a large number of physicochemical and technologic implementations in the design of dosage forms. The complexities in the design of dosage forms necessitate the development of an elaborate system to evaluate dosage forms and systems on the basis of the attributes listed at the beginning of this chapter.

Evaluation of Drug Delivery Systems

It is not possible to predict if the administered will result in a consistent desirable therapeutic response. However, several tests can be conducted to assure some measure of reliability in dosage form functions. These include the following.

Chemical Content

It is essential that dosage forms contain the labeled amount of the active drug. Chemicals which are biologically active are also highly chemically reactive and can therefore undergo chemical decomposition reactions which result in a loss of content. For example, aspirin decomposes to salicylic acid and acetic acid. Salicylic acid is undesirable because it causes more gastrointestinal irritation than aspirin and also because it may not possess a therapeutic activity equivalent to aspirin. *Para*-aminosalicylic acid decomposes via decarboxylation to meta-amino phenol, resulting in discoloration and enhanced toxicity. Tetracycline converts to epianhydrotetracycline, which is highly toxic to the kidneys.

Although not all chemical decomposition reactions result in a toxic product, a change in the color or the consistency of a preparation will quite often make it unacceptable to the patient. It is therefore necessary to provide a shelf life or expiration date on the products. A three to five year expiration date is rather common for a relatively stable product, and sometimes a shelf life of only a few days or weeks is assigned to highly reactive drugs or radio-labeled compounds.

In order to account for the loss of drug during shelf life, overage additions are often made at the time of manufacture. This overage addition is necessary to compensate for the high reactivity of the active components. However, large overage additions cannot be allowed for drugs used internally or for those for which a narrow plasma concentration fluctuation has to be maintained, as with cardiac glycosides and anticoagulants, which have narrow therapeutic indices.

Chemical decomposition is not the only way in which active ingredients are lost from dosage forms. For some compounds with a low boiling point the active principal can be lost by evaporation or volatilization. For instance, nitroglycerin tablets, if dispensed in a plastic container, have been reported to lose up to 80% of their active components in two years. Nitroglycerin tablets are therefore required by law to be dispensed in the original glass container and even then there can be a significant drug loss within the unopened container.

The container also plays an important role in determining dosage form effectiveness. For example, the increasing use of plastic large-volume parenteral containers has created an unanticipated problem of loss of drugs due to absorption onto the plastic and absorption through it, resulting in a significant loss of drugs such as vitamin A.

Chemical content evaluations are therefore fundamental in determining dosage form effectiveness. Hundreds of drugs have been recalled by the Food and Drug Administration (FDA) due to subpotent or in some instances superpotent products, making potency one of the primary criteria in the evaluation of dosage forms.

Content Uniformity

The chemical equivalence testing described above is generally performed on a large number of dosage form units (e.g., 20 tablets) at one time. This testing determines the average amount of active ingredients(s). It will not, however, reveal variations in drug content among the units. For example, the oral contraceptive Ortho-Novum 1/50 contains 1 mg of norethindrone and 0.05 mg of mestranol per tablet. What if one tablet contains 0.1 mg of mestranol while another

TABLE 1 Selected Examples of Tablets Containing Small Amounts of Active Drug Component Drug Available Tablet Strength (mg)

Atroping sulfate	0.3
Colchicine	0.5
Dexamethasone	0.25
Diethylstilbesterol	0.1
Ethinyl estradiol	0.05
Digitoxin	0.05
Digoxin	0.125
Reserpine	0.1

tablet contains none? Although the two tablets combines will pass the chemical equivalence test, a course of therapy with tablets of this quality might result in an unanticipated pregnancy. The problem of content uniformity, therefore, exists for all products containing minute amounts of active ingredients, as is shown in Table 1.

The problems of content uniformity arise mainly from the mixing of small amounts of drugs into large batches where a uniform distribution must be assured. Again, the FDA has recalled many products in the last few years due to noncompliance with the United States Pharmacopoeia (USP) content uniformity requirements of 5%.

Presence of Contaminants

Contaminant is defined as any undesirable substances contained in a formulation. Contamination of the drug product may occur during processing from impurities in raw materials, heavy metal ions from manufacturing equipment, microorganisms, or chemical decomposition products, which may be toxic as noted above or inactive, as the product of reaction between isoproterenol and bisulfite preservatives. Another source of contamination is dust spreading during the manufacturing process, when several products are handled simultaneously in a manufacturing facility. Although the presence of contaminants may not always be deleterious, it is always desirable to have as few as possible to prevent changes in the physical or esthetic appearance of a product as well as unanticipated adverse reactions.

Disintegration Test

The disintegration test ascertains the time required for a compressed tablet to break up into granules. The first official disintegration test was included in pharmacopeia Helvetica in 1934. Since then most official pharmacopeias have included these test to formulate a basis for prediction of the availability of drugs from dosage forms. Up until 1950s, disintegration was the key word and any dosage form that disintegrated within a prescribed time was assumed to provide adequate bioavailability.

A large number of formulation factors can affect the rate of tablet or capsule disintegration, including.

Diluents or Fillers

Manufacturing methods, such as dry or wet granulation, etc.
Compression pressure in capsulation.

Hardness

1. Concentration of disintegrant and the method of its addition
2. Types and concentrations of lubricants, surfactants, and binders
3. Drug properties such as particle size, surface characteristics, solubility, and crystallinity
4. Composition and properties of capsule shell
5. Type and composition of coating
6. Age of finished product and storage conditions.

The USP disintegration method involves a basket-rack assembly which is moved up and down 30 times a minute. At specific times, the number of tablets or capsules disintegrated is

determined. The disintegration time allowed varies from five minutes to one hour. For example, aspirin tablets have a time limit of five minutes.

The present USP and National Formulary disintegration tests measure only the physical break-up of the tablet or capsule, which may not necessarily correlate with drug bioavailability. In order for a drug to be absorbed, it must be present in a solution form. It is possible that the particles from disintegrated tablets might not further disintegrate or dissolve and thus no bioavailability assurance can be obtained from formulations meeting only the official disintegration tests.

Dissolution Test

A dissolution is much more discriminating than the disintegration test. It is a better estimate of bioavailability, though it is still not fool proof. Dissolution rate test can be used to predict bioavailability if these two conditions are met:

- The dissolved drug remains free and intact in the gastrointestinal tract. If the dissolved drug complexes with a component of the gastrointestinal tract, and if drug decomposition occurs in the gastrointestinal tract then the dissolution test cannot be a very good index of bioavailability.
- Absorption is not the rate-limiting step. If the solution formed is quickly absorbed, then the amount absorbed can be correlated with the in vitro dissolution rate. However, when absorption is slow or limited, bioavailability may not be proportional to the dissolution rate.

The formulation factors listed as affecting the disintegration rates also affect the dissolution rates. A large volume of data has been reported which correlates various formulation factors and the dissolution rates. For example, the particle size of a drug is most clearly related to the dissolution rate. Addition of surfactants quite often substantially increases the dissolution rates of hydrophobic drugs by the removal of air pockets around the particles, thus facilitating the contact of the dissolution medium with the drug. An important source of surface activity is the gastric fluid, where the surface tension varies between 38 and 52 dynes/cm. This lower surface tension allows better wetting of particles and promotes dissolution. The primary cause of surface activity in the gastric fluids is the reflux of intestinal contents into the stomach. The intestinal fluids have significant surface activity, as may be expected because of the lecithins, bile salts, etc.

The fillers and diluents used in a formulation have a significant effect on its dissolution. If the drug is hydrophobic, a hydrophilic filler will tend to enhance dissolution, especially if this filler is at the same time a disintegrant. Starch has hydrophilic properties and is an effective disintegrant and thus proves to be an excellent filler.

The lubricants used may have varying effects. If the granule particles are hydrophilic and disintegrate quickly, a surface active lubricant will have little effect. If the granule particles are less hydrophilic and do not disintegrate as quickly, a surface active lubricant may enhance dissolution. The use of such hydrophobic lubricants as stearates decreases dissolution rates, but this effect is minimal if their concentration is less than l%.

The effect of compression pressure on dissolution rates is the most difficult to predict. Dissolution rates will generally decrease with increasing compression pressure due to a closer binding of the granules to each other. At higher pressure a crushing of the granules and perhaps even of the drug crystals would occur, resulting in an increased surface area and an increased dissolution rate. A further increase in pressure may make the bonding more important than the crushing, resulting in a decrease in the dissolution rates. Where the bonding is not significant, a direct increase in the dissolution rates can be expected with increasing compression pressure at higher pressures.

The effect of tablet storage on the dissolution rate can also be important and reports have been made suggesting both increasing and decreasing dissolution rates.

In view of the importance of dissolution tests in predicting drug bioavailability, the official compendia continues to require dissolution test as part of the regulatory requirements,

such as for: acetohexamide, nitrofurantoin, digoxin, phenylbutazone, ergotamine tartarate and caffeine tablets, Prednisolone, Hydrochlorothiazide, Prednisone, Lithium carbonate, sulfamethoxazole, meprobamate, sulfisoxazole, methaqualone, theophylline, ephedrine hydrochloride and phenobarbital tablets, methylprednisolone, tolbutamide. Appendix 3 lists the dissolution conditions for various approved drugs.

In those instances where a relatively insoluble drug is given orally, the role of dissolution rates can be ascertained from the blood levels achieved as a function of dose.

Absorption Principles

When a drug is introduced into the gastrointestinal tract and is present in a forms which can be absorbed, the process of absorption may be categorized as either passive diffusion or active transport.

Passive Diffusion

This process describes the movement of drug moleculcs from a region of high-relative concentration to a region of lower relative concentration. It also includes the movement of ions from a region of high-ionic charge of one type to a region of lower charge of the same type or of opposite charge:

$$\frac{dX_a}{dt} = -DA(C_{gut} - C) \tag{4}$$

where X_a, amount of drug at the absorption site; D, diffusion coefficient; A, area of absorption surface; C_{gut}, concentration of drug in the gastrointestinal tract; C, concentration of drug in the plasma.

The driving force for passive diffusion is the concentration or the electrical gradient across the membrane which separates the gastrointestinal lumen from the circulating blood. The concentration gradient is, however, more appropriately viewed as the chemical potential, represented by the number of molecules or ions which are free to move across a membrane and not by the total concentration in the lumen or plasma. In many instances, the plasma concentration is much lower than the concentration in the gastrointestinal tract due to the rapid removal of the absorbed drug by the circulating blood, making the rate of transport across the membrane proportional to the chemical potential only in the gastrointestinal tract.

Apart from the concentration gradient, diffusion rates depend also on the permeability characteristics of the membrane. The gastrointestinal membrane acts like lipid barrier which permits the passage of lipid-soluble drugs, but across which lipid-insoluble but water-soluble molecules pass with difficulty; some of them may pass across the membrane through numerous pores which are too small to be seen even with the aid of an electron microscope, but for which strong evidence exists.

Active Transport

Active transport is a specialized process which requires the expenditure of energy. The various active transport processes found in the gastrointestinal tract are relatively structure-specific and serve primarily in the absorption of natural substances, such as monosaccharides, 1-amino acids, pyrimidines, bile salts, and certain vitamins. However, there is evidence that certain drugs may also be absorbed by one of these active processes, if their chemical structures are sufficiently similar to that of the natural substrate. The anticancer drug 5-fluorouracil is an example of an actively transported drug. It is similar in structure to the natural substance, uracil, which is absorbed by means of the pyrimidine transport system.

Active transport is specific not only in terms of chemical structure but also with respect to direction, transporting molecules mainly from the mucosal side to the serosal side of the gastrointestinal tract. The transport can also take place against the concentration gradient, i.e., from a region of lower concentration or activity to the region of higher concentration or activity. Since active transport involves enzymes, these can be saturated at higher concentrations of the drug, and may be subject to competitive inhibition in the presence of other drugs. Since active transport processes consume energy, they can be inhibited by various metabolic poisons such as fluoride or dinitrophenol, as well as by lack of oxygen.

Quite often an active transport of drug molecules occurs concomitantly with passive diffusion. Faster absorption rates can generally be expected at lower concentrations due to the contributions of the active process but the passive diffusion becomes more important due to possible saturation of the active transport process at higher concentrations. Poor absorption or permeation is more likely when there are more than 5 H-bond donors, 10 H-bond acceptors, the molecular weight is greater than 500, and the calculated Log *P* is greater than 5. This is also often referred to as Rule 5 of Lipinski. However, Lipinski specifically states that the Rule of 5 only holds for compounds that are not substrates for active transporters. Since almost all drugs are substrates for some transporter, much remains to be studied about the Lipinski's rule. In addition, unless a drug molecule can passively gain intracellular access, it is not possible to simply investigate whether the molecule is a substrate for efflux transporters.

Solvent Drag

There are some variants of the two major types of transport processes described above. Water flux, in the same direction as drug movement, can increase the diffusion rate of a substance across the gastrointestinal membrane. This is known as solvent drag.

Facilitated Transport

Some substances are transported by a process which does not take place against a concentration gradient, but which involves a carrier which is subject to competition by other substances of similar structure and is affected by the metabolic inhibitors. This absorption process appears to be an active one and is referred to as facilitated transport. The classical example of facilitated transport is the absorption of vitamin B_{12}. The vitamin B_{12} forms a complex with the intrinsic factor produced by the stomach wall and is transported in the form of this complex.

The observed low permeability of some drug substances in humans could be caused by efflux of drugs via membrane transporters such as P-glycoprotein (P-gp). When the efflux transporters are absent in these models, or their degree of expression is low compared to that in humans, there may be a greater likelihood of misclassification of permeability class for a drug subject to efflux compared to a drug transported passively. Expression of known transporters in selected study systems should be characterized. Functional expression of efflux systems (e.g., P-gp) can be demonstrated with techniques such as bidirectional transport studies, demonstrating a higher rate of transport in the basolateral-to-apical direction as compared to apical-to-basolateral direction using selected model drugs or chemicals at concentrations that do not saturate the efflux system (e.g., cyclosporin A, vinblastine, rhodamine 123). An acceptance criterion for intestinal efflux that should be present in a test system cannot be set at this time. Instead, this guidance recommends limiting the use of nonhuman permeability test methods for drug substances that are transported by passive mechanisms. Pharmacokinetic studies on dose linearity or proportionality may provide useful information for evaluating the relevance of observed in vitro efflux of a drug. For example, there may be fewer concerns associated with the use of in vitro methods for a drug that has a higher rate of transport in the basolateral-to-apical direction at low drug concentrations but exhibits linear pharmacokinetics in humans.

Ion-Pair Transport

The absorption of highly ionized compounds at gastrointestinal pH cannot be explained by passive diffusion or other mechanisms. A hypothesis has been suggested whereby highly ionized compounds (such as quaternary structures and sulfonic acids) form neutral complexes with other ions in the gastrointestinal tract (such as mucin) and these ion–air complexes are then absorbed by passive diffusion, since the complex has both the required lipid and aqueous solubility. This mechanism is referred to as ion-pair transport.

Pinocytisis

Another mechanism of absorption is that of pinocytisis, a process of physical absorption whereby an invagination of the cell membrane engulfs the particulate or droplet material. It is the only transport mechanism whereby a drug does not have to be in aqueous solution in order

to be absorbed. Only a few compounds are absorbed by this mechanism, including Vitamins A, D, E, and K. Pinocytosis is of significant importance in the uptake of nutrients.

Absorption Factors

The gastrointestinal tract is composed of heterogeneous anatomic regions. As drug molecules descend through the gastrointestinal tract, they encounter different environments which vary in pH, nature and concentration of enzymes, and fluidity of contents, as well as in the area available for absorption.

While the differences between the pH of the gastric and the intestinal fluids can account to some extent for the different rates of absorption of certain drugs from these two zones, the main reason is the difference in the absorption surface areas. Anatomically, the small intestine is much better designed for absorption than the stomach. The intestinal mucosa is covered by numerous villi and microvilli, providing a large surface area of approximately 120 m^2 (the intestine without the villi and microvilli would have a surface area of only 4 m^2). The large intestine has no villi and little drug absorption takes place from this region.

As the drug passes through the small intestine, the consistency of gastrointestinal contents changes from fluid to paste due to the absorption of water. Thus the drug particles which have not been dissolved in the stomach or upper small intestine will encounter difficulty in their dissolution in the lower intestine. Even if the drug is dissolved, it may not be absorbed quickly from the lower part of the intestine due to the retarded diffusion of molecules through pasty contents. Thus in addition to the differences in absorption rates in different regions of the gastrointestinal tract due to pH differences and absorption surface area, the consistency of the contents is also an important factor. In general, therefore, the upper part of the small intestine is the most important zone for the absorption of drugs, whether acids or bases.

Except for the colon, all other regions of the gastrointestinal tract have areas for the specific transport of compounds. For example, iron absorption occurs mainly in the proximal part of the small intestine and decreases progressively in the intestine; thiamine absorption occurs mainly in the proximal region; and vitamin 12 is absorbed from the ileum. Therefore, if a drug is absorbed primarily through a specific gastrointestinal area, it should not be administered by the rectal route.

For most drugs in general, and especially for those which are absorbed from a specific part of the gastrointestinal tract, the extent and rates of absorption are dependent on the rate of passage of contents through the gastrointestinal tract. Depending on the rate of passage, there may be only a limited time available for the dissolution of a solid particle and for the modification of its molecules into absorbable forms. This is exceptionally critical if the optimum absorption site is the proximal section of the small intestine. The rate of passage of intestinal contents through the upper small intestine is higher than it is through the lower part. Thus, if a drug is not present in an absorbable form within indicated time limits, it may be propelled past its absorption site and excreted totally or in part in the feces.

Gastrointestinal Fluids

Drugs must dissolve in the gastrointestinal fluids before they can be absorbed; poorly water soluble drugs have therefore inherent problems in their bioavailability. Any changes in the composition of gastrointestinal fluids such as increased viscosity due to meal ingestion can reduce the dissolution of drugs. A moderate volume of fluid is also essential for optimal absorption since in addition to providing dissolution it also helps spread the drug over a larger area for absorption. It should be noted that larger fluid volumes also decrease the concentration gradient, yet the effect of increased contact area with the intestine overcomes this loss of driving force (concentration gradient). It is therefore advisable to take drugs with moderate volumes of fluids.

The pH of the gastrointestinal fluids varies from about 1 to 3 in the stomach to about eight in the large intestine. The factors which affect the pH include:

- type of diet
- use of soft drinks
- stress

- gastrointestinal disease
- general health.

Since the unionized forms of the drug is generally more lipid soluble, higher rates of absorption are observed at pH where the drug molecules are present predominantly in an unionized form. Several studies have confirmed this theory referred to as pH Partition Theory. In some instances, lack of conformation with this theory has been explained on the basis of a virtual membrane pH which may be different from the pH of the lumen.

Whereas the pH Partition Theory holds in principle, majority of drugs whether acids or basis are primarily absorbed from the small intestine where much larger surface area is provided for absorption compared to the stomach a large intestine. It is interesting to note that the pH factors which make the drug molecules more absorbable, can reduce the dissolution of drug molecules; For example, if tetracycline hydrochloride is administered with sodium bicarbonate in a capsule form, the total absorption is significantly decreased due to decreased dissolutions of tetracycline at the alkaline pH due to sodium bicarbonate. If a solution of tetracycline hydrochloride is administered with sodium bicarbonate no such effects are noted.

It should be noted that several formulation requires exposures to specific pH for their disintegration and changes in their gastric residence time can significantly alter the absorption. Exposure to intestinal pH may result in reduced absorption for some drugs due to the formation of insoluble hydroxides such as demonstrated when aluminum aspirin was used in chewable formulations and in the absorption of various iron preparations.

Drugs which are unstable at acidic pH show reduced absorption if the gastric residence times are prolonged such as shown for penicillin and erythromycin. In some instances use of a chemical modification such as erythromycin in an ester form helps to overcome this problem.

Intestinal fluids also contain a variety of components that may interact with drug molecules such as bile salts which may increase drug absorption by solubilization of drugs, increasing the diffusion of drug molecules across the lumen of the tract and by modifying the permeability of the intestinal membrane. Besides bile salts there are several other naturally occurring surfactants of the intestinal fluid which regurgitate and are into stomach is primarily responsible for the lower surface tension (ca 35–50 dynes/cm) of gastric fluid. Drugs generally dissolve faster in gastric fluid than in 0.1 N hydrochloric acid.

Since the secretion of bile is not a continuous phenomenon, a variety of factors can affect the total content of bile and thus absorption of various drugs. For example, food causes increased secretion of bile and thus increased absorption of drugs like griseofulvin is observed. In addition to their solubilization effects, bile salts and synthetic derivatives such as dehydrochloric acid increase intestinal membrane permeability by a mycolytic action which reduces the barrier effect of intestinal mucins, and by increasing biliary secretion due to their hydrocholerectic effects. A good example is the increased absorption of certain quaternary hypotensive agents which bind to mucin. Another drug which seems to bind with mucin is tetracyline. It has been suggested to use pharmacologically inert quaternary compounds that can competively bind to mucin and thus increase absorption of the active quaternary compounds.

Gastric Emptying

The gastric emptying rate affects the absorption rate primarily because of the pH differences between stomach and intestine. For example, weakly basic drug such as amphetamine and codeine will be absorbed primarily from the small intestine rather than from the stomach, and any delay in gastric emptying will tend to delay the absorption and thus the therapeutic response. Slow gastric emptying can also affect the bioavailability of drugs that are unstable in gastric fluids, e.g., l-dopa, since the extent of degradation is proportional to the time for which the drug is exposed to the low pH and the enzymes of the stomach.

Some of the factors which affect the gastric emptying rates are as follows.

TYPE OF FOOD

The type of food will affect the stomach emptying rate significantly. For example, fats decrease the rate; proteins affect a lesser decrease; and carbohydrates retard gastric emptying the least. A fatty meal can therefore retard absorption rates of the drug and delay the onset of action. However, with such water-insoluble drugs as griseofulvin the absorption can be increased as a result of retarded gastric emptying. The reason is that griseofulvin passes slowly to the small intestine, and therefore the longer duration of contact of griseofulvin with the intestine results in a greater chance for it to be dissolved and absorbed through a specific region. Table 2 highlights some examples where food affects absorption of various drugs.

A faster gastric emptying rate is desirable for drug which is not absorbed in the stomach. These should be taken either on an empty stomach or an hour before or two hours after meals (Table 3).

Volume of Fluid or Food

The volume of fluid or food has a definite influence on the gastric emptying rate. The rate with which gastric contents leave the stomach is proportional to their volume. With small volumes, there is an initial lag time before gastric emptying begins, while with higher volumes, there is a initial phase of more rapid emptying. The fluid intake also affects the dissolution rate and forms the integral part of certain drug actions, e.g., the use of bulk laxatives.

Osmotic Pressure

The emptying rates are also dependent on the osmotic pressure of the liquids. For example, water leaves the stomach with a half life of about five minutes (a glass of water leaves the stomach in about 5 to 20 minutes). Hypertonic or hypotonic solutions generally leave the stomach at a slower rate than isotonic solutions.

Acidity

Gastric emptying is also retarded by increased acidity of gastric fluids. The use of antacid compounds increases gastric emptying rates. An interesting application of this property is the administration of l-dopa with sodium bicarbonate. Since l-dopa decomposes in the stomach, its administration with sodium bicarbonate increases its bioavailability due to decreased decomposition in the stomach and the result of both decreased acidity and increased emptying of the contents into the intestine.

TABLE 2 Influence of Food on the Absorption of Various Drugs in Man

Drug	Influence on absorption
Acetaminophen	Reduction in rate but not extent of absorption
Aspirin	Reduction in rate but not extent of absorption
Bretylium tosylate	Reduction in rate and extent of absorption
Capuride	Reduction in rate but not extent of absorption
Cephradine and Cephalexin	Reduction in rate but not extent of absorption
Clindamycin	Reduction in rate but not extent of absorption
Digoxin	Reduction in rate but not extent of absorption
L-Dopa	Any factor reducing emptying rate will reduce rate and extent of absorption
Ethanol	Milk reduces the rate of absorption
Ethanol	Reduction in rate of absorption
Fenoprofen	Reduction in rate of absorption
Lincomycin	Reduction in rate and extent of absorption
Nitrofurantoin	Reduction in rate but increase in extent of absorption
Propantheline	Reduction in magnitude of pharmacologic response
Rifampicin	Reduction in rate and extent of absorption
Theophylline	No noticeable influence on absorption

TABLE 3 Examples of Relationships Between Food Intake and Drug Regimens

On empty stomach	1 hr before or 2 hr after meals	1 hr before meals
Piperazine citrate	Tetracycline	Anticholinergic agents
Bephenium hydroxynapthoate	Ampicillin	Methantheline bromide
Castor oil	Cefaxolin sodium	Mepenzolate bromide
Penaerythritol tetranitrate	Sulfisoxazole	Pancrelipase
Lincomycin	Trimethoprim	Sitosterols
Isosorbide dinitrate	Demeclocycline hydrochloride	Chlordiazepoxide hydrochloride
Dicloxacillin sodium	Fenfluramine hydrochloride	Anisotropine methylbromide
	Erythromycin	Diethylpropion hydrochloride
	Penicillin	Phenmetrazine hydrochloride
	Cholestyramine	Hexocyclium methylsulfate
	Rifampin	Propantheline bromide
	Methacycline	Glycopyrrolate
	Troleandomycin	Mazindol
	Nafcillin	
	Oxytetracycline	
	Hetacillin	

Food Temperatures

Hot or cold foods or fluids prolong the gastric emptying. For example, water taken at the temperature of 25°C leaves the stomach at one-third the rate water taken at 37°C.

Viscosity

Liquids of low VISCOSITY are emptied faster than liquids of higher viscosity. Solutions or suspensions of fine particles leave the stomach at a higher rate than lumpy substances.

Psychologic State

The psychologic state of an individual also affects gastric emptying. Depression, injury, and trauma lead to prolonged emptying. Agitation and excitement increase the peristaltic movement, thus increasing the rate of gastric emptying.

Body Posture

Body posture can also significantly affect gastric emptying. Lying on the right side and standing may facilitate emptying, whereas the supine position may retard emptying.

Drugs

A number of drugs are capable of affecting the gastric emptying rate, usually through some central mechanism, such as anticholinergic drugs atropine, antihistamines, tranquilizers, aspirin, and morphine derivatives. Table 4 summarizes of the various factors affecting gastric emptying.

Intestinal Transit

The residence time in the intestine has a direct bearing on the amount of drug absorbed, however, absorption may be reached if the drug is unstable in intestinal fluids or binds irreversibly to the intestinal contents. For dosage forms where the drug is released only in the small intestine, e.g., enteric coated forms intestinal transit times are of utmost importance.

The peristaltic and mixing movements of intestine are also important in affecting the dissolution of the drug. Even though food greatly increases intestinal movements, administration of drug with food is generally not recommended because of other interactions with food.

TABLE 4 The Influence of Various Factors on Gastric Emptying in Man

1. Volume	The larger the starting volume the greater the initial rate of emptying. After this initial period, the larger the original volume, the slower the rate of emptying
2. Type of meal	
a) Fatty acids	Reduction in rate of emptying in direct proprotion to their concentration and carbon chain length. Little difference from acetic to octanoic acids. Major inhibitory influence seen in chain length greater than 10 carbons (decanoic to steric acids)
b) Triglycerides	Reduction in rate of emptying. Unsaturated triglycerides are more effective than saturated ones. The most effective in reducing emptying rate were linseed and olive oils
c) Carbohydrates	Reduction in rate of emptying primarily as a result of osmotic pressure. Inhibition of emptying increases as concentration increases
d) Amino acids	Reduction in rate of emptying to an extent directly dependent upon concentration. Probably as a result of osmotic pressure
3. Osmotic pressure	Reduction in rate emptying to an extent dependent upon concentration for salts and nonelectrolytes. Rate of emptying may increase at lower concentrations and then decrease at higher concentrations
4. Physical state of gastric contents	Solutions or suspensions of small particles empty more rapidly than chunks of material which must first be reduced in size prior to emptying
5. Chemicals	
a) Acids	Reduction in rate of emptying dependent upon concentration and molecular weight of the acid. Lower molecular weight acids are more effective than those of higher molecular weight. (In order of decreasing effectiveness: HCl, acetic, lactic, tartaric, citric acids.)
b) Alkali ($NaHCO_3$)	Increased rate of emptying at low concentrations (1%) and decreased rate at higher concentrations (5%)
6. Drugs	
a) Anticholinergics	Reduction in rate of emptying
b) Narcotic analgesics	Reduction in rate of emptying
c) Metoclopramide	Increase in rate of emptying
d) Ethanol	Reduction in rate of emptying
7. Miscellaneous	
a) Body position	Rate of emptying is reduced in a patient lying on the left side
b) Viscosity	Rate of emptying is reduced with viscous solutions
c) Emotional state	Aggressive or stressful emotional states increase stomach contractions and emptying rate. Depression reduces stomach contraction and emptying
d) Bile salts	Rate of emptying is reduced
e) Disease states	Rate of emptying is reduced in some diabetics, local pyloric lesions (pyloric ulcers, pyloric stenosis), and hypothyroidism. Gastric emptying rate is increased in hyperthyroidism and in the presence of duodenal ulcers
f) Exercise	Vigorous exercise reduces emptying rate
g) Gastric surgery	Gastric emptying difficulties often encountered after gastric surgery

Once the drug passes through to colon very little absorption can take place since the main function of this part of the intestine is to absorb water.

Blood Flow

The splanchnic circulation receives about 28% of the cardiac output which passes thorough liver via portal vein to the general circulation thus a significant metabolism of drug can take place in the liver before reaching the general circulation. The high perfusion of the gastrointestinal tract creates a "sink" for the diffusion of drug molecules across the membrane. For most drugs, blood flow does not effect absorption rates unless;

- the drug is actively absorbed where blood flow provides the energy for absorption process.
- the absorption is very fast where it is more dependent on blood flow rate than on the transit across the membrane.

Ingestion of meal increases flow rates whereas extraneous exercise reduces blood flow rates to the gastrointestinal tract.

Gastrointestinal Drug Biotransformation

The bioavailability of orally administered drugs can be affected due to biotransformation the in the gastrointestinal tract, and the various organs (e.g., the liver) through which the drug molecules pass before reaching the general circulation. For example, the chromotropic activity of isoproterenol is about 1000 times greater when administered intravenously than through oral administration, largely due to the biotransformation of isoproterenol into an inactive sulfate during the transfer across the gut wall and passage through the liver. Similarly, some of the steroids are also extensively biotransformed during absorption. Since biotransformation reactions require the presence of enzymes, the saturation of these enzymes at higher drug concentrations results in dose-dependent effects which have been noted for l-dopa, and *para*-amino hippuric acid.

The intestinal microflora also play an important role, causing biotransformation of such drugs as methotrexate, succinylsulfathiazole, and certain coumarin derivatives. Generally, the microflora has little effect on drug absorption except for drugs dissolving slowly or contained in slow-release dosage forms since these forms reach the distal end where most of the biotransformation takes place. The conjugates of many drugs can be cleaved by micro-organisms which may cause recycling of drug molecules, a phenomenon which is altered in antibiotic therapy. However, for drugs which may generally be biotransformed in the gut, antibiotic therapy can increase their bioavailability. The following are some of interesting examples:

- antibiotic therapy alters the pattern of 1-dopa metobolites excreted in urine.
- conjugates of isonicotinic acid are hydrolyzed by intestinal bacteria with subsequent reabsorption of isonicotinic acid.
- lantoside C is converted to digoxin by intestinal bacteria.
- cyclamate is converted to toxic metabolites cyclohexamine in the intestine.
- gut wall metabolism of salicylic acid and various steroids results in glucuronidation.
- nonspecific esterase hydrolyze aspirin in the gut wall.
- 1-dopa is metabolized by decarboxylase enzyme in gastric mucosa.

The overall impact of gut metabolism on drug bioavailability and toxicity has not been fully evaluated due to lack of awareness of these mechanisms and analytic methods to monitor the metabolites.

FOOD INTERACTIONS

Food affects drug bioavailability by several mechanism including:

- changes in gastric and intestinal transit times.
- increased gastrointestinal secretions.
- adsorption of drug onto food.
- competition of food components with drug for absorption.
- physicochemical interactions between food and drug.
- increased viscosity of gastrointestinal fluids.

Drugs which are actively absorbed from the gastrointestinal tract may show competitive absorption with food components such as amino acids. Examples include l-dopa and several anticancer drugs.

It is often taken for granted that food impairs the absorption of drugs and that the drugs should be taken on an empty stomach. Some misleading assumptions include: (*i*) drugs should be administered with food only if they are irritant; (*ii*) a reduction in drug absorption with food intake occurs due to decreased gastric emptying and; (*iii*) most drugs are absorbed by a passive diffusion process. Recent findings dispute these assumptions and show that food can improve the bioavailability of several drugs. It has also been observed that food may influence the rate of drug absorption without affecting the extent of absorption. Furthermore, active intestinal

transport mechanisms may be more important than hitherto recognized. Food may also affect the first pass biotransformation of drugs in the gut and in the liver. The effect of food composition such as carbohydrate/protein ratios may change the elimination rates of some drugs.

Several aspects must be considered in studying the effect of food on the bioavailability of drugs:

- food induces changes in the gastric emptying rate, intestinal transit time, and/or in gastroenterohepatic secretion of hydrochloric acid, bicarbonate, enzymes, and bile.
- specific food components and contaminants can alter metabolic transformation of drugs in the gut and in the liver.
- food refers to different kind of meals and that one type of meal or food component may have both qualitatively and quantitatively different effects on drug bioavailability than other.
- different preparations of same drug may interact differently with food.
- findings based on single meal, single dose studies in healthy volunteers may not necessarily be relevant as to food effects on the steady plasma level of drug during its long term use in patients.

Rifampicin absorption is also reduced when given with food. Since this drug is mostly given once a day, generally an hour before breakfast, food interaction does not present a problem in therapeutic management. Thus all those drugs which are generally given once a day, food interactions are of less significance unless an increased absorption is possible, in which case the dosing must be carefully monitored.

The absorption interactions of tetracyclines are well known. The absorption of first generation tetracylines such as oxytetracycline or tetracyline is drastically reduced by intake of antacids, or of calcium containing food items, such as milk and cheese. It is a well established fact that nonabsorbable chelates are formed between the metals (AI, Mg, Ca, and Fe) and the tetracyclines. In addition, the pH raising influence of food and antacids is important, as the solubility of tetracycline is reduced with increasing pH. Absorption of newer tetracycline analogs, doxycycline and minocycline, is not significantly affected by food but is inhibited by antacids and iron preparations.

Absorption of penicillin, ampicillin, oxacillin, dicloxacillin, lincomycin, and some erythromycin preparations is reduced when taken with food. However, bioavailability of amoxicillin or that of ampicillin when given in esterified form is not affected by food and recent studies indicates increased absorption of erythromycin stearate when given with food. In view of the irritant properties of ampicillin and erythromycin, these should be administered with food.

The bioavailability of nitrofurantoin is increased from both macro and microcrystalline forms when given with food due to reduced gastric emptying which allows greater time for the dissolution of the drug.

PATHOPYHSIOLOGIC DISORDERS

Drug bioavailability is significantly altered in the presence of various pathophysiologic disorders. The following are some specific observations:Alterations in gastric pH have following implications:

- pH partitioning and dissolution of poorly soluble drugs can be significantly affected, e.g., aspirin is better absorbed in achlorhydric patients.
- changes secondary to pH change may include epithelium integrity and blood flow rates which can directly affect rate and extent of drug absorption.
- stability of acid labile drugs cab be significantly altered.
- several disease states including gastric cancer have been identified when the gastric pH is elevated.

Gastric emptying is hampered after gastric surgery and gastrectomy increases gastric emptying. The various effects observed are as follows:

- drugs requiring exposure to gastric environment for dissolution show reduced bioavailability in gastrectomy.
- enteric coated tablets will show specific absorption problem in pyloric stenosis.
- gastric emptying is delayed in labor and further exacerbated due to narcotic analgesics, serious consequences may result due to regurgitation of gastric fluid into respiratory tract.

The effect of intestinal transit on drug bioavailability is also very pronounced. Intestinal transit rate is decreased when:

- digestive juice secretion is reduced.
- thyroxine secretion is reduced.
- hypothyroidism exists.
- insulin hypoglycemia exists.
- chronic diarrhea exists.

A variety of malabsorption syndromes affect drug absorption as well:

- in steatorrhea absorption of phenoxymethyl penicillin is reduced.
- ampicillin and nalidixic acid are less absorbed in shigellosis.
- propranolol availability is increased in celiac diseases due to reduced intestinal metabolism.
- riboflavin absorption is impaired in biliary artesia.

A variety of drugs administered to treat pathophysiologic orders show interactions resulting in alteration of absorption. Table 5 lists some examples of these interactions.

AGE

Several gastrointestinal functions mature with age including specialized absorption mechanisms. For example, sugar absorption is very inefficient in younger children. Whereas significant changes in the structural and the functional properties of gastrointestinal tract and blood flow occur in the elderly, no studies have demonstrated changes in the bioavailability of drugs in the elderly.

FIRST PASS BIOTRANSFORMATION

A distinction can often be made between the biotransformation in the intestine and that in the liver during the first pass by administering the drugs either intraperitoneally or directly into portal vein. Some mathematical approaches have also been used and will be discussed later. Table 6 lists the drugs which are suspected of first pass or gastric hepatic biotransformation. Whereas 100% of the orally administered dose goes through the liver, only about 25% to 30% of the intravenously or intramuscularly administered dose passes through the liver, which may partly explain the differences in responses observed as a function of route of administration. The hepatic clearance of drugs depends on two factors:

- blood flow to liver
- capacity of liver to remove drug.

Sublingual/Buccal Administration

Some drugs are administered by placing them beneath the tongue or in the cheek pouch. A rapid absorption of drugs is thereby generally expected due to the high vascularity of this

TABLE 5 Drug Interactant Influence on Drug Absorption

1. pH		
Folic acid	$NaHCO_3$	Reduced rate and possibly extent of absorption. Mechanism unknown but may be related to drug ionization
Diphenylhydantoin	Reduced absorption. Possibly due to alkalinization of gut fluids by the anticonvulsant	
Tetracycline	$NaHCO_3$	Reduced rate and extent of absorption from capsules. No influence on absorption from solution. Effect due to reduce drug dissolution rate from capsules
2. Gastric emptying and intestinal motility		
Acetaminophen	Diacetylmorphine (i.m.)	Reduced rate but not extent of absorption
	Meperidine (i.m.)	Reduced rate but not extent of absorption
	Metoclopramide (i.v.)	Increased rate but not extent absorption
	Propantheline (i.v.)	Reduced rate but not extent of absorption
Bishydroxycoumarin	Heptabarbital	Reduced absorption possibly due to increased intestinal transit rate
Chlordiazepoxide	Antacid	Reduced rate but not extent of absorption. Possibly due to decreased gastric emptying rate
Diazepam	Metoclopramide (i.v.)	Increased rate of absorption
Digoxin	Propantheline	Increased rate and extent of absorption from a slowly dissolving tablet. No influence on absorption from a rapidly dissolving product
	Metoclopramide	Reduced rate and extent of absorption
L-Dopa	Antacid	Increased rate and extent of absorption possibly due to increased gastric emptying rate. Effect seems variable
	Imipramine	Reduced rate of absorption
	Metoclopramide	Increased rate and extent of absorption
Ethanol	Metoclopramide (i.v. and p.o.)	Increased rate and possibly extent of absorption
	Propantheline (i.v. and p.o.)	Decreased rate and possibly extent of absorption after i.v. propantheline. Oral propantheline has no influence on ethanol absorption
Griseofulvin	Phenobarbital	Reduced absorption possibly due to increased intestinal transit rate
Isoniazid	Antacid	Reduced rate and possibly extent of absorption possibly due to decreased gastric emptying rate
Nitrofurantoin	Propantheline	Increased extent of absorption
Phenolsufonphthalein	Propantheline	Reduced rate but increased extent of absorption
Phenylbutazone	Desmethylimipramine	Reduced rate of absorption
Pivampicillin	Metoclopramide (i.m.)	Increased rate of absorption
	Atropine (s.c.)	Reduced rate of absorption
Riboflavin	Propantheline	Reduced rate but increased extent of absorption
Sulfamethoxazole	Propantheline	Reduced rate of absorption
Tetracycline	Atropine (s.c.)	Reduced rate of absorption
	Metoclopramide (i.m.)	Increased rate of absorption
3. Adsorption		
Acetaminophen	Charcoal	Reduced extent of absorption
Aspirin	Charcoal	Reduced rate and extent of absorption
	Cholestyramine	Reduced rate and possibly extent of absorption
Chlorpromazine	Antacid	Reduced rate of absorption
Chlorothiazide	Colestipol	Reduced extent of absorption
Lincomycin	Antidiarrheal preparation	Reduced extent of absorption
Nortriptyline	Charcoal	Reduced extent of absorption
Phenylpropanolamine	Charcoal	Reduced extent of absorption
Promazine	Charcoal	Reduced rate and extent of absorption
	Antidiarrheal preparation	Reduced rate and extent of absorption
Propantheline	Charcoal	Reduced response
Propoxyphene	Charcoal	Reduced extent of absorption

(Continued)

TABLE 5 Drug Interactant Influence on Drug Absorption *(Continued)*

Pseudoephedrine	Kaolin	Reduced rate of absorption
Salicylamide	Charcoal	Reduced extent of absorption
Thyroxine	Cholestyramine	Reduced extent of absorption
Vitamin B_{12}	Cholestyramine	Reduced extent of absorption
Varfarin	Cholestyramine	Reduced rate and extent of absorption
4. Complexation		
Bishydroxycoumarin	Milk of magnesia	Increased rate and extent of absorption
Chlortetracycline	Antacids	Reduced extent of absorption
Tetracycline	Antacids	Reduced extent of absorption
5. Miscellaneous		
Ergotamine	Caffeine	Increased rate and extent of absorption possibly due to complexation
Folic acid	Salicylazosulfapyridine	Reduced extent of absorption mechanism not known
Iron	Hexocyclium	Reduced absorption possibly methosulfate due to decreased secretion of a gastric factor needed for absorption
Pseudoephedrine	Antacid	Increased rate of absorption. Effect may be due to changes in gut pH or gastric emptying
Rifampicin	Para-aminosalicylic acid	Reduced rate and extent of absorption. Mechanism not known
Vitamin B_{12}	Para-aminosalicylic acid	Reduced absorption by an unknown mechanism

region. The pH of saliva is about six, and drugs are absorbed by passive diffusion with a slightly higher requirement for lipid solubility than is needed for intestinal absorption.

A significant advantage of this route is that gastrointestinal degradation and biotransformation are bypassed along with hepatic first pass biotransformation. A variety of drugs can be administered by this route, including nitrates, such hormones like methyltestosterone, testosterone propionate, and oxytocin. Few studies have reported on the effective use of this route of administration. One such reports significantly higher blood levels of methyltestosterone from sublingual tablets than are obtained from other routes. Absorption properties of sympathomimetic amines, methadone, meperidine, lidocaine, chlorpheniramine, imipramine, desipramine, and barbiturates have also been studied. Recently, chewing gum-based drug delivery systems have been developed for several drugs.

Rectal Administration

Some drugs are administered rectally either in suppository or in solution form, e.g., retention enema. The solution yield better absorption provided that they are retained for a sufficient length of time in the rectum. The suppositories are the most commonly used dosage forms for both local and systemic effect. Examples of drugs administered rectally for systemic action include aspirin, acetaminophen, indomethacin, diazepam, theophylline, prochlorperazine, cyclizine, promethazine, and barbiturates.

The absorption mechanism mainly involves passive diffusion with no sites for active transport. The absorption rate and bioavailability are more erratic than observed with oral administration, due to such added factors as the presence of feces retarding absorption or irritant suppository bases such as carbowaxes causing early evacuation. The use of an enema before drug administration generally increase the absorption significantly.

TABLE 6 Drugs for Which First Pass Hepatic Biotransformation Is Suspected, Possibly in Addition to Gastrointestinal Biotransformation

Alprenolol	Pheniprazine
Desmethylimipramine	Propranolol
Dopamine	Reserpine
Lidocaine	Serotonin
Nortriptyline	Tryptophan
Oxyphenbutazone	

The rectal route of administration is not suitable for irritant drugs such as tetracycline or penicillin. A large number of studies have attempted to develop an "ideal" base for suppositories or formulation for a microenema, but little has been reported regarding their comparative bioavailability in humans. Thus a conclusion cannot be drawn regarding the relative merits of this route of administration when compared with other routes.

Intravenous Administration

The direct administration of drugs into veins is the only route where bioavailability considerations are not relevant. This route provides an almost instantaneous response with controllability of the rate of drug input into the body. This route is especially suitable for those drugs which cannot be absorbed adequately from the gastrointestinal tract or tissues depots (e.g., intramuscular administration) or where there is a significant first pass effect upon oral administration. The drugs which would be intolerably painful in the subcutaneous or muscle tissues by virtue of their irritant properties may be injected slowly into a vein without much difficulty, e.g., nitrogen mustard in cancer chemotherapy.

There are however, several disadvantages with the use of the intravenous route. A drug administered intravenously cannot be recalled, whereas some such measures can be taken with other routes. Rapid intravenous injection may evoke catastropic effects in the circulatory and the respiratory systems due to the transient wave of concentrated solute suddenly reaching the myocardium and the chemoreceptors in the aortic arch and carotid sinus. Intravenous injections should, therefore, be administered slowly, preferably over a period of one minute or more, during which time the blood completes its circulation. The possibility of anaphylactoid reactions is much greater than with any other route of administration.

The tonicity of solution is also important since hypotonic or hypertonic solution can cause hemolysis or agglutination of erythrocytes. The damage of the vascular wall also leads to local reactions, especially after prolonged infusions.

The possibility of microbiologic contamination and pyrexia due to pyrogens is a serious concern in the use of intravenous administration.

The intravenous route is especially suitable when a rapid response is required, as in the treatment of epileptic seizures, acute asthmatic attacks, cardiac arrhythmias, etc. The fluctuation of plasma concentration is generally very small if a drug is administered by slow intravenous infusion, as is employed for lidocaine, theophylline, and many antibiotics. A caution is needed for drugs with poor water solubility which can precipitate resulting in thrombosis, an removal of drug from circulation and deposition of the precipitate in various tissues resulting in reduced apparent bioavailability. In addition, drugs which bind to plasma proteins extensively may show altered response depending on rate of injection since the initial binding and concentration at site of action can vary significantly.

Intra-arterial Administration

This route is used for the injection of substances used in diagnosis. A typical example is the injection of a radiopaque compound into the carotid artery to trace the circulation of the brain by roentgenography. In addition, certain specialized techniques in cancer chemotherapy call for regional infusion of drugs by arterial routes, which may provide a significant advantage over other routes.

Intramuscular Administration

More than 50% of hospitalized patients receive intramuscular drug administration. The popularity of this route is due to the decreased hazard of administration when compared with the intravenous route. Large volumes of solution can be injected (2–10 mL) by this route, generally with less pain and irritation than is encountered with the subcutaneous route.

Aqueous solution of drugs are usually absorbed from intramuscular administration sites within 10 to 30 minutes, but faster or slower absorption rates are possible depending on the

vascularity of the site (blood flow rates range from 0.02 to 0.07 mL/min), the ionization and lipid solubility of the drug, the volume of injection, the osmolality of the solution, and other variables, including coadministered drugs and adjuvants in the formulation.

The small molecules are absorbed directly into the capillaries from the intramuscular administration sites, whereas large molecules gain access to the circulation by way of the lymphatic channels.

The drugs which are poorly water-soluble, such as digoxin and diazepam, or those drugs which dissolve at pH values far above the physiologic range are often administered in nonaqueous media such as propylene glycol or in strongly acid or alkaline aqueous solutions. However, after intramuscular administration these drugs may not stay in solution, resulting in slow or incomplete absorption. In some instances the total bioavailability may be less than that from oral administration, as is demonstrated with phenytoin, diazepam and cefamandol.

The high lipid-soluble molecules are quickly absorbed from intramuscular administration sites, whereas lipid-insoluble molecules diffuse between interstitial fluid and plasma only through the pores in the capillary membrane; this is generally not the rate-limiting step in the absorption. Only very large lipid-insoluble molecules which must be absorbed through the lymphatic system have a rate limitation in their absorption, due to the slow rate of lymph flow (0.1% of the plasma flow).

The concentration of the injected solution can also affect the rate of absorption. For example, atropine is absorbed more rapidly administered in a smaller volume of more concentrated solution. Absorption rates can be accelerated by spreading the solution over large tissue areas, e.g., by massaging or using high-pressure injection devices.

The blood flow to the administration site is often the rate-limiting step in the absorption of drugs. Absorption is more rapid after injection into the deltoid than into the vastus lateralis, and is slowest after gluteal muscle injection. The drugs can be absorbed faster after administration into a buttock in males due to greater adipose tissue in females. Absorption rates increase during exercise regardless of the site of intramuscular administration since this results in increased blood flow to skeletal muscles. Conversely, absorption rates decrease in circulatory shocks, hypotension, congestive heart failure, myxedema, and other disturbances of the circulatory system.

Absorption rates can often be quite erratic upon intramuscular administration of drugs. This is due to increased membrane contact as the solution spreads, change in drug concentration as a result of absorption, a possible hypertonic effect drawing water to the site, or to the precipitation of the drugs. The precipitation can lead to incomplete absorption due to extremely slow redissolution or to phagocytosis of the drug particles. Examples of these incompletely absorbed drugs are ampicillin, cephaloridin, cephradine, phenytoin, and quinidine. Conversely, the slow absorption of drugs can itself be exploited to produce prolonged administration. The slow absorption can be accomplished by the use of injection vehicles of high viscosity, such as glycerin, cottonseed oil, sesame oil, or polyethylene glycols. Another technique involves preparation of fatty acid ester derivatives, such as decanoate derivative of

TABLE 7 Drugs That Can Undergo Biotransformation in the Lumen or During Absorption in the Mucosa

Acetylsalicylic acid	Meperidine
Aldosterone	Methadone
Aminobenzoic acid	α-Methyl dopa
Aminohippuric acid	Nitrates, organic
Chlorpromazine	Pentazocine
Cortisone	Progesterone
Dexamethasone	Propoxyphene
l-Dopa	Salicylamide
Estrogens	Stilbesterol
Hippuric acid	Sulfonamides
Hydrocortisone	Testosterone
Isoproterenol Terbutaline	

fluphenazine, which hydrolyzes slowly and provides gradual release. Benzathine penicillin and procaine penicillin are injected as water insoluble suspensions for the same purpose. Slowly released preparations of antipsychotic agents have been useful in the maintenance therapy of schizophrenia.

The side effects of intramuscular administration include pain, elevation of serum creatine phosphokinase as a result of trauma, and often sciatic nerve damage following gluteal injections. Other complications include skin pigmentation, hemorrhage, septic or sterile abscesses, cellulitis, muscular fibrosis, tissue necrosis, and gangrene.

Subcutaneous Administration

The factors affecting intramuscular drug absorption also determine subcutaneous drug availability. The blood flow rates are poorer than in muscles and so are the rates of absorption. Yet some drugs are absorbed as rapidly from a subcutaneous site as from intramuscular administration, e.g., anionic dye, phenosulfonphthalein, and insulin.

A prime determinant of the absorption rate of a subcutaneous depot is the total surface area over which the absorption can occur. Although the subcutaneous tissues are somewhat loose, and moderate amounts of fluids can be administered, the normal connective tissue prevents indefinite lateral spread of the injected solutions. These barriers can be bypassed with the aid of hyaluronidase, an enzyme that breaks down mucopolysaccharides of the connective tissue matrix and results in wider spreading of solutions and faster absorption rates. The absorption rates can also be increased by massage or by application of heat to increase blood flow. Quite frequently drugs affect their own rates of absorption if they alter the blood supply or capillary permeability. For example, methacholine, a cholinergic drug, causes vasodilation, which results in an immediate systemic response following subcutaneous administration.

The absorption of drugs from the depots formed following subcutaneous administration can be retarded to provide prolonged effect by such techniques as immobilization of the limb, local cooling to cause vasoconstriction, and the application of a tourniquet proximal to the injection site to block the superficial venous drainage and lymphatic flow. Inclusion of minute amounts of epinephrine (1:100,000 or 1:2,000,000) in the subcutaneous injection may retard absorption by constricting the veins to elicit local rather than systemic effects are desired, e.g., in the administration of local anesthetics.

The subcutaneous route of administration has frequently been used to provide prolonged release of drugs by incorporating the drugs into compressed pellets that can be implanted under the skin. The drug must be present in a relatively insoluble form and the pellet must resist disintegration by the subcutaneous fluid environment and mechanical stress. These conditions have been achieved with certain steroid hormones. For example, cylindrical pellets of testosterone, about 5 mm in thickness and diameter and weighing about 100 mg, are implanted subcutaneously in humans. The absorption of about l% per day is generally obtained during the steady state for up to two months.

An ideal shape for achieving constant rates of absorption is a flat disc. A change in the weight of the disc due to absorption results in very little change in the total surface area exposed since the release of the drug takes place from the flat surfaces.

Another example is that of estradiol (Progynon, 24mg) and testosterone (Oreton 75 mg) where subcutaneous implantation is used. For spherical pellets the ratio of surface area to volume increases with decreasing diameter. Thus, when drugs are prepared as spheres of known diameter the rate of absorption can be predicted: the larger the sphere the slower the rate of absorption. This principle has been used in the design of long-acting insulin preparations. Prompt insulin consists of small particles, and extended insulin is made up of relatively large particles. Two examples of pellets used for subcutaneous implantation are Oreton pellets (75 mg testosterone) and Progynon pellets (24 mg estradiol).

Some drugs produce severe pain when injected subcutaneously. Local necrosis and sterile abscesses may also occur. Such drugs may have to be administered intravenously because no solution concentrated enough to be useful can be given subcutaneously or intramuscularly.

Percutaneous Administration

The absorption of drugs through the skin should be a difficult matter since the function of the skin is to act as a barrier between the outside environment and the vulnerable tissues under the skin. Yet drugs are absorbed, sometimes quite efficiently, from the skin.

A major function of skin is to retard the diffusion and evaporation of water from within the body, except at the sweat glands. The stratum corneum, also known as the horny layer, which is densely packed with keratin, is responsible for this retardation. Beneath the horny layer, separating it from the underlying granular layer of epithelial cells, is the so-called barrier area, a clear dense region which is quite different from the horny layer both in microscopic appearance and in chemical properties. If the horny layer is stripped but the barrier area is left intact, little change in permeability occurs although water loss increases. However, removal of the barrier area leads to an abrupt increase in permeability for all kinds of molecules, large or small, lipid- or water-soluble. The dermis is generally freely permeable to all types of molecules.

The penetration rates of drugs through the skin are determined largely by their lipid/water partition coefficients excluding significant absorption of ions or water-soluble structures, except for very small molecules. Highly lipid soluble molecules also penetrate skin slowly compared to their penetration through other membranes.

Drugs may be applied to the skin for a local effect, especially on the superficial layers of the epidermis. The drugs are incorporated into vehicles which adhere to the skin, allowing diffusion of drug molecules out of the vehicle and into the epidermis. If a pathologic condition exists in the deeper layers of the skin the systemic administration may be more desirable, especially if the drug is water-soluble. For example, antifungal and antibacterial agents are often much more effective in skin infections when given orally or by injection than when applied to the skin. Highly lipid-soluble drugs, such as griseofluvin, are also effective in systemic administration for local skin infections.

Some recent studies suggest the use of pharmacologically inactive solvents, such as dimethyl sulfoxide, to facilitate the absorption of drugs through the skin. Examples of drugs whose absorption has been increased are corticosteroids, antineoplastics, antibiotics, carcinogens, and insulin. There is, however, great controversy on the toxicity of these solvents in topical formulations and it is difficult to justify their use at the present time.

A recent approach to utilizing Therapeutic Systems consists of a multilaminate structure of small size which is worn in the postauricular region, providing optimum drug permeability. Scopolamine is the first drug applied in this way for prevention or treatment of motion induced nausea with reduced parasympatholytic effects. A large number of "patches" are currently available for delivery of nitroglycerin through skin. These dosage forms are designed such that the rate-limiting factor in the absorption is the release form and dosage forms and not the skin permeability. This is necessary to reduce variability in absorption due to biologic factors pertaining to skin permeability.

The ionic drugs can often be administered through the skin by applying electrical gradients to the skin. This method of iontophoresis involves applying galvanic current to electrodes placed at the absorption site and at other parts of the body.

The fast absorption of lipid-soluble molecules through the skin indicates an environmental hazard which continues to grow with increasing pollution in the atmosphere. For example, carbon tetrachloride and other organic solvents prevents the body through the skin and cause serious toxic effects.

Organic phosphates [diisopropyl fluorophosphate (DFP), parathion, and malathion] and nicotine insecticides have caused deaths in agricultural workers as a result of percutaneous absorption upon in-field contact. Chlorovinyl arsine dichloride (lewisite), a mustard gas, is readily absorbed through the skin and has been used in chemical warfare. Most of the carcinogens in the atmosphere can be efficiently absorbed through the skin and it is no wonder that there is a higher cancer incidence rate in people living around the industrial centers, even though these people may not be directly exposed to these chemicals.

Topical delivery of drugs using semisolid, controlled release patches and many other delivery systems dosage forms offers advantages including reduced blood level fluctuation,

obviating the first pass effect and protection from gastrointestinal pH. Where localized action is desire, this dosage form offers remarkable opportunity for drug action. However, skin is a poor medium to deliver drugs because by its very design, it is supposed to prevent entry of chemicals (though it fails miserably as we know from chemical warfare agents). Generally, large polar molecules do not penetrate the stratum corneum well. The intrinsic physicochemical properties of candidate drugs important in expediting delivery across skin include molecular weight and volume, aqueous solubility, melting point and log *P*. For weakly acidic or basic drugs, the skin pH will play a strong role in their transport. Drugs that form zwitterions can be made more penetrable by using appropriate salt forms.

The formulation additives strongly impact on transdermal delivery as the variety of dosage forms such as creams, ointments, lotions, gels, and patches offer a wide variety of formulation additives. The problems related to crystallization of drugs as discussed under suspension dosage forms also apply here just as do considerations that optimize physical and chemical stability. Entire textbooks have been dedicated to formulating semisolid and topical delivery dosage forms that describe in detail how the choice of basic drugs structure and additives affects stability. Where salt forms are available, it is often difficult to predict the stability profile including such factors as photostability, a test that must be conducted for all dosage forms. It is known that different salt forms can show differences in their photostability profile.

Pulmonary Administration

Drugs can be introduced into the pulmonary system as gases or in aerosol forms. An almost instantaneous absorption can be expected due to the extremely large surface area available for absorption. The primary mechanism of absorption is passive diffusion but the lipid solubility tends to play a smaller role than in gastrointestinal absorption. The main limiting step in the utilization of this route has been the need to design dosage forms which accurately deliver the drugs. Most of these drugs are administered as aerosols, and their delivery to a great extent is dependent on the particles size distribution. Particles greater than 10 U are almost completely removed by impaction in the nasal passages. IMPACTION refers to the deposition of particles in the respiratory tract. The precipitation of particles arises from the tendency of a particle moving in a stream of air to continue in its original direction when the air current changes direction at bronchial branch points and at curves in the bronchial tree. Impaction due to diffusion is negligible except for very small particles. Particles below 10 U in diameter are of great significance since these include bacteria, viruses, smoke, industrial fumes, dust laden with fission product, pollens, insecticide dusts and sprays, and inhalant sprays used in the therapy of pulmonary diseases.

In order for a drug to be absorbed from an aerosol its particles must impact, preferably in the alveolar sacs, and dissolve in the available fluids. Larger particles are retained in the upper respiratory tract and smaller particles penetrate deeper into the pulmonary tree. Particles larger than 2 U in diameter probably do not reach the alveolar sacs. Particles sizes approximating l U are most desirable, but there is a greater tendency for these particles to be exhaled without being impacted. Thus many formulations include hygroscopic substances in the formulation to increase the size of particles deeper into the trachea. The tidal volume is also an important consideration. At a given respiratory rate, the air stream velocity is greater at high-tidal volumes and thus particles of all sizes tend to be driven deeper into the pulmonary tree before impaction.

Pulmonary administration has been used mainly for local therapy. For example, aerosols of epinephrine, isoproterenol, and dexamethasone are commonly used for acute asthmatic attacks, and antibiotics are sometimes incorporated for the treatment of complicated bronchopulmonary infections. In some instances, the systemic absorption of drugs administered for local action may be appreciable. For example, isoproterenol in a 0.5% aerosol is an effective bronchodilator, but a l% aerosol is apt to cause undesirable cardioacceleratory and hypertensive actions after only a few inhalations. The quick responses can, however, be beneficial in the treatment of anaphylactic episodes, as in the use of epinephrine.

Although the pulmonary route is used mainly for local effects, several drugs have been successfully administered in this way for systemic effect, including penicillin, glycosides, diuretics, and tranquilizers. More recently, an inhalation form of insulin has been marketed where the primary mechanism is impaction of very fine particles.

The problem of accurate dosing in pulmonary dosing in pulmonary administration remains a serious obstacle to greater use of this route. The use of metered dose devices is certainly an improvement and some products use the drug as a powder aerosol. The powder particles sizes range primarily between 2 and 6 U. This device, currently used for disodium cromoglycate (cromolyn sodium: Aarane® inhaler) provides a greater and more consistent absorption than can be obtained from other metered dose devices.

The pressurized MDIs the use of environmentally friendly propellants means choice of hydrofluoroalkanes wherein the dosage form can be a suspension of solution form. The problems of formulating suspensions as discussed above apply here as well but particularly with respect to interactions with the formulation components specific to pressurized inhaler systems. Solution dosage forms require selection of propellants wherein the drug can dissolve without crystallizing and may require addition of surfactants and cosolvents. However, there are toxicological issues with the use of surfactants. The solubility of drugs in solvents is determined by filtering the suspension in pressurized can into another can and then evaporating the clear solution (bringing to room temperature) and determining the amount of drug in it. High solubility in propellants can lead to crystal growth as propellants evaporate. Ostwald ripening common to suspensions applies to inhalation suspensions; the changes in the property of suspension can be studied using microscopy and observing changes in the axial ratio of crystal.

Drugs for inhalation therapy in a powder form required particular particle size which is achieved by the process of micronization between 1 to 6 μm to allow deep penetration through the lung alveoli system. There are number of devices which can deliver drugs to the lungs as dry powders, e.g., Turbuhaler™ or Diskhaler™. These dosage forms rely on a larger carrier particle, such as α-lactose monohydrate, to which the drug is attached. The lactose is usually fractionated such that it lies in the size range 63–90 μm. Upon delivery, the drug detaches from the lactose and, because the drug is micronized, it is delivered to the lung, whereas the lactose is eventually swallowed. It should be realized that the polymorphic form of the lactose used could affect the aersosolization properties of the formulation. The β-forms were easily entrained, but held onto the drug particles most strongly when flow properties are studied. The anhydrous α-form shows an opposite behavior and the monohydrate α-form demonstrates intermediate behavior. Interactions with packaging materials can also alter powder characteristics; for example, long contact times with PVC, polyethylene or aluminum should be avoided since the adhesion force between the drug and these surfaces is much higher than between it and the lactose carrier. Thus, detachment and loss of drug in the formulation could occur. Because lactose is widely used as a carrier, its compatibility with the new drugs should be studied in detail specially if there are any amino groups in the structure. The surface property of lactose is also important. With increasing specific surface area and roughness, the effective index of inhalation decreases due the drug being held more tightly in the inhaled airstreams. Therefore, characterization of the carrier particles by, e.g., surface area measurements, SEM and other solid-state techniques are recommended preformulation activities.

The recent approval by the U.S. FDA of Exubera®, an inhalation form of insulin is a classical example where the dosage form is an integral part of drug action. Using the Nektar company's delivery system to create a fine powder mist, insulin in Exubera is absorbed as the mist of fine reaches into deep portions of lung structure without getting impacted. Whereas reduction in particle size is pivotal to pulmonary delivery of drugs, micronization makes powders difficult to flow and these changes should be studied using such techniques as DVS, microcalorimetry and inverse gas chromatography (IGC). The high energy at the surface of micronized powders can often be relieved by exposing it to higher humidity air which can crystallize the amorphous high-energy regions. As a result, the common preformulation stage evaluations include measurements of the micromeritic, RH and electrostatic properties of the powder. Different salt forms show variant flow properties; for example, stearate salts generally are better for aerosol formulation.

Nebulizer formulations are normally solutions but suspensions (particle size of less than 2 μm) are also used. Important preformulation considerations include stability, solubility, viscosity, and surface tension of the solution of suspension.

Ophthalmic Administration

As with permeability in most other routes of administration, the permeability of drugs into and through the cornea is function of their lipoid and aqueous solubility. The cornea is composed of three distinct layers: the outer epithelium, an inner stroma, and the endothelium. The epithelium and endothelium are much more lipoidal than stroma. Therefore drugs must possess biphasic solubility characteristics in order to be absorbed through this route.

Weakly basic drugs, such as tropicanade, epinephrine, pilocarpine, atropine, homatropine, or cyclopentolate, freely penetrate the cornea because of rapid equilibration between their lipid-soluble unionized forms and their water-soluble ionized forms. The penetration of quaternary ammonium compounds, such as carbachol, echothiophate iodide, and demecarium bromide, which are charged and water-soluble at all pH values, is postulated on a binding mechanism which permits a small but sufficient quantity of these potent antiglaucomic agents to reach aqueous humor and evoke a therapeutic response. Tetracycline, gentamicin, carbenicillin, and methicillin do not penetrate the cornea because of their low lipid solubility, but chloramphenicol shows good penetration.

Fluorescein is used for diagnostic purposed because of its high-lipid solubility, which prevents its entry into the stroma unless there is abrasion. If there is an abrasion, fluorescein enters the stroma and possibly the aqueous humor, giving a brilliant green color due to its alkaline pH. In the precorneal film fluorescein exists in a yellow or orange form.

A variety of physiologic factors influence corneal drug absorption. Lacrimal drainage of an instilled drug solution competes for drug with corneal penetration and can account for a considerable loss of drug. When a drop of solution is applied to the eye, two processes occur simultaneously: the solution is diluted by reflex tearing and the added volume in excess of the normal lacrimal volume is drained from the eye, which is partly facilitated by reflex blinking. In humans, administration of 25 μL of solution to the eye at three-minute intervals will minimize volume build-up, dilution, excess drainage, and overflow. Shorter intervals of administration would reduce ophthalmic bioavailability. The normal lacrimal volume in humans is about 7 μL, and if blinking does not occur the human eye can hold approximately 10 μL. Since the size of commercial ophthalmic drops is between 50 and 75 μL, the loss of drug due to spillage out of the eye can be considered a significant factor in the reduction of bioavailability.

Ophthalmic dosage forms include solutions, ointments, suspensions, lyophilized powders, and oily solutions. Several new dosage forms have recently been introduced to the market. One is an ophthalmic insert, an elliptical device consisting of a drug-containing core surrounded by a flexible copolymer membrane through which pilocarpine diffuses while the ocular delivery system remains in contact with conjunctiva (Occusert). A spray device has also been designed for accurate delivery of drugs.

Polymers such as methylcellulose, hydroxypropyl methylcellulose, and polyvinyl alcohol decrease the surface tension and increase the viscosity of solutions, thus enhancing bioavailability. Soft contact lenses soaked in pilocarpine have also been used. Biodegradable polymers have been employed for the controlled delivery of hydrocortisone and tetracycline.

Nasal Administration

The nasal cavity provides and ideal opportunity for the delivery of drugs. The nasal mucose has high vascularity and offers very little formability of local biotransformation. The pH of the surface is shown 7.2 and drugs are generally absorbed by passive diffusion based on their lipid solubility. A number of drugs are administered intranasally for their local affects such an antibiotics, decongestants, and antihistamines. The systemic delivery has generally been limited to only a few preparations such as extracts of the posterior lobe of pituitary gland to treat diabetics insipidus. Drug developers and researchers are discovering that the accessibility and the vascular structure of the nose make nasal drug delivery an attractive method for

delivering both small molecule drugs and biologics, systemically as well as across the blood–brain barrier to the central nervous system. Nasal delivery offers the potential for faster onset of action and less frequent dosing relative to oral drugs. Nasal delivery of systemic drugs will grow at the expense of the predominant drug delivery methods (oral and parenteral), which cannot be readily optimized for the delivery and dosing of a significant portion of biologically derived drug substances. Recent developments have suggested that insulin, contraceptives, promabotol, lorazepam, midazolam, butorphanol, hydromorphone, and several steroid hormones, vaccines can be administered intranasally for their systemic effects. Significant problems in nasal delivery include the use of aerosol particles, the mucociliary clearance and clearance to the lung. Generally particles larger than 4 μm do not pass into the lung when given nasally. It is expected that in the near future, several drugs may be administered intranasally; especially those which undergo first pass metabolism or show poor stability in the GI tract. However, the recent developments in nanoparticle research are likely to make intranasal drug delivery one of the most prominent areas of pharmaceutical research.

Miscellaneous Routes of Administration

Drugs are also administered through such routes as the urethra, vagina, and spinal cord. For example, urethral suppositories are frequently used for treatment of localized infections. Anesthetics are often administered in the spinal fluid, as are other drugs on occasion for localized effect.

Recent studies suggest that vaginal administration of drugs for systemic effect may be a valid alternative to rectal or even oral routes of administration because of fast and complete absorption from this site. Direct controlled delivery of fertility-controlling hormones has also been successfully made.

Smart dosage forms embedded with electronic sensors are likely to make drug delivery systems more controllable from outside of the body opening up an entirely new area of bioequivalence testing.

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9 Bioanalytical Method Validation

Analytical methods used in the testing of biological fluids must be validated, and although any approach that establishes compliance with the requirements given below is acceptable to regulatory authorities, it is a good idea to follow a formal protocol to the development of these methods.

BACKGROUND

The bioanalytical testing procedures involve such methods as gas chromatography (GC), high-pressure liquid chromatography (LC), combined GC and LC mass spectrometric (MS) procedures, such as LC–MS, LC–MS–MS, GC–MS, and GC–MS–MS, performed for the quantitative determination of drugs and/or metabolites in biological matrices, such as blood, serum, plasma, or urine. Selective and sensitive analytical methods for the quantitative evaluation of drugs and their metabolites (analytes) are critical for the successful conduct of preclinical and/or biopharmaceutics and clinical pharmacology studies. Bioanalytical method validation includes all of the procedures that demonstrate that a particular method used for quantitative measurement of analytes in a given biological matrix, such as blood, plasma, serum, or urine, is reliable and reproducible for the intended use. The fundamental parameters for this validation include (*i*) accuracy, (*ii*) precision, (*iii*) selectivity, (*iv*) sensitivity, (*v*) reproducibility, and (*vi*) stability. Validation involves documenting, through the use of specific laboratory investigations, that the performance characteristics of the method are suitable and reliable for the intended analytical applications. The acceptability of analytical data corresponds directly to the criteria used to validate the method.

Published methods of analysis are often modified to suit the requirements of the laboratory performing the assay. These modifications should be validated to ensure suitable performance of the analytical method. When changes are made to a previously validated method, the analyst should exercise judgment as to how much additional validation is needed. During the course of a typical drug development program, a defined bioanalytical method undergoes many modifications. The evolutionary changes to support specific studies and different levels of validation demonstrate the validity of an assay's performance. Different types and levels of validation are defined and characterized as follows.

FULL VALIDATION

Full validation is important when developing and implementing a bioanalytical method for the first time and is particularly important for a new drug entity or where metabolites are added to an existing assay for quantification.

Partial Validation

Partial validations are modifications of already validated bioanalytical methods. Partial validation can range from as little as one intraassay accuracy and precision determination to

a nearly full validation. Typical bioanalytical method changes that fall into this category include, but are not limited to:

- Bioanalytical method transfers between laboratories or analysts
- Change in analytical methodology (e.g., change in detection systems)
- Change in anticoagulant in harvesting biological fluid
- Change in matrix within species (e.g., human plasma to human urine)
- Change in sample processing procedures
- Change in species within matrix (e.g., rat plasma to mouse plasma)
- Change in relevant concentration range
- Changes in instruments and/or software platforms
- Limited sample volume (e.g., pediatric study)
- Rare matrices
- Selectivity demonstration of an analyte in the presence of concomitant medications
- Selectivity demonstration of an analyte in the presence of specific metabolites

Cross-Validation

Cross-validation is a comparison of validation parameters when two or more bioanalytical methods are used to generate data within the same study or across different studies. An example of cross-validation would be a situation where an original validated bioanalytical method serves as the *reference* and the revised bioanalytical method is the *comparator*. The comparisons should be done both ways.

When sample analyses within a single study are conducted at more than one site or more than one laboratory, cross-validation with spiked matrix standards and subject samples should be conducted at each site or laboratory to establish interlaboratory reliability. Cross-validation should also be considered when data generated using different analytical techniques (e.g., LC–MS–MS vs. ELISA) in different studies are included in a regulatory submission.

All modifications should be assessed to determine the recommended degree of validation. The analytical laboratory conducting pharmacology/toxicology and other preclinical studies for regulatory submissions should adhere to Food and Drug Administration's good laboratory practices (GLPs) (21 CFR part 58) and to sound principles of quality assurance throughout the testing process. The bioanalytical method for human bioavailability, bioequivalence, pharmacokinetic, and drug interaction studies must meet the criteria in 21 CFR 320.29. The analytical laboratory should have a written set of standard operating procedures (SOPs) to ensure a complete system of quality control (QC) and assurance. The SOPs should cover all aspects of analysis from the time the sample is collected and reaches the laboratory until the results of the analysis are reported. The SOPs also should include record keeping, security and chain of sample custody (accountability systems that ensure integrity of test articles), sample preparation, and analytical tools, such as methods, reagents, equipment, instrumentation, and procedures for QC and verification of results.

The process by which a specific bioanalytical method is developed, validated, and used in routine sample analysis can be divided into (*i*) reference standard preparation, (*ii*) bioanalytical method development and establishment of assay procedure, and (*iii*) application of validated bioanalytical method to routine drug analysis and acceptance criteria for the analytical run and/or batch. These three processes are described later in this chapter.

REFERENCE STANDARD

Analysis of drugs and their metabolites in a biological matrix is carried out using samples spiked with calibration (reference) standards and QC samples. The purity of the reference standard used to prepare spiked samples can affect study data. For this reason, an authenticated analytical reference standard of known identity and purity should be used to prepare solutions of known concentrations. If possible, the reference standard should be identical to the analyte. When this is not possible, an established chemical form (free base or acid, salt or ester)

of known purity can be used. Three types of reference standards are usually used: (*i*) certified reference standards [e.g., USP (U.S. Pharmacopoeia) compendial standards], (*ii*) commercially supplied reference standards obtained from a reputable commercial source, and/or (*iii*) other materials of documented purity custom-synthesized by an analytical laboratory or other noncommercial establishment. The source and lot number, expiration date, certificates of analyses when available, and/or internally or externally generated evidence of identity and purity should be furnished for each reference standard.

METHOD DEVELOPMENT

The method development and establishment phase defines the chemical assay. The fundamental parameters for a bioanalytical method validation are accuracy, precision, selectivity, sensitivity, reproducibility, and stability. Measurements for each analyte in the biological matrix should be validated. In addition, the stability of the analyte in spiked samples should be determined. Typical method development and establishment for a bioanalytical method include determination of (*i*) selectivity, (*ii*) accuracy, precision, recovery, (*iii*) calibration curve, and (*iv*) stability of analyte in spiked samples.

Selectivity is the ability of an analytical method to differentiate and quantify the analyte in the presence of other components in the sample. For selectivity, analyses of blank samples of the appropriate biological matrix (plasma, urine, or other matrix) should be obtained from at least six sources. Each blank sample should be tested for interference, and selectivity should be ensured at the lower limit of quantification (LLOQ). Potential interfering substances in a biological matrix include endogenous matrix components, metabolites, decomposition products, and in the actual study, concomitant medication and other exogenous xenobiotics. If the method is intended to quantify more than one analyte, each analyte should be tested to ensure that there is no interference.

The accuracy of an analytical method describes the closeness of mean test results obtained by the method to the true value (concentration) of the analyte. Accuracy is determined by replicate analysis of samples containing known amounts of the analyte. It should be measured using a minimum of five determinations per concentration. A minimum of three concentrations in the range of expected concentrations is recommended. The mean value should be within 15% of the actual value except at LLOQ, where it should not deviate by more than 20%. The deviation of the mean from the true value serves as the measure of accuracy.

The precision of an analytical method describes the closeness of individual measures of an analyte when the procedure is applied repeatedly to multiple aliquots of a single homogeneous volume of biological matrix. Precision should be measured using a minimum of five determinations per concentration. A minimum of three concentrations in the range of expected concentrations is recommended. The precision determined at each concentration level should not exceed 15% of the coefficient of variation (CV) except for the LLOQ, where it should not exceed 20% of the CV. Precision is further subdivided into within-run, intra-batch precision or repeatability, which assesses precision during a single analytical run, and between-run, inter-batch precision or repeatability, which measures precision with time, and may involve different analysts, equipment, reagents, and laboratories.

The recovery of an analyte in an assay is the detector response obtained from an amount of the analyte added to and extracted from the biological matrix compared to the detector response obtained for the true concentration of the pure authentic standard. Recovery pertains to the extraction efficiency of an analytical method within the limits of variability. Recovery of the analyte need not be 100%, but the extent of recovery of an analyte and of the internal standard should be consistent, precise, and reproducible. Recovery experiments should be performed by comparing the analytical results for extracted samples at three concentrations (low, medium, and high) with unextracted standards that represent 100% recovery.

Calibration

A calibration (standard) curve is the relationship between instrument response and known concentrations of the analyte. A calibration curve should be generated for each analyte in the

sample. A sufficient number of standards should be used to adequately define the relationship between concentration and response. A calibration curve should be prepared in the same biological matrix as the samples in the intended study by spiking the matrix with known concentrations of the analyte. The number of standards used in constructing a calibration curve will be a function of the anticipated range of analytical values and the nature of the analyte/response relationship. Concentrations of standards should be chosen on the basis of the concentration range expected in a particular study. A calibration curve should consist of a blank sample (matrix sample processed without internal standard), a zero sample (matrix sample processed with internal standard), and six to eight nonzero samples covering the expected range, including LLOQ.

- *LLOQ*: The lowest standard on the calibration curve should be accepted as the limit of quantification if the following conditions are met:
  - The analyte response at the LLOQ should be at least five times the response compared to blank response.
  - Analyte peak (response) should be identifiable, discrete, and reproducible with a precision of 20% and accuracy of 80% to 120%.
- *Calibration curve/standard curve/concentration–response*: The simplest model that adequately describes the concentration–response relationship should be used. Selection of weighting and use of a complex regression equation should be justified. The following conditions should be met in developing a calibration curve:
  - 20% deviation of the LLOQ from nominal concentration
  - 15% deviation of standards other than LLOQ from nominal concentration

At least four out of six nonzero standards should meet the above criteria, including the LLOQ and the calibration standard at the highest concentration. Excluding the standards should not change the model used.

Stability

Drug stability in a biological fluid is a function of the storage conditions, the chemical properties of the drug, the matrix, and the container system. The stability of an analyte in a particular matrix and container system is relevant only to that matrix and container system and should not be extrapolated to other matrices and container systems. Stability procedures should evaluate the stability of the analytes during sample collection and handling, after long-term (frozen at the intended storage temperature) and short-term [bench top, room temperature (RT)] storage, and after going through freeze and thaw cycles and the analytical process. Conditions used in stability experiments should reflect situations likely to be encountered during actual sample handling and analysis. The procedure should also include an evaluation of analyte stability in stock solution.

All stability determinations should use a set of samples prepared from a freshly made stock solution of the analyte in the appropriate analyte-free, interference-free biological matrix. Stock solutions of the analyte for stability evaluation should be prepared in an appropriate solvent at known concentrations.

- *Freeze and thaw stability*: Analyte stability should be determined after three freeze and thaw cycles. At least three aliquots at each of the low and high concentrations should be stored at the intended storage temperature for 24 hours and thawed unassisted at RT. When completely thawed, the samples should be refrozen for 12 to 24 hours under the same conditions. The freeze–thaw cycle should be repeated two more times then analyzed on the third cycle. If an analyte is unstable at the intended storage temperature, the stability sample should be frozen at −70°C during the three freeze and thaw cycles.
- *Short-term temperature stability*: Three aliquots of each of the low and high concentrations should be thawed at RT and kept at this temperature from 4 to 24 hours (based on the expected duration that samples will be maintained at RT in the intended study) and analyzed.

- *Long-term stability*: The storage time in a long-term stability evaluation should exceed the time between the date of first sample collection and the date of last sample analysis. Long-term stability should be determined by storing at least three aliquots of each of the low and high concentrations under the same conditions as the study samples. The volume of samples should be sufficient for analysis on three separate occasions. The concentrations of all the stability samples should be compared to the mean of back-calculated values for the standards at the appropriate concentrations from the first day of long-term stability testing.
- *Stock solution stability*: The stability of stock solutions of drug and the internal standard should be evaluated at RT for at least six hours. If the stock solutions are refrigerated or frozen for the relevant period, the stability should be documented. After completion of the desired storage time, the stability should be tested by comparing the instrument response with that of freshly prepared solutions.
- *Postpreparative stability*: The stability of processed samples, including the resident time in the autosampler, should be determined. The stability of the drug and the internal standard should be assessed over the anticipated run time for the batch size in validation samples by determining concentrations on the basis of original calibration standards.

Although the traditional approach of comparing analytical results for stored samples with those for freshly prepared samples has been referred to in this chapter, other statistical approaches based on confidence limits for evaluation of an analyte's stability in a biological matrix can be used. SOPs should clearly describe the statistical method and rules used. Additional validation may include investigation of samples from dosed subjects.

Principles of Bioanalytical Method Validation and Establishment

The fundamental parameters to ensure the acceptability of the performance of a bioanalytical method validation are accuracy, precision, selectivity, sensitivity, reproducibility, and stability. A specific, detailed description of the bioanalytical method should be written. This can be in the form of a protocol, study plan, report, and/or SOP. Each step in the method should be investigated to determine the extent to which environmental, matrix, material, or procedural variables can affect the estimation of analyte in the matrix from the time of collection of the material up to and including the time of analysis. It may be important to consider the variability of the matrix due to the physiological nature of the sample. In the case of LC–MS–MS-based procedures, appropriate steps should be taken to ensure the lack of matrix effects throughout the application of the method, especially if the nature of the matrix changes from the matrix used during method validation. A bioanalytical method should be validated for the intended use or application. All experiments used to make claims or draw conclusions about the validity of the method should be presented in a report (method validation report). Whenever possible, the same biological matrix as the matrix in the intended samples should be used for validation purposes. (For tissues of limited availability, such as bone marrow, physiologically appropriate proxy matrices can be substituted.)

The stability of the analyte (drug and/or metabolite) in the matrix during the collection process and the sample storage period should be assessed, preferably prior to sample analysis. For compounds with potentially labile metabolites, the stability of analyte in matrix from dosed subjects (or species) should be confirmed. The accuracy, precision, reproducibility, response function, and selectivity of the method for endogenous substances, metabolites, and known degradation products should be established for the biological matrix. For selectivity, there should be evidence that the substance being quantified is the intended analyte. The concentration range over which the analyte will be determined should be defined in the bioanalytical method, based on evaluation of actual standard samples over the range, including their statistical variation. This defines the standard curve. A sufficient number of standards should be used to adequately define the relationship between concentration and response. The relationship between response and concentration should be demonstrated to be continuous and reproducible. The number of standards used should be a function of the dynamic range and nature of the concentration–response relationship. In many cases, six to eight concentrations (excluding blank values) can define the standard curve. More standard concentrations may be

recommended for nonlinear than for linear relationships. The ability to dilute samples originally above the upper limit of the standard curve should be demonstrated by accuracy and precision parameters in the validation.

In consideration of high-throughput analyses, including but not limited to multiplexing, multicolumn, and parallel systems, sufficient QC samples should be used to ensure control of the assay. The number of QC samples to ensure proper control of the assay should be determined based on the run size. The placement of QC samples should be judiciously considered in the run. For a bioanalytical method to be considered valid, specific acceptance criteria should be set in advance and achieved for accuracy and precision for the validation of QC samples over the range of the standards.

Specific Recommendations for Method Validation

The matrix-based standard curve should consist of a minimum of six standard points, excluding blanks, using single or replicate samples. The standard curve should cover the entire range of expected concentrations. Standard curve fitting is determined by applying the simplest model that adequately describes the concentration–response relationship using appropriate weighting and statistical tests for *goodness of fit*. LLOQ is the lowest concentration of the standard curve that can be measured with acceptable accuracy and precision. The LLOQ should be established using at least five samples independent of standards and determining the CV and/or appropriate confidence interval. The LLOQ should serve as the lowest concentration on the standard curve and should not be confused with the limit of detection and/or the low QC sample. The highest standard will define the upper limit of quantification (ULOQ) of an analytical method.

For validation of the bioanalytical method, accuracy and precision should be determined using a minimum of five determinations per concentration level (excluding blank samples). The mean value should be within $\pm 15\%$ of the theoretical value, except at LLOQ, where it should not deviate by more than $\pm 20\%$. The precision around the mean value should not exceed 15% of the CV, except for LLOQ, where it should not exceed 20% of the CV. Other methods of assessing accuracy and precision that meet these limits may be equally acceptable.

The accuracy and precision with which known concentrations of analyte in biological matrix can be determined should be demonstrated. This can be accomplished through analysis of replicate sets of analyte samples of known concentrations (QC samples) from an equivalent biological matrix. At a minimum, three concentrations representing the entire range of the standard curve should be studied: one within three times the LLOQ (low QC sample), one near the center (middle QC), and one near the upper boundary of the standard curve (high QC).

Reported method validation data and the determination of accuracy and precision should include all outliers; however, calculations of accuracy and precision excluding values that are statistically determined as outliers can also be reported.

The stability of the analyte in biological matrix at intended storage temperatures should be established. The influence of freeze–thaw cycles (a minimum of three cycles at two concentrations in triplicate) should be studied. The stability of the analyte in matrix at ambient temperature should be evaluated over a time period equal to the typical sample preparation, sample handling, and analytical run times.

Reinjection reproducibility should be evaluated to determine if an analytical run could be reanalyzed in the case of instrument failure. The specificity of the assay methodology should be established using a minimum of six independent sources of the same matrix. For hyphenated MS-based methods, however, testing six independent matrices for interference may not be important. In the case of LC–MS and LC–MS–MS-based procedures, matrix effects should be investigated to ensure that precision, selectivity, and sensitivity will not be compromised. Method selectivity should be evaluated during method development and throughout method validation and can continue throughout application of the method to actual study samples.

Acceptance/rejection criteria for spiked, matrix-based calibration standards, and validation QC samples should be based on the nominal (theoretical) concentration of analytes. Specific criteria can be set up in advance and achieved for accuracy and precision over the range of the standards, if so desired.

Microbiological and Ligand-Binding Assays

Many of the bioanalytical validation parameters and principles previously discussed are also applicable to microbiological and ligand-binding assays. However, these assays possess some unique characteristics that should be considered during method validation.

Selectivity Issues

As with chromatographic methods, microbiological and ligand-binding assays should be shown to be selective for the analyte. The following recommendations for dealing with two selectivity issues should be considered:

- Interference from substances physiochemically similar to the analyte
  - Cross-reactivity of metabolites, concomitant medications, or endogenous compounds should be evaluated individually and in combination with the analyte of interest.
  - When possible, the immunoassay should be compared with a validated reference method (such as LC–MS) using incurred samples and predetermined criteria for agreement of accuracy of immunoassay and reference method.
  - The dilutional linearity to the reference standard should be assessed using study (incurred) samples.
  - Selectivity may be improved for some analytes by incorporation of separation steps prior to immunoassay.
- Matrix effects unrelated to the analyte
  - The standard curve in biological fluids should be compared with standard in buffer to detect matrix effects.
  - Parallelism of diluted study samples should be evaluated with diluted standards to detect matrix effects.
  - Nonspecific binding should be determined.

Quantification Issues

Microbiological and immunoassay standard curves are inherently nonlinear and, in general, more concentration points may be recommended to define the fit over the standard curve range than for chemical assays. In addition to their nonlinear characteristics, the response–error relationship for immunoassay standard curves is a nonconstant function of the mean response (heteroscadisticity). For these reasons, a minimum of six nonzero calibrator concentrations, run in duplicate, is recommended. The concentration–response relationship is most often fitted to a four- or five-parameter logistic model, although others may be used with suitable validation. The use of anchoring points in the asymptotic high- and low-concentration ends of the standard curve may improve the overall curve fit. Generally, these anchoring points will be at concentrations that are below the established LLOQ and above the established ULOQ. Whenever possible, calibrators should be prepared in the same matrix as the study samples or in an alternate matrix of equivalent performance. Both ULOQ and LLOQ should be defined by acceptable accuracy, precision, or confidence interval criteria based on the study requirements.

For all assays, the key factor is the accuracy of the reported results. This accuracy can be improved by the use of replicate samples. In the case where replicate samples should be measured during the validation to improve accuracy, the same procedure should be followed as for unknown samples. The following recommendations apply to quantification issues:

- If separation is used prior to assay for study samples but not for standards, it is important to establish recovery and use it in determining results. Possible approaches to assess efficiency

and reproducibility of recovery are (*i*) the use of radiolabeled tracer analyte (quantity too small to affect the assay), (*ii*) the advance establishment of reproducible recovery, (*iii*) the use of an internal standard that is not recognized by the antibody but can be measured by another technique.

- Key reagents, such as antibody, tracer, reference standard, and matrix should be characterized appropriately and stored under defined conditions.
- Assessments of analyte stability should be conducted in true study matrix (e.g., should not use a matrix stripped to remove endogenous interferences).
- Acceptance criteria: At least 67% (four out of six) of QC samples should be within 15% of their respective nominal value, 33% of the QC samples (not all replicates at the same concentration) may be outside 15% of nominal value. In certain situations, wider acceptance criteria may be justified.
- Assay reoptimization or validation may be important when there are changes in key reagents, as follows:
  - Labeled analyte (tracer)
  - Binding should be reoptimized
  - Performance should be verified with standard curve and QCs
  - Antibody
  - Key cross-reactivities should be checked
  - Tracer experiments above should be repeated
  - Matrix
  - Tracer experiments above should be repeated.

Method development experiments should include a minimum of six runs conducted over several days, with at least four concentrations (LLOQ, low, medium, and high) analyzed in duplicate in each run.

Application of Validated Method to Routine Analysis

Assays of all samples of an analyte in a biological matrix should be completed within the time period for which stability data are available. In general, biological samples can be analyzed with a single determination without duplicate or replicate analysis if the assay method has acceptable variability as defined by validation data. This is true for procedures where precision and accuracy variabilities routinely fall within acceptable tolerance limits. For a difficult procedure with a labile analyte where high precision and accuracy specifications may be difficult to achieve, duplicate or even triplicate analyses can be performed for a better estimate of analyte.

A calibration curve should be generated for each analyte to assay samples in each analytical run and should be used to calculate the concentration of the analyte in the unknown samples in the run. The spiked samples can contain more than one analyte. An analytical run can consist of QC samples, calibration standards, and either (*i*) all the processed samples to be analyzed as one batch or (*ii*) a batch composed of processed unknown samples of one or more volunteers in a study. The calibration (standard) curve should cover the expected unknown sample concentration range in addition to a calibrator sample at LLOQ. Estimation of concentration in unknown samples by extrapolation of standard curves below LLOQ or above the highest standard is not recommended. Instead, the standard curve should be redefined or samples with higher concentration should be diluted and reassayed. It is preferable to analyze all study samples from a subject in a single run.

Once the analytical method has been validated for routine use, its accuracy and precision should be monitored regularly to ensure that the method continues to perform satisfactorily. To achieve this objective, a number of QC samples prepared separately should be analyzed with processed test samples at intervals based on the total number of samples. The QC samples in duplicate at three concentrations [one near the LLOQ (i.e., $\pm 3\times$LLOQ], one in midrange, and one close to the high end of the range) should be incorporated in each assay run. The number of QC samples (in multiples of three) will depend on the total number of samples in the run. The results of the QC samples provide the basis of accepting or rejecting the run. At least four of

every six QC samples should be within ±15% of their respective nominal value. Two out of the six QC samples may be outside the ±15% of their respective nominal value, but not both at the same concentration.

The following recommendations should be noted in applying a bioanalytical method to routine drug analysis:

- A matrix-based standard curve should consist of a minimum of six standard points, excluding blanks (either single or replicate), covering the entire range.
- Response function: Typically, the same curve fitting, weighting, and goodness of fit determined during prestudy validation should be used for the standard curve within the study. Response function is determined by appropriate statistical tests based on the actual standard points during each run in the validation. Changes in the response function relationship between prestudy validation and routine run validation indicate potential problems.
- The QC samples should be used to accept or reject the run. These QC samples are matrix spiked with analyte.
- System suitability: Based on the analyte and technique, a specific SOP (or sample) should be identified to ensure optimum operation of the system used.
- Any required sample dilutions should use like matrix (e.g., human-to-human) obviating the need to incorporate actual within-study dilution matrix QC samples.
- Repeat analysis: It is important to establish an SOP or guideline for repeat analysis and acceptance criteria. This SOP or guideline should explain the reasons for repeating sample analysis. Reasons for repeat analyses could include repeat analysis of clinical or preclinical samples for regulatory purposes, inconsistent replicate analysis, samples outside of the assay range, sample processing errors, equipment failure, poor chromatography, and inconsistent pharmacokinetic data. Reassays should be done in triplicate if sample volume allows. The rationale for the repeat analysis and the reporting of the repeat analysis should be clearly documented.
- Sample data reintegration: An SOP or guideline for sample data reintegration should be established. This SOP or guideline should explain the reasons for reintegration and how the reintegration is to be performed. The rationale for the reintegration should be clearly described and documented. Original and reintegration data should be reported.

Acceptance Criteria for the Run

The following acceptance criteria should be considered for accepting the analytical run:

- Standards and QC samples can be prepared from the same spiking stock solution, provided the solution stability and accuracy have been verified. A single source of matrix may also be used, provided selectivity has been verified.
- Standard curve samples, blanks, QCs, and study samples can be arranged as considered appropriate within the run.
- Placement of standards and QC samples within a run should be designed to detect assay drift over the run.
- Matrix-based standard calibration samples: 75%, or a minimum of six standards, when back-calculated (including ULOQ) should fall within 15%, except for LLOQ, when it should be 20% of the nominal value. Values falling outside these limits can be discarded, provided they do not change the established model.
- Acceptance criteria for accuracy and precision as outlined in the section "Specific Recommendations for Method Validation," should be provided for both the intraday and intra-run experiment.
- QC samples: The QC samples replicated (at least once) at a minimum of three concentrations [one within three times of the LLOQ (low QC), one in the midrange (middle QC), and one approaching the high end of the range (high QC)] should be incorporated into each run. The results of the QC samples provide the basis of accepting or rejecting the run. At least 67% (four out of six) of the QC samples should be within 15% of their respective

nominal (theoretical) values; 33% of the QC samples (not all replicates at the same concentration) can be outside the 15% of the nominal value. A confidence interval approach yielding comparable accuracy and precision is an appropriate alternative.
- The minimum number of samples (in multiples of three) should be at least 5% of the number of unknown samples or six total QCs, whichever is greater.
- Samples involving multiple analytes should not be rejected based on the data from one analyte failing the acceptance criteria.
- The data from rejected runs need not be documented, but the fact that a run was rejected and the reason for failure should be recorded.

Documentation

The validity of an analytical method should be established and verified by laboratory studies, and documentation of successful completion of such studies should be provided in the assay validation report. General and specific SOPs and good record keeping are an essential part of a validated analytical method. The data generated for bioanalytical method establishment and the QCs should be documented and available for data audit and inspection. Documentation for submission to the agency should include (*i*) summary information, (*ii*) method development and establishment, (*iii*) bioanalytical reports of the application of any methods to routine sample analysis, and (*iv*) other information applicable to method development and establishment and/or to routine sample analysis.

Summary Information

Summary information should include:

- Summary table of validation reports, including analytical method validation, partial revalidation, and cross-validation reports. The table should be in chronological sequence, and include assay method identification code, type of assay, and the reason for the new method or additional validation (e.g., to lower the limit of quantitation).
- Summary table with a list, by protocol, of assay methods used. The protocol number, protocol title, assay type, assay method identification code, and bioanalytic report code should be provided.
- A summary table allowing cross-referencing of multiple identification codes should be provided (e.g., when an assay has different codes for the assay method, validation reports, and bioanalytical reports, especially when the sponsor and a contract laboratory assign different codes).

Documentation for Method Establishment

Documentation for method development and establishment should include:

- An operational description of the analytical method
- Evidence of purity and identity of drug standards, metabolite standards, and internal standards used in validation experiments
- A description of stability studies and supporting data
- A description of experiments conducted to determine accuracy, precision, recovery, selectivity, limit of quantification, calibration curve (equations and weighting functions used, if any), and relevant data obtained from these studies
- Documentation of intra- and interassay precision and accuracy
- In new drug application submissions, information about cross-validation study data, if applicable
- Legible annotated chromatograms or mass spectrograms, if applicable
- Any deviations from SOPs, protocols, or GLPs (if applicable), and justifications for deviations

Application to Routine Drug Analysis

Documentation of the application of validated bioanalytical methods to routine drug analysis should include:

- Evidence of purity and identity of drug standards, metabolite standards, and internal standards used during routine analyses.
- Summary tables containing information on sample processing and storage. Tables should include sample identification, collection dates, storage prior to shipment, information on shipment batch, and storage prior to analysis. Information should include dates, times, sample condition, and any deviation from protocols.
- Summary tables of analytical runs of clinical or preclinical samples. Information should include assay run identification, date and time of analysis, assay method, analysts, start and stop times, duration, significant equipment and material changes, and any potential issues or deviation from the established method.
- Equations used for back-calculation of results.
- Tables of calibration curve data used in analyzing samples and calibration curve summary data.
- Summary information on intra- and interassay values of QC samples and data on intra- and interassay accuracy and precision from calibration curves and QC samples used for accepting the analytical run. QC graphs and trend analyses in addition to raw data and summary statistics are encouraged.
- Data tables from analytical runs of clinical or preclinical samples. Tables should include assay run identification, sample identification, raw data and back-calculated results, integration codes, and/or other reporting codes.
- Complete serial chromatograms from 5% to 20% of subjects, with standards and QC samples from those analytical runs. For pivotal bioequivalence studies for marketing, chromatograms from 20% of serially selected subjects should be included. In other studies, chromatograms from 5% of randomly selected subjects in each study should be included. Subjects whose chromatograms are to be submitted should be defined prior to the analysis of any clinical samples.
- Reasons for missing samples.
- Documentation for repeat analyses. Documentation should include the initial and repeat analysis results, the reported result, assay run identification, the reason for the repeat analysis, the requestor of the repeat analysis, and the manager authorizing reanalysis. Repeat analysis of a clinical or preclinical sample should be performed only under a predefined SOP.
- Documentation for reintegrated data. Documentation should include the initial and repeat integration results, the method used for reintegration, the reported result, assay run identification, the reason for the reintegration, the requestor of the reintegration, and the manager authorizing reintegration. Reintegration of a clinical or preclinical sample should be performed only under a predefined SOP.
- Deviations from the analysis protocol or SOP, with reasons and justifications for the deviations.

Other Information

Other information applicable to both method development and establishment and/or to routine sample analysis could include:

- Lists of abbreviations and any additional codes used, including sample condition codes, integration codes, and reporting codes
- Reference lists and legible copies of any references
- SOPs or protocols covering the following areas:
  - Calibration standard acceptance or rejection criteria
  - Calibration curve acceptance or rejection criteria

- The QC sample and assay run acceptance or rejection criteria
- Acceptance criteria for reported values when all unknown samples are assayed in duplicate
- Sample code designations, including clinical or preclinical sample codes and bioassay sample code
- Assignment of clinical or preclinical samples to assay batches
- Sample collection, processing, and storage
- Repeat analyses of samples
- Reintegration of samples

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APPENDIX A: VALIDATION REPORT

A high performance liquid chromatographic (HPLC) method for the determination of niazin in human plasma. (Note: niazin and sarfarin are drugs yet to be developed.)

INTRODUCTION

Niazin is a new modulator of leptin. Common clinical uses of niazin include appetite control and weight loss.

SUMMARY

An accurate, sensitive, and reproducible HPLC method for the quantitation of niazin in plasma using sarfarin as an internal standard has been developed and validated. The procedure involves protein precipitation with 10% trichloroacetic acid. The drug and the internal standard were eluted from a symmetry C_{18} stainless steel column (5 μm, 3.9 mm i.d.$\times$150 mm) at RT with a mobile phase consisting of acetonitrile and potassium dihydrogen phosphate buffer (12:88), % (v/v) at pH 3.00 and at a flow rate of 2.0 ml/min. A UV detector set at 280 nm was used to monitor the effluent. Each analysis required no longer than 10 minutes. Quantitation

was achieved by measurement of the peak area ratio of the drug to the internal standard. The limit of quantification of niazin in plasma was 0.25 μg/mL.

The intraday accuracy of the method for niazin ranged from 97.08% to 102.00%, while the intraday precision ranged from 1.719% to 3.137%. The interday accuracy ranged from 98.00% to 99.87%, while the interday precision ranged from 1.928% to 5.306%. The absolute analytical recovery of niazin was 93.23% and for the internal standard (sarfarin) was 85.13%. The relative analytical recovery of niazin ranged from 98.56% to 100.11%. Stability study showed that niazin is stable (short-term RT) for 4 hours, and for 16 hours (after preparation) at RT and for 48 hours at −20°C. Niazin is stable for three cycles of freeze and thaw, when stored at −20°C and thawed at RT. The stock solution of niazin is stable at RT for six hours and at −20°C for four weeks. The stock solutions of the internal standard are stable at RT for six hours and at 20°C for two weeks. Niazin is stable in plasma (long-term) for four weeks when stored at −20°C.

DESCRIPTION OF THE COMPOUNDS

Niazin:
Molecular formula:
Molecular weight:
Sarfarin (I.S.):
Molecular formula:
Molecular weight:

EXPERIMENTAL

HPLC method has been developed for the quantification of niazin in human plasma.

Instrumentation

HPLC System, Alliance, consisted of a solvent delivery pump and Autosampler (2690), UV detector (2487), and Millennium Software for Chromatographic determination and evaluation (Waters Associates, Bedford, Massachusetts, U.S.A.).

Column

A symmetry C_{18} stainless steel (5 μm, 3.9 mm i.d.×150 mm) (Waters Associates, Bedford, Massachusetts, U.S.A.). A guard column of the same material was used (Waters Associates, Bedford, Massachusetts, U.S.A.).

Reagents

All solvents used were of HPLC grade, while other chemicals and reagents were of spectro-quality or analytical grade. Niazin and sarfarin were obtained from Sigma-Aldrich Chemical Company (St Louis, Missouri, U.S.A.).

Stock and Working Standard Solutions Preparation

Niazin

Stock standard solution: Amount equivalent to 10.00 mg niazin was weighed using niazin standard powder and dissolved in 100 mL deionized water (Milli-Q. water) to produce a concentration of 100 μg/mL. This solution was stable at 20°C for at least four weeks.

Sarfarin

Stock standard solution: Amount equivalent to 10.00 mg sarfarin was weighed and dissolved in 10.0 mL deionized water (Milli-Q. water) to produce a concentration of 1 mg/mL. This solution was stable at 20°C for at least two weeks.

Working internal standard solution: 1 mL of the stock solution was completed to 10 mL with deionized water (Milli-Q. water) to produce a working concentration of 100.00 μg/mL. The working standard solution was freshly prepared daily.

Standard Calibration Curve Samples Preparation

Eleven volumetric flasks (25 mL) were labeled as blank sample, standard zero sample, 0.25, 0.50, 1.00, 2.00, 3.00, 4.00, 6.00, 8.00, and 10.00 μg/mL. The following volume of niazin's stock standard solution (100 μg/mL), shown in the following table, were diluted up to 25 mL with blank human plasma and vortexed for five minutes.

Concentration (μg/mL)	Volume of stock standard solution (μL)
Blank sample	0
Standard zero sample	0
0.25	62.50
0.50	125.00
1.00	250.00
2.00	500.00
3.00	750.00
4.00	1000.00
6.00	1500.00
8.00	2000.00
10.00	2500.00

Aliquot of 0.400 mL samples from each volumetric flask were then transferred into a 1.5 mL Eppendorf centrifuge tubes and stored at −20°C.

QC Samples Preparation

Four volumetric flasks (25 mL) were labeled as 0.25, 0.75, 5.00, and 9.00 μg/mL and to each volumetric flask, the volume of niazin's stock standard solution shown in the below table were diluted up to 25 mL with blank human plasma and vortexed for five minutes.

QC label	Concentration (μg/mL)	Stock standard solution (μL)
LLOQ	0.25	62.50
Low QC	0.75	187.50
Medium QC	5.00	1250.00
High QC	9.00	2250.00

Abbreviations: LLOQ, lower limit of quantification; QC, quality control.

Aliquot of 0.400 mL samples from each volumetric flask were then transferred into a 1.5 mL Eppendorf centrifuge tubes and stored at −20°C.

Description of Method

Sample Preparation

A 100 μL of internal standard (sarfarin) working solution was added to 400 μL plasma sample (standard sample, control sample, or volunteer sample), then 100 μL of trichloroacetic acid solution (10%) was spiked. The samples were vortex for 30 seconds, centrifuged for 10 minutes at 13,000 rpm, then 100 μL aliquot sample of the supernatant were injected and chromatographed onto a symmetry C_{18} (5 μm) (150×3.9 mm) column. Niazin and the internal standard were separated from endogenous plasma substances.

Column
A symmetry (5 μm) (150×3.9 mm) column (Waters Associates, Bedford, Massachusetts, U.S.A.).

Mobile Phase
The mobile phase consisted of acetonitrile and potassium dihydrogen phosphate buffer (12:88), % (v/v) was used. The mobile phase pH was adjusted to 3.00 using phosphoric acid. The mobile phase was degassed daily by passing it through a 0.22 μm membrane filter (Millipore, Bedford, Massachusetts, U.S.A.).

Injection volume: 100 μL.
Flow rate: 2.00 mL/min.
Temperature: ambient temperature.

Standardization and Calculation
The standard calibration curve lines were shown to be linear from 0.25 to 10 μg/mL for niazin in human plasma. Calibration lines of peak area ratios (peak area analyte/peak area internal standard) versus concentration were determined by single-level calibration curve (linear regression equation, $Y=BX+A$), where X is the concentration (μg/mL), B is the slope, Y is the peak area ratio, and A is the intercept.

Terms of quantification:

Mean was calculated using the equation:

$$\text{Mean} = \frac{\sum_{i=1}^{N}(x_i)}{N},$$

where N is the number of samples.

Standard deviation (SD) was evaluated according to the equation:

$$\text{Standard deviation (SD)} = \sqrt{\frac{\sum_{i=1}^{N}(x_i-\bar{x})^2}{N-1}}$$

The CVs were evaluated according to the equation:

$$\text{CV (\%)} = \frac{\text{Standard deviation}}{\text{Mean}} \times 100$$

The accuracy was evaluated according to the equation:

$$\text{Accuracy (\%)} = \frac{\text{Mean value}}{\text{Nominal value}} \times 100$$

RESULTS AND DISCUSSION

Method Development

A HPLC method for the quantification of niazin concentrations in human plasma was developed. The analysis was performed on a symmetry C_{18} (5 μm) (150×3.9 mm) HPLC column using acetonitrile:potassium dihydrogen phosphate buffer (12:88), % (v/v), at pH 3.00. The analytes were monitored by the measurement of the peak area of both niazin and the internal standard, and the peak area ratio was calculated.

Validation Study

The validation of this procedure was performed in order to evaluate the method in terms of:

1. Linearity, accuracy, precision, and sensitivity
2. Specificity
3. Recovery
4. Stability

Linearity, Accuracy, Precision, and Sensitivity

For the determination of linearity, accuracy, precision, and sensitivity standard calibration curves of 9 points (nonzero standards) and 10 sets of four spiked QC samples were prepared and analyzed on day 1, for days 2 and 3, the validation included the analysis of plasma sample representing two complete standard calibration curves plus five sets of four QC samples. The calibration curves were evaluated individually by linear regression. The results of peak area ratio of 10 measured calibration curves for niazin are given in Table A.1. The coefficient of correlation was consistently greater than or equal to 0.9991 during the course of validation, data are presented in Table A.1. The concentrations of the calibration standards were back-calculated, the means, SD, CV (%), and accuracy (%) were calculated for the back-calculated concentrations of each standard calibration curve, data are presented in Table A.2. A representative standard calibration curve plot is shown in Figures A.1.

Intraday Accuracy and Precision

The intraday accuracy and precision of the assay were measured by analyzing 10 samples of each spiked QC sample of niazin, the concentrations were calculated by the average of the two standard calibration curves analyzed in that day. The statistical summary includes means, SD, CVs (%), and accuracy (%). Intraday accuracy of the method for niazin ranged from 97.08% to 102.00%, while the precision ranged from 1.719% to 3.137% at the concentrations of 0.25, 0.75, 5.00, and 9.00 μg/mL. Data are presented in Table A.3.

Interday Accuracy and Precision

The interday accuracy and precision of the assay were measured by analyzing 20 samples of each spiked QC of niazin obtained from days 1, 2, and 3 over two-week period, the concentrations were calculated by the average of the two calibration curves analyzed in that day. The statistical summary includes means, SD, CVs (%), and accuracy (%). Interday accuracy of the method for niazin ranged from 98.00% to 99.87%, while the precision ranged from 1.928% to 5.306%, at the concentrations of 0.25, 0.75, 5.00, and 9.00 (g/mL. Data are presented in Table A.4.

TABLE A.1 Peak Area Ratio Values of 10 Standard Calibration Curves of Niazin in Human Plasma

Concentration (μg/mL)	Peak area ratio									
	# 1	# 2	# 3	# 4	# 5	# 6	# 7	# 8	# 9	# 10
0.25	0.027	0.028	0.029	0.024	0.024	0.027	0.025	0.024	0.026	0.026
0.50	0.053	0.057	0.066	0.047	0.047	0.045	0.045	0.057	0.054	0.045
1.00	0.111	0.116	0.129	0.098	0.093	0.096	0.098	0.108	0.098	0.096
2.00	0.220	0.220	0.203	0.187	0.184	0.183	0.198	0.214	0.210	0.173
3.00	0.328	0.320	0.294	0.288	0.272	0.302	0.296	0.329	0.318	0.274
4.00	0.435	0.425	0.401	0.394	0.386	0.381	0.376	0.410	0.411	0.393
6.00	0.654	0.619	0.598	0.582	0.576	0.594	0.576	0.623	0.621	0.599
8.00	0.879	0.831	0.812	0.784	0.774	0.786	0.773	0.806	0.814	0.767
10.00	1.060	1.031	1.033	0.961	0.975	0.961	0.946	1.068	1.020	0.942
Intercept	0.0041	0.0099	0.0061	−0.0001	−0.0069	−0.0006	0.0030	0.0022	0.0035	−0.0025
Slope	0.1073	0.1024	0.1010	0.0973	0.0976	0.0973	0.0951	0.1042	0.1019	0.0960
R	0.9997	0.9999	0.9993	0.9997	0.9998	0.9997	0.9998	0.9991	0.9999	0.9992

TABLE A.2 Back-Calculated Niazin Concentrations of the Calibration Standards in Human Plasma

Concentration (μg/mL)	# 1	# 2	# 3	# 4	# 5	# 6	# 7	# 8	# 9	# 10
0.25	0.2008	0.2000	0.2647	0.2142	0.2847	0.3054	0.2247	0.2147	0.2525	0.2525
0.50	0.4384	0.4766	0.5385	0.4466	0.5207	0.5001	0.4254	0.5459	0.5357	0.4446
1.00	0.9916	1.0393	1.0729	0.9618	0.9927	1.0234	0.9573	1.0577	0.9807	0.9605
2.00	2.0311	2.0311	2.0225	1.8609	1.9264	1.9161	1.9609	2.1215	2.1135	1.7393
3.00	3.0611	2.9848	2.9418	2.8812	2.8293	3.1372	2.9445	3.2757	3.2059	2.7609
4.00	4.0815	3.9862	4.0228	3.9520	3.9991	3.9478	3.7474	4.0886	4.1466	3.9645
6.00	6.1701	5.8363	6.0129	5.8512	5.9486	6.1333	5.7546	6.2263	6.2706	6.0481
8.00	8.3159	7.8581	8.1748	7.8919	7.9802	8.1034	7.7317	8.0629	8.2228	7.7474
10.00	10.0420	9.7655	10.4073	9.6799	10.0427	9.8990	9.4680	10.6924	10.3064	9.5174

Concentration (μg/mL)	Mean	SD	Precision as CV (%)	Accuracy (%)
0.25	0.2414	0.0344	14.250	96.56
0.50	0.4873	0.0442	9.070	97.46
1.00	1.0038	0.0399	3.975	100.38
2.00	1.9723	0.1112	5.638	98.62
3.00	3.0022	0.1575	5.246	100.07
4.00	3.9937	0.1031	2.582	99.84
6.00	6.0252	0.1671	2.773	100.42
8.00	8.0089	0.1902	2.375	100.11
10.00	9.9821	0.3773	3.780	99.82

Abbreviations: CV, coefficient of variation; SD, standard deviation.

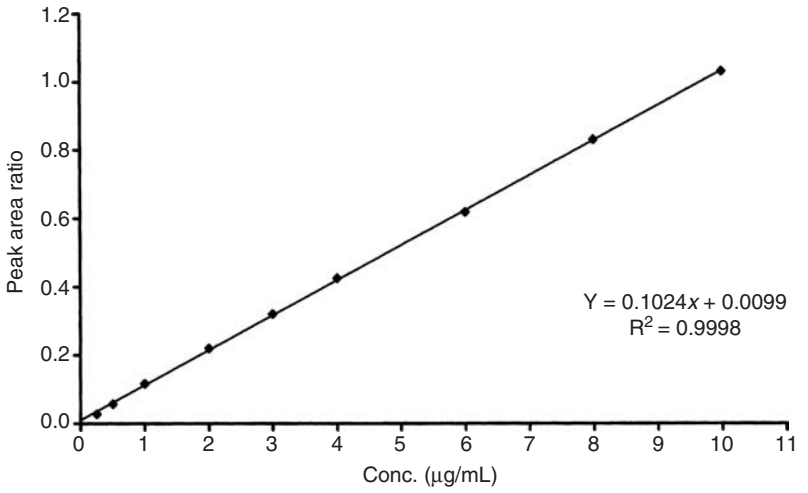

FIGURE A.1 Niazin means standard calibration curve in human plasma.

Sensitivity
The LLOQ is 0.25 μg/mL.

Recovery

Absolute Analytical Recovery
The percentage absolute analytical recovery was determined by comparing the absolute peak area of niazin and sarfarin from a plasma sample prepared according to the method, to the absolute peak area of an equivalent aqueous standard, which was prepared to contain a concentration of drug and internal standard assuming 100% recovery. Data are presented in Tables A.5 and A.6.

Relative Analytical Recovery
Relative analytical recovery of niazin was determined by comparing the measured concentration with actual added ones using three different QC samples (low, medium, and high QCs). Data are presented in Table A.7.

Specificity

The specificity of the method was determined by screening six different batches of controlled human blank plasma, which were free from interfering endogenous plasma components. This is evidenced by the lack of interfering peaks in the chromatograms of plasma samples. Figures A.2–A.7showed representative chromatograms from validation. Solutions containing,

TABLE A.3 Intraday Accuracy and Precision of the Niazin Spiked Quality Control Samples in Human Plasma

Day	Theo-concentration (μg/mL)	Measured niazin concentration (μg/mL)					Mean (μg/mL)	SD (μg/mL)	Precision as CV (%)	Accuracy (%)
1	0.25	0.250	0.257	0.268	0.267	0.247	0.255	0.008	3.137	102.00
		0.254	0.254	0.261	0.247	0.245				
	0.75	0.741	0.765	0.769	0.769	0.764	0.757	0.014	1.849	100.93
		0.739	0.741	0.756	0.745	0.781				
		4.835	4.812	4.608	4.671	4.978	4.854	0.134	2.761	97.08
		4.941	5.074	4.792	4.887	4.942				
	9.0	8.920	8.847	8.686	8.892	8.761	8.841	0.152	1.719	98.23
		9.108	9.059	8.602	8.726	8.804				

Abbreviations: CV, coefficient of variation; SD, standard deviation.

TABLE A.4 Interday Accuracy and Precision of Niazin Spiked Quality Control Samples in Human Plasma

	Measured niazin concentration in human plasma (μg/mL)							
Analyzed on day	**0.25**		**0.75**		**5.0**		**9.0**	
1	0.250	0.257	0.741	0.765	4.835	4.941	8.920	9.108
	0.254	0.254	0.739	0.741	4.812	5.074	8.847	9.059
	0.268	0.267	0.769	0.745	4.608	4.792	8.686	8.602
	0.247	0.245	0.764	0.781	4.671	4.978	8.892	8.726
	0.261	0.247	0.769	0.756	4.887	4.942	8.761	8.804
2	0.254		0.745		5.073		8.944	
	0.232		0.740		5.104		8.887	
	0.228		0.744		4.974		8.954	
	0.236		0.735		4.792		8.652	
	0.245		0.708		4.921		9.008	
3	0.236		0.750		4.849		9.062	
	0.234		0.771		4.844		9.201	
	0.218		0.734		4.902		8.947	
	0.234		0.756		4.949		8.542	
	0.239		0.768		5.083		8.796	
Mean (μg/mL)	0.245		0.749		4.902		8.870	
SD (μg/mL)	0.013		0.019		0.129		0.171	
Precision as CV (%)	5.306		2.537		2.632		1.928	
Accuracy (%)	98.00		99.87		98.04		98.56	

Abbreviations: CV, coefficient of variation; SD, standard deviation.

aspirin, acetaminophen, ascorbic acid, caffeine, nicotine, and ibuprofen were prepared in mobile phase and then they were injected to check for interference from these commonly used drugs and all of them showed no interference, with the peaks of niazin and sarfarin.

Stability

Short-Term RT Stability (Counter Stability)

Fifteen samples of each concentration of two different QCs were prepared (the low QC and the high QC); five of each concentration were processed and analyzed for initial concentration

TABLE A.5 Absolute Analytical Recovery of Niazin

Concentration (μg/mL)	**Peak area of niazin from plasma standard sample**					**Mean peak area**
	A	**B**	**C**	**D**	**E**	
0.75	33,599	33,378	35,354	35,325	36,237	34,779
5.00	214,369	212,027	208,027	202,565	206,131	208,624
9.00	391,487	389,405	383,881	406,997	375,111	389,376

Concentration (μg/mL)	**Peak area of niazin from standard aqueous sample**					**Mean peak area**
	A	**B**	**C**	**D**	**E**	
0.75	36,388	37,200	38,231	37,482	37,912	37,443
5.00	224,731	222,750	223,417	219,347	220,814	222,212
9.00	417,478	416,516	416,380	423,412	419,988	418,755

Concentration (μg/mL)	**Mean peak area**		**Recovery (%)**
	Plasma sample	**Aqueous sample**	
0.75	34,779	37,443	92.89
5.00	208,624	222,212	93.89
9.00	389,376	418,755	92.99

TABLE A.6 Absolute Analytical Recovery of the Internal Standard (Sarfarin)

	Peak area of internal standard						
Matrix	**# 1**	**# 2**	**# 3**	**# 4**	**# 5**	**Mean**	**Recovery (%)**
Plasma	409,609	401,978	409,727	407,273	414,007	408,519	85.13
Aqueous	482,744	479,334	480,724	475,327	481,377	479,901	

TABLE A.7 Relative Analytical Recovery of Niazin

Actual concentration (0.75 μg/mL)		**Actual concentration (5.00 μg/mL)**		**Actual concentration (9.00 μg/mL)**	
Measured concentration (μg/mL)	**Relative recovery (%)**	**Measured concentration (μg/mL)**	**Relative recovery (%)**	**Measured concentration (μg/mL)**	**Relative recovery (%)**
0.749	99.87	4.812	96.24	8.924	99.16
0.747	99.60	4.835	96.70	8.892	98.80
0.757	100.93	4.978	99.56	8.760	97.33
0.753	100.40	4.942	98.84	9.060	100.67
0.748	99.73	5.074	101.48	8.808	97.87
Mean	100.11		98.56		98.77
SD	0.494		1.92		1.15
CV (%)	0.493		1.95		1.16

Abbreviations: CV, coefficient of variation; SD, standard deviation.

SAMPLE INFORMATION

Sample Name:	unk4	Acquired By:	System
Sample Type:	unknown	Date Acquired:	10/5/2003 2:18:01 PM
Vial:	14	Acq. Method Set:	
Injection #:	1	Date Processed:	10/5/2003 2:30:13 PM
Injection Volume:	100.00 μl	Processing Method:	
Run Time:	12.0 min	Channel Name:	2487 Channel 1
Sample Set Name:		Proc. Chnl. Descr.:	

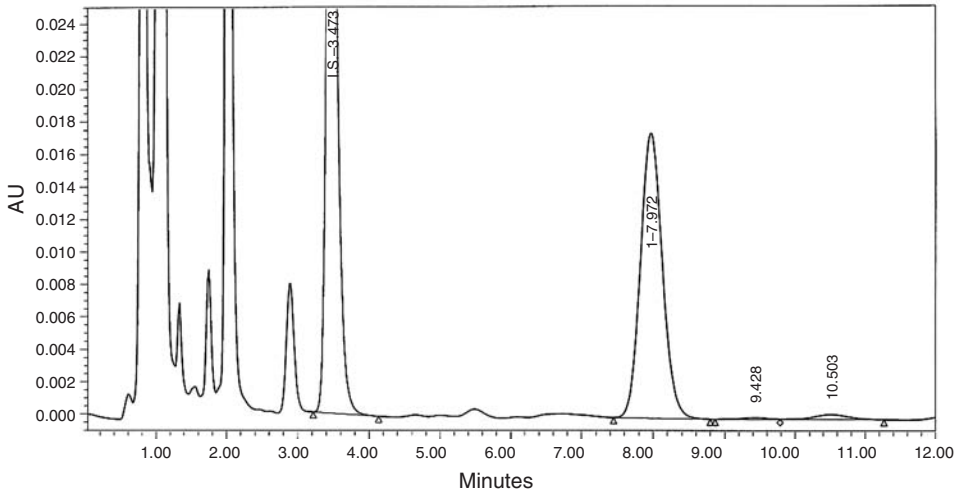

FIGURE A.2 Liquid chromatogram of a blank plasma sample (for 12 minutes).

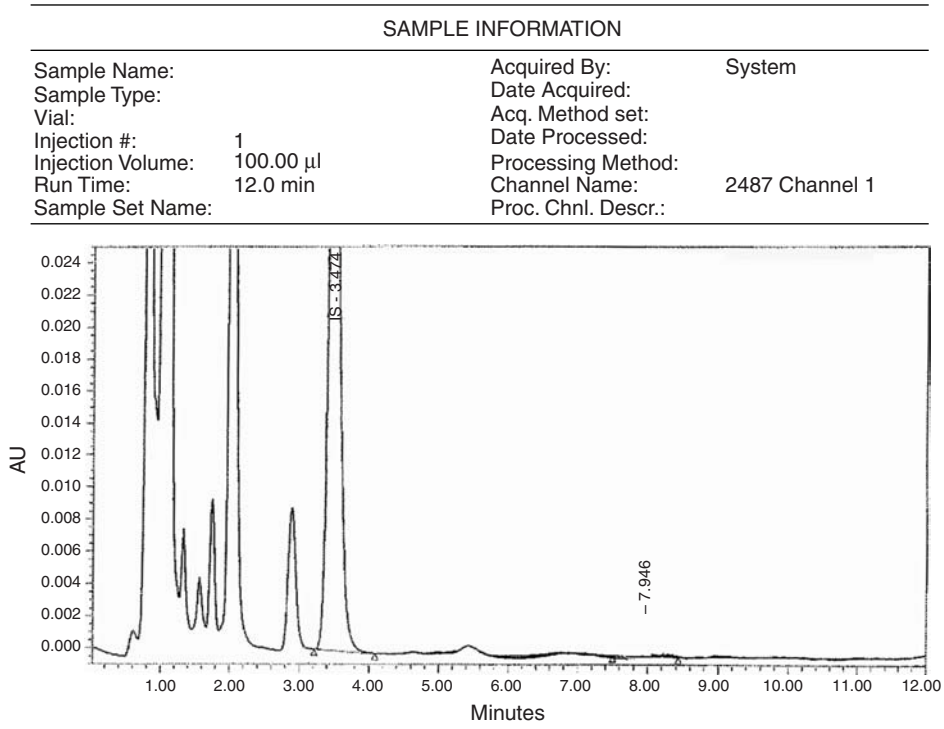

FIGURE A.3 Liquid chromatogram of a blank plasma sample containing the internal standard (niazin) at a concentration of 16.6 µg/mL.

determination at zero time. The other 10 plasma samples of each concentration were allowed to stand on the bench top at RT without further treatment. Five plasma samples of the remaining of each concentration were processed as described in section, "Sample Preparation" and analyzed after two hours. The other five plasma samples of each concentration were processed and analyzed after four hours; data are presented in Tables A.8 and A.9. Stability study showed that niazin is stable (short-term) for at least four hours at RT.

Sample Stability After Preparation Procedure (Autosampler Stability)

On a validation day, 15 samples of each concentration of two different QC samples (the low QC and the high QC) were prepared as in section, "Sample Preparation," the supernatant of samples was pooled. Five samples of each concentration were analyzed immediately after preparation, another five processed samples of each concentration were stored at RT for 16 hours and at 20°C for 48 hours after preparation, data are presented in Table A.10. Stability study showed that niazin is stable (after preparation) for 16 hours at RT and for 48 hours at 20°C.

Internal Standard Stability After Preparation Procedure (Autosampler Stability)

On a validation day, 15 samples of internal standard (sarfarin) with the medium QC sample were prepared as in "Sample Preparation," the supernatant of samples was pooled. Five samples were analyzed immediately after preparation, another five processed samples were stored at RT for 16 hours and at 20°C for 48 hours after preparation, data are presented in Table A.11. Stability study showed that sarfarin is stable (after preparation) for 16 hours at RT and for 48 hours at 20°C.

SAMPLE INFORMATION

Sample Name:	unk 1	Acquired By:	System
Sample Type:	Unknown	Date Acquired:	10/5/2003 1:34:40 PM
Vial:	11	Acq. Method Set:	
Injection #:	1	Date Processed:	10/5/2003 1:46:52 PM
Injection Volume:	100.00 µl	Processing Method:	
Run Time:	12.0 min	Channel Name:	2487 Channel 1
Sample Set Name:		Proc. Chnl. Descr.:	

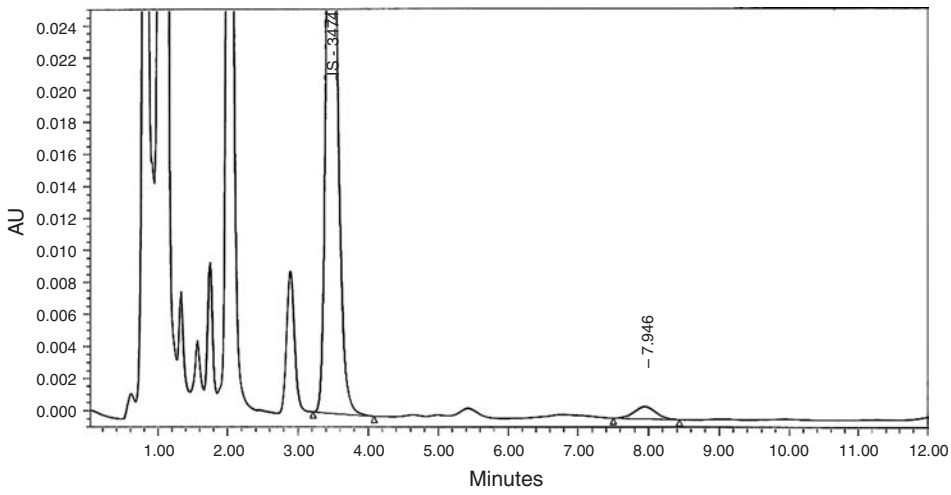

FIGURE A.4 Liquid chromatogram of standard plasma containing the drug (cefuroxime) at a concentration of 0.25 µg/mL (lower limit of quantification) and the internal standard (niazin) at a concentration of 16.6 µg/mL.

Freeze–Thaw Stability of Niazin in Human Plasma

Testing for freeze and thaw analyte stability was determined during three freeze and thaw cycles. Fifteen samples of each spiked QCs of two different concentrations (low QC and high QC) were prepared and stored at −20°C for 24 hours. All samples were thawed unassisted at RT, when completely thawed five samples of each control concentration were analyzed and the rest were returned to the freezer and kept frozen for 24 hours. The same procedure was repeated for remaining samples of the controls for testing cycle # 2 and cycle # 3. The freeze–thaw stability samples were compared with freshly prepared samples (day 1), data are presented in Table A.12. Stability data showed that niazin is stable for three cycles of freeze and thaw.

Stock Solution Stability

The stability of stock solution of niazin and the internal standard (sarfarin) was evaluated at RT for six hours and at −20°C for four weeks for niazin and two weeks for sarfarin. After completion of the desired storage time, the stability was tested by comparing the peak area with that of freshly prepared solutions, data are presented in Table A.13. Stability study showed that niazin and sarfarin (internal standard) are stable for six hours at RT and for four weeks at −20°C for niazin and for two weeks at −20°C for sarfarin.

Long-Term Stability (Freezer Storage Stability)

Ten samples of each concentration of the low and the high QC samples (0.75 and 9.00 µg/mL) were prepared and stored at 20°C. Five plasma samples of each concentration were prepared as described in section, "Sample preparation" and analyzed at the end of the second week, another five plasma samples of each concentration were prepared and analyzed at the end of the fourth week. The data are presented in Table A.14. Stability study showed that niazin is stable (freezer storage) in plasma for four weeks.

SAMPLE INFORMATION			
Sample Name:	unk 2	Acquired By:	System
Sample Type:	Unknown	Date Acquired:	10/5/2003 1:49:05 PM
Vial:	12	Acq. Method Set:	
Injection #:	1	Date Processed:	10/5/2003 2:01:18 PM
Injection Volume:	100.00 µl	Processing Method:	
Run Time:	12.0 min	Channel Name:	2487 Channel 1
Sample Set Name:		Proc. Chnl. Descr.:	

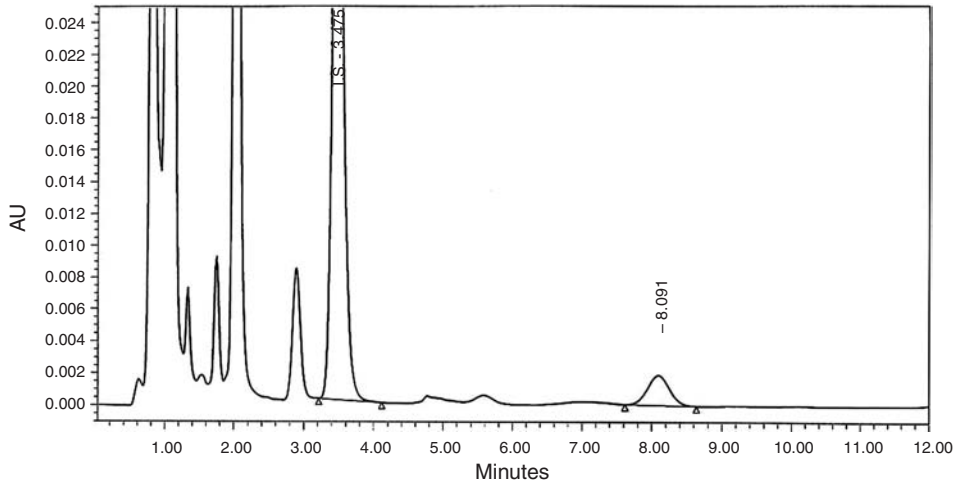

FIGURE A.5 Liquid chromatogram of standard plasma sample containing the drug (cefuroxime) at a concentration of 0.75 µg/mL (low QC) and the internal standard (niazin) at a concentration of 16.6 µg/mL.

SAMPLE INFORMATION			
Sample Name:	unk 3	Acquired By:	System
Sample Type:	Unknown	Date Acquired:	10/5/2003 2:03:34 PM
Vial:	13	Acq. Method Set:	
Injection #:	1	Date Processed:	10/5/2003 2:15:46 PM
Injection Volume:	100.00 µl	Processing Method:	
Run Time:	12.0 min	Channel Name:	2487 Channel 1
Sample Set Name:		Proc. Chnl. Descr.:	

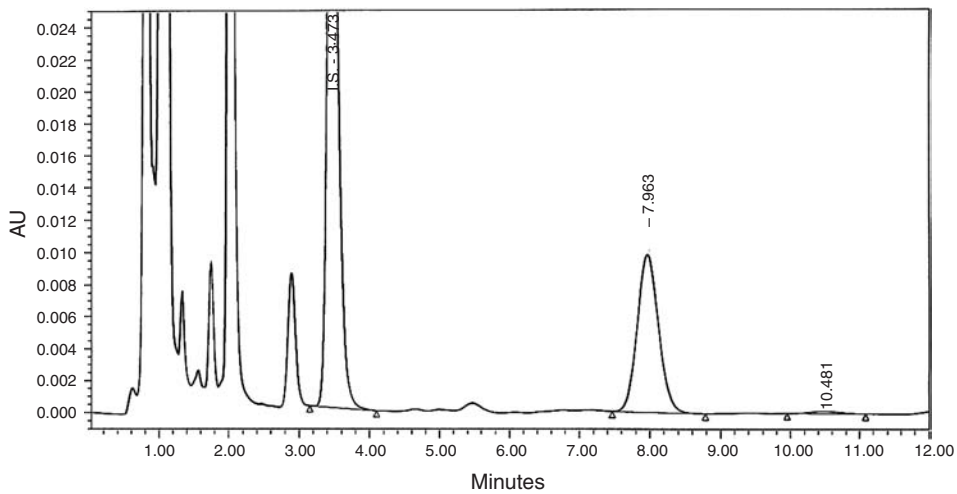

FIGURE A.6 Liquid chromatogram of standard plasma sample containing the drug (sarfarin) at a concentration of 5 µg/mL (medium QC) and the internal standard (niazin) at a concentration of 16.6 µg/mL.

SAMPLE INFORMATION			
Sample Name:	unk 4	Acquired By:	System
Sample Type:	Unknown	Date Acquired:	10/5/2003 2:18:01 PM
Vial:	14	Acq. Method Set:	
Injection #:	1	Date Processed:	10/5/2003 2:30:13 PM
Injection Volume:	100.00 µl	Processing Method:	
Run Time:	12.0 min	Channel Name:	2487 Channel 1
Sample Set Name:		Proc. Chnl. Descr.:	

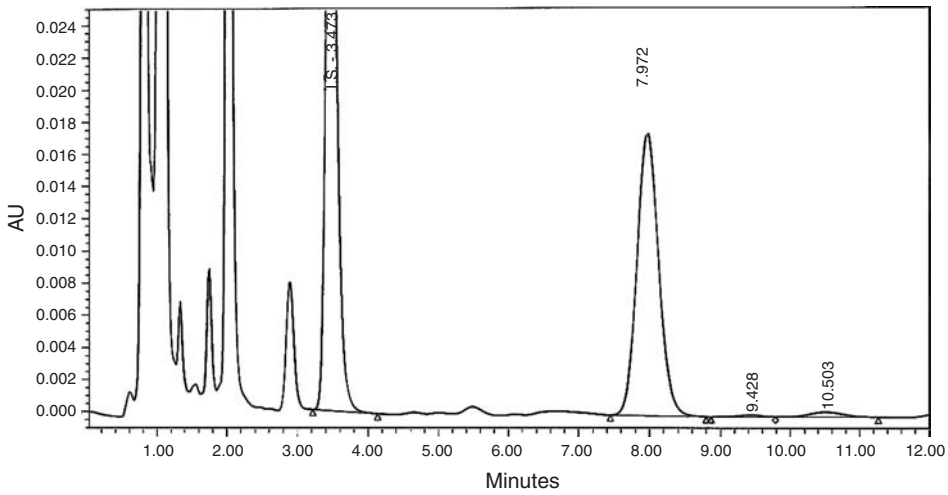

FIGURE A.7 Liquid chromatogram of standard plasma sample containing the drug (sarfarin) at a concentration of 9.00 µg/mL (high QC) and the internal standard (niazin) at concentration of 16.6 µg/mL.

TABLE A.8 Short-Term RT Stability for the Low QC (0.75 µg/mL)

QC sample 0.75 µg/mL	Initial analyzed concentration (µg/mL)	Analyzed concentration after 2 hr (µg/mL)	Ratio of analyzed 2 hr/initial	Stability (%)
After 2 hr				
A	0.736	0.742	1.008	100.82
B	0.744	0.740	0.995	99.46
C	0.741	0.735	0.992	99.19
D	0.765	0.734	0.960	95.95
E	0.739	0.696	0.942	94.18
Mean	0.745	0.729		97.85
SD	0.010	0.017		2.612
CV (%)	1.342	2.332		2.678

QC sample 0.75 µg/mL	Initial analyzed concentration (µg/mL)	Analyzed concentration after 4 hr (µg/mL)	Ratio of analyzed 4 hr/initial	Stability (%)
After 4 hr				
A	0.736	0.734	0.997	99.73
B	0.744	0.711	0.956	95.57
C	0.741	0.726	0.980	97.98
D	0.765	0.751	0.982	98.17
E	0.739	0.748	1.012	101.22
Mean	0.745	0.734		98.52
SD	0.010	0.015		1.891
CV (%)	1.342	2.044		1.919

(Analyzed concentration after two hours at RT/Initial analyzed concentration) × 100 = 97.85.
(Analyzed concentration after four hours at RT/Initial analyzed concentration) × 100 = 98.52.
Abbreviations: CV, coefficient of variation; QC, quality control; RT, room temperature; SD, standard deviation.

TABLE A.9 Short-Term RT Stability for the High QC (9.00 μg/mL)

QC sample 9.00 μg/mL	Initial analyzed concentration (μg/mL)	Analyzed concentration after 2 hr (μg/mL)	Ratio of analyzed 2 hr/initial	Stability (%)
After 2 hr				
A	8.920	8.804	0.987	98.70
B	8.847	8.726	0.986	98.63
C	8.686	8.602	0.990	99.03
D	8.892	9.059	1.019	101.88
E	8.761	8.655	0.988	98.79
Mean	8.821	8.769		99.41
SD	0.086	0.160		1.244
CV (%)	0.975	1.825		1.251
QC sample 9.00 μg/mL	**Initial analyzed concentration (μg/mL)**	**Analyzed concentration after 4 hr (μg/mL)**	**Ratio of analyzed 4 hr/initial**	**Stability (%)**
After 4 hr				
A	8.920	8.847	0.992	99.19
B	8.847	8.567	0.968	96.84
C	8.686	8.711	1.003	100.29
D	8.892	9.062	1.019	101.91
E	8.761	8.797	1.004	100.41
Mean	8.821	8.797		99.73
SD	0.086	0.163		1.684
CV (%)	0.975	1.853		1.689

(Analyzed concentration after two hours at RT/Initial analyzed concentration) × 100 = 99.41.
(Analyzed concentration after four hours at RT/Initial analyzed concentration) × 100 = 99.73.
Abbreviations: CV, coefficient of variation; QC, quality control; RT, room temperature; SD, standard deviation.

TABLE A.10 Sample Stability After Preparation (Autosampler Stability)

QC sample 0.75 μg/mL	Initial analyzed concentration (μg/mL)	Analyzed concentration (μg/mL) after 16 hr RT	Analyzed concentration (μg/mL) after 48 hr at −20°C
(a) For the Low QC (0.75 μg/mL)			
A	0.723	0.698	0.706
B	0.733	0.728	0.709
C	0.741	0.746	0.734
D	0.744	0.732	0.729
E	0.756	0.746	0.722
Mean	0.739	0.730	0.720
SD	0.011	0.018	0.011
CV (%)	1.489	2.466	1.528

QC sample 9.00 μg/mL	Initial analyzed concentration (μg/mL)	Analyzed concentration (μg/mL) after 16 hr RT	Analyzed concentration (μg/mL) after 48 hr at −20°C
(b) For the high QC (9.00 μg/mL)			
A	8.718	8.672	8.417
B	8.797	8.747	8.637
C	8.544	8.464	8.376
D	8.665	8.542	8.655
E	8.904	8.537	8.602
Mean	8.726	8.592	8.537
SD	0.121	0.102	0.117
CV (%)	1.387	1.187	1.371

(Analyzed concentration after 16 hours at RT/Initial analyzed concentration) × 100 = 98.78. (Analyzed concentration after 48 hours at −20°C/Initial analyzed concentration) × 100 = 97.43.
(Analyzed concentration after 16 hours at RT/Initial analyzed concentration) × 100 = 98.46. (Analyzed concentration after 48 hours at −20°C/Initial analyzed concentration) × 100 = 97.83.
Abbreviations: CV, coefficient of variation; QC, quality control; RT, room temperature; SD, standard deviation.

TABLE A.11 Internal Standard Stability After Preparation (Autosampler Stability)

	Peak area of I.S./peak area of niazin[a]		
Internal standard + M. QC sample	**Initial analyzed peak area ratio**	**Analyzed peak area ratio after 16 hr RT**	**Analyzed peak area ratio after 48 hr at −20°C**
A	1.9817	1.9993	1.9125
B	1.9782	1.9613	1.9263
C	2.1494	1.9398	1.9024
D	2.0685	1.9774	1.9144
E	2.2499	2.0057	1.9217
Mean	2.0855	1.9767	1.9155
SD	0.104	0.024	0.008
CV (%)	4.987	1.214	0.418

(Analyzed peak area ratio after 16 hours at RT/Initial analyzed peak area ratio) × 100 = 94.78. (Analyzed peak area ratio after 48 hours at −20°C/Initial analyzed peak area ratio) × 100 = 91.85.
[a] Medium quality control concentration.
Abbreviations: CV, coefficient of variation; QC, quality control; RT, room temperature; SD, standard deviation.

TABLE A.12 Freeze–Thaw Stability of Niazin

	Concentration of niazin (μg/mL)			
QC 0.75 μg/mL	**Initial concentration (fresh samples—day 1)**	**FT once**	**FT twice**	**FT thrice**
(a) For low QC (0.75 μg/mL)				
A	0.738	0.723	0.712	0.710
B	0.692	0.680	0.684	0.676
C	0.712	0.704	0.712	0.742
D	0.720	0.722	0.718	0.712
E	0.735	0.731	0.724	0.718
Mean	0.719	0.712	0.710	0.712
SD	0.017	0.018	0.014	0.021
CV (%)	2.364	2.528	1.972	2.949
	Concentration of niazin (μg/mL)			
QC 9.00 μg/mL	**Initial concentration (fresh samples—day 1)**	**FT once**	**FT twice**	**FT thrice**
(b) For high QC (9.00 μg/mL)				
A	8.971	8.954	8.716	8.847
B	8.544	8.726	8.763	8.686
C	8.992	8.886	8.843	8.789
D	8.665	8.592	8.674	8.590
E	8.417	8.367	8.519	8.604
Mean	8.718	8.705	8.703	8.703
SD	0.229	0.211	0.108	0.101
CV (%)	2.627	2.424	1.241	1.161

(Analyzed concentration FT once/initial analyzed concentration) × 100 = 99.03. (Analyzed concentration FT twice/initial analyzed concentration) × 100 = 98.75. (Analyzed concentration FT thrice/initial analyzed concentration) × 100 = 99.03.
(Analyzed concentration FT once/initial analyzed concentration) × 100 = 99.85. (Analyzed concentration FT twice/initial analyzed concentration) × 100 = 99.83. (Analyzed concentration FT thrice/initial analyzed concentration) × 100 = 99.83.
Abbreviations: CV, coefficient of variation; FT, freeze–thaw; QC, quality control, SD, standard deviation.

TABLE A.13 Stock Solution Stability

	Analyzed peak area of niazin			
	At zero time	**After 6 hr at room temperature**	**After 2 wk at −20°C**	**After 4 wk at −20°C**
(a) For niazin 100 μg/mL niazin was prepared in deionized water; 100 μL of the solution was injected (five replicates)				
A	4,867,867	4,846,725	4,756,302	4,506,332
B	4,798,327	4,804,563	4,722,817	4,487,448
C	4,947,834	4,917,241	4,683,984	4,438,719
D	4,824,377	4,895,324	4,692,718	4,389,347
E	4,905,846	4,828,654	4,575,442	4,402,797
Mean	4,869,850	4,858,501	4,686,253	4,444,929
SD	53,169	41,821	60,942	45,785
CV (%)	1.092	0.861	1.300	1.030

	Analyzed peak area of sarfarin		
	At zero time	**After 6 hr at room temperature**	**After 2 wk at −20°C**
(b) For sarfarin 100 μg/mL sarfarin was prepared in deionized water; 100 μL of the solution was injected (five replicates)			
A	2,827,834	2,846,482	2,716,362
B	2,938,457	2,801,716	2,601,494
C	2,847,201	2,747,984	2,583,947
D	3,004,722	2,734,112	2,574,156
E	2,967,873	2,708,204	2,543,734
Mean	2,917,217	2,767,700	2,603,939
SD	68,653	49,840	59,256
CV (%)	2.323	1.801	2.276

(Analyzed peak area after six hours at room temperature/initial analyzed peak area) × 100 = 99.77. (Analyzed peak area after two weeks at −20°C/initial analyzed peak area) × 100 = 96.23. (Analyzed peak area after four weeks at −20°C/initial analyzed peak area) × 100 = 91.28.
(Analyzed peak area after six hours at room temperature/initial analyzed peak area) × 100 = 94.88. (Analyzed peak area after two weeks at −20°C/initial analyzed peak area) × 100 = 89.26.
Abbreviations: CV, coefficient of variation; SD, standard deviation.

TABLE A.14 Long-Term Stability (Freezer Storage Stability)

QC sample 0.75 μg/mL	Initial concentration (μg/mL) (fresh samples—day 1)	Analyzed concentration (μg/mL) after 2 wk at −20°C	Analyzed concentration (μg/mL) after 4 wk at −20°C
(a) For low QC (0.75 μg/mL)			
A	0.755	0.745	0.740
B	0.781	0.749	0.738
C	0.763	0.754	0.748
D	0.769	0.758	0.746
E	0.741	0.753	0.742
Mean	0.762	0.752	0.743
SD	0.013	0.005	0.004
CV (%)	1.706	0.665	0.538

QC sample 9.00 μg/mL	Initial concentration (μg/mL) (fresh samples—day 1)	Analyzed concentration (μg/mL) after 2 wk at −20°C	Analyzed concentration (μg/mL) after 4 wk at −20°C
(b) For high QC (9.00 μg/mL)			
A	9.060	8.892	8.888
B	8.868	8.804	8.790
C	8.944	8.726	8.747
D	8.798	8.694	8.827
E	8.904	8.868	8.781
Mean	8.915	8.797	8.807
SD	0.087	0.077	0.048
CV (%)	0.976	0.875	0.545

(Analyzed concentration after two weeks at −20°C/initial analyzed concentration)×100=98.69. (Analyzed concentration after four weeks at −20°C/initial analyzed concentration)×100=97.51.
(Analyzed concentration after two weeks at −20°C/initial analyzed concentration)×100=98.68. (Analyzed concentration after four weeks at −20°C/initial analyzed concentration)×100=98.79.
Abbreviations: CV, coefficient of variation; QC, quality control; SD, standard deviation.

10 Good Clinical Practice

Good Clinical Practice (GCP) is an international ethical and scientific quality standard for designing, conducting, recording, and reporting trials that involve the participation of human subjects. Compliance with this standard provides public assurance that the rights, safety and well-being of trial subjects are protected, consistent with the principles that have their origin in the Declaration of Helsinki, and that the clinical trial data are credible.

1. DECLARATION OF HELSINKI[a]

1.1 Basic Principles

1.1.1 Biomedical research involving human subjects must conform to generally accepted scientific principles and should be based on adequately performed laboratory and animal experimentation and on a thorough knowledge of the scientific literature.

1.1.2 The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol which should be transmitted to a specially appointed independent committee for consideration, comment and guidance.

1.1.3 Biomedical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given his or her consent.

1.1.4 Biomedical research involving human subjects cannot legitimately be carried out unless the importance of the objective is in proportion to the inherent risk to the subject.

1.1.5 Every biomedical research project involving human subjects should be preceded by careful assessment of predictable risks in comparison with foreseeable benefits to the subject or to others. Concern for the interests of the subject must always prevail over the interests of science and society.

1.1.6 The right of the research subject to safeguard his or her integrity must always be respected. Every precaution should be taken to respect the privacy of the subject and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.

1.1.7 Physicians should abstain from engaging in research projects involving human subjects unless they are satisfied that the hazards involved are believed to be predictable. Physicians should cease any investigation if the hazards are found to outweigh the potential benefits.

1.1.8 In publication of the results of his or her research, the physician is obliged to preserve the accuracy of the results. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.

1.1.9 In any research on human beings, each potential subject must be adequately informed of the aims, methods, anticipated benefits and potential hazards of the study and

[a] From World Medical Organization. Declaration of Helsinki. *British Medical Journal* (7 December) 1996; 313(7070):1448–1449.

the discomfort it may entail. He or she should be informed that he or she is at liberty to abstain from participation in the study and that he or she is free to withdraw visor her consent to participation at any time. The physician should then obtain the subject's freely given informed consent, preferably inheriting.

1.1.10 When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in dependent relationship to him or her or may consent under duress. In that case the informed consent should be obtained by a physician who is not engaged in the investigation and who is completely independent of this official relationship.

1.1.11 In case of legal incompetence, informed consent should be obtained from the legal guardian in accordance with national legislation. Where physical or mental incapacity makes it impossible to obtain informed consent, or when the subject is a minor, permission from the responsible relative replaces that of the subject in accordance with national legislation. Whenever the minor child is in fact able to give consent, the minor's consent must be obtained in addition to the consent of the minor's legal guardian.

1.1.12 The research protocol should always contain a statement of the ethical considerations involved and should indicate that the principles enunciated in the present declaration are complied with.

1.2 Non-Therapeutic Biomedical Research Involving Human Subjects (Non-Clinical Biomedical Research)

1.2.1 In the purely scientific application of medical research carried out on a human being, it is the duty of the physician to remain the protector of the life and health of that person on whom biomedical research is being carried out.

1.2.2 The subjects should be volunteers—either healthy persons or patients for whom the experimental design is not related to the patient's illness.

1.2.3 The investigator or the investigating team should discontinue the research if in his/her or their judgment it may, if continued, be harmful to the individual.

1.2.4 In research on man, the interest of science and society should never take precedence over considerations related to the well-being of the subject.

2. THE PRINCIPLES OF ICH GCP

2.1 Clinical trials should be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, and that are consistent with GCP and the applicable regulatory requirement(s).

2.2 Before a trial is initiated, foreseeable risks and inconveniences should be weighed against the anticipated benefit for the individual trial subject and society. A trial should be initiated and continued only if the anticipated benefits justify the risks.

2.3 The rights, safety, and well-being of the trial subjects are the most important considerations and should prevail over interests of science and society.

2.4 The available nonclinical and clinical information on an investigational product should be adequate to support the proposed clinical trial.

2.5 Clinical trials should be scientifically sound, and described in a clear, detailed protocol.

2.6 A trial should be conducted in compliance with the protocol that has received prior institutional review board (IRB)/independent ethics committee (IEC) approval/favorable opinion.

2.7 The medical care given to, and medical decisions made on behalf of, subjects should always be the responsibility of a qualified physician or, when appropriate, of a qualified dentist.

2.8 Each individual involved in conducting a trial should be qualified by education, training, and experience to perform his or her respective task(s).

2.9 Freely given informed consent should be obtained from every subject prior to clinical trial participation.
2.10 All clinical trial information should be recorded, handled, and stored in a way that allows its accurate reporting, interpretation, and verification.
2.11 The confidentiality of records that could identify subjects should be protected, respecting the privacy and confidentiality rules in accordance with the applicable regulatory requirement(s).
2.12 Investigational products should be manufactured, handled, and stored in accordance with applicable good manufacturing practice (GMP). They should be used in accordance with the approved protocol.
2.13 Systems with procedures that assure the quality of every aspect of the trial should be implemented.

3. INSTITUTIONAL REVIEW BOARD/INDEPENDENT ETHICS COMMITTEE (IRB/IEC)

3.1 Responsibilities

3.1.1 An IRB/IEC should safeguard the rights, safety, and well-being of all trial subjects. Special attention should be paid to trials that may include vulnerable subjects.
3.1.2 The IRB/IEC should obtain the following documents: trial protocol(s)/amendment(s), written informed consent form(s) and consent form updates that the investigator proposes for use in the trial, subject recruitment procedures (e.g., advertisements), written information to be provided to subjects, Investigator's Brochure (IB), available safety information, information about payments and compensation available to subjects, the investigator's current curriculum vitae and/or other documentation evidencing qualifications, and any other documents that the IRB/IEC may need to fulfil its responsibilities. The IRB/IEC should review a proposed clinical trial within a reasonable time and document its views in writing, clearly identifying the trial, the documents reviewed and the dates for the following:
   a. approval/favorable opinion;
   b. modifications required prior to its approval/favorable opinion;
   c. disapproval/negative opinion; and
   d. termination/suspension of any prior approval/favorable opinion.
3.1.3 The IRB/IEC should consider the qualifications of the investigator for the proposed trial, as documented by a current curriculum vitae and/or by any other relevant documentation the IRB/IEC requests.
3.1.4 The IRB/IEC should conduct continuing review of each ongoing trial at intervals appropriate to the degree of risk to human subjects, but at least once per year.
3.1.5 The IRB/IEC may request more information than is outlined in paragraph 4.8.10 be given to subjects when, in the judgment of the IRB/IEC, the additional information would add meaningfully to the protection of the rights, safety and/or well-being of the subjects.
3.1.6 When a non-therapeutic trial is to be carried out with the consent of the subjects' legally acceptable representative (see 4.8.12 and 4.8.14), the IRB/IEC should determine that the proposed protocol and/or other document(s) adequately addresses relevant ethical concerns and meets applicable regulatory requirements for such trials.
3.1.7 Where the protocol indicates that prior consent of the trial subject or the subjects' legally acceptable representative is not possible (see 4.8.15), the IRB/IEC should determine that the proposed protocol and/or other document(s) adequately addresses relevant ethical concerns and meets applicable regulatory requirements for such trials (i.e., in emergency situations).
3.1.8 The IRB/IEC should review both the amount and method of payment to subjects to assure that neither presents problems of coercion or undue influence on the trial subjects. Payments to a subject should be prorated and not wholly contingent on completion of the trial by the subject.

3.1.9 The IRB/IEC should ensure that information regarding payment to subjects, including the methods, amounts, and schedule of payment to trial subjects, is set forth in the written informed consent form and any other written information to be provided to subjects. The way payment will be prorated should be specified.

3.2 Composition, Functions, and Operations

3.2.1 The IRB/IEC should consist of a reasonable number of members, who collectively have the qualifications and experience to review and evaluate the science, medical aspects, and ethics of the proposed trial. It is recommended that the IRB/IEC should include:
   a. At least five members.
   b. At least one member whose primary area of interest is in a nonscientific area.
   c. At least one member who is independent of the institution/trial site.

Only those IRB/IEC members who are independent of the investigator and the sponsor of the trial should vote/provide opinion on a trial-related matter.

A list of IRB/IEC members and their qualifications should be maintained.

3.2.2 The IRB/IEC should perform its functions according to written operating procedures, should maintain written records of its activities and minutes of its meetings, and should comply with GCP and with the applicable regulatory requirement(s).
3.2.3 An IRB/IEC should make its decisions at announced meetings at which at least a quorum, as stipulated in its written operating procedures, is present.
3.2.4 Only members who participate in the IRB/IEC review and discussion should vote/provide their opinion and/or advice.
3.2.5 The investigator may provide information on any aspect of the trial, but should not participate in the deliberations of the IRB/IEC or in the vote/opinion of the IRB/IEC.
3.2.6 An IRB/IEC may invite nonmembers with expertise in special areas for assistance.

3.3 Procedures

The IRB/IEC should establish, document in writing, and follow its procedures, which should include:

3.3.1 Determining its composition (names and qualifications of the members) and the authority under which it is established.
3.3.2 Scheduling, notifying its members of, and conducting its meetings.
3.3.3 Conducting initial and continuing review of trials.
3.3.4 Determining the frequency of continuing review, as appropriate.
3.3.5 Providing, according to the applicable regulatory requirements, expedited review and approval/favorable opinion of minor change(s) in ongoing trials that have the approval/favorable opinion of the IRB/IEC.
3.3.6 Specifying that no subject should be admitted to a trial before the IRB/IEC issues its written approval/favorable opinion of the trial.
3.3.7 Specifying that no deviations from, or changes of, the protocol should be initiated without prior written IRB/IEC approval/favorable opinion of an appropriate amendment, except when necessary to eliminate immediate hazards to the subjects or when the change(s) involves only logistical or administrative aspects of the trial [e.g., change of monitor(s), telephone number(s)] (see 4.5.2).
3.3.8 Specifying that the investigator should promptly report to the IRB/IEC: (*i*) Deviations from, or changes of, the protocol to eliminate immediate hazards to the trial subjects (see 3.3.7, 4.5.2, and 4.5.4). (*ii*) Changes increasing the risk to subjects and/or affecting significantly the conduct of the trial (see 4.10.2). (*iii*) All adverse drug reactions (ADRs) that are both serious and unexpected. (*iv*) New information that may affect adversely the safety of the subjects or the conduct of the trial.

3.3.9 Ensuring that the IRB/IEC promptly notify in writing the investigator/institution concerning:
   a. Its trial-related decisions/opinions.
   b. The reasons for its decisions/opinions.
   c. Procedures for appeal of its decisions/opinions.

3.4 Records

The IRB/IEC should retain all relevant records (e.g., written procedures, membership lists, lists of occupations/affiliations of members, submitted documents, minutes of meetings and correspondence) for a period of at least 3 years after completion of the trial and make them available upon request from the regulatory authority(ies).

The IRB/IEC may be asked by investigators, sponsors or regulatory authorities to provide its written procedures and membership lists.

4. INVESTIGATOR

4.1 Investigator's Qualifications and Agreements

4.1.1 The investigator(s) should be qualified by education, training, and experience to assume responsibility for the proper conduct of the trial, should meet all the qualifications specified by the applicable regulatory requirement(s), and should provide evidence of such qualifications through up-to-date curriculum vitae and/or other relevant documentation requested by the sponsor, the IRB/IEC, and/or the regulatory authority(ies).
4.1.2 The investigator should be thoroughly familiar with the appropriate use of the investigational product(s), as described in the protocol, in the current Investigator's Brochure, in the product information and in other information sources provided by the sponsor.
4.1.3 The investigator should be aware of, and should comply with, GCP and the applicable regulatory requirements.
4.1.4 The investigator/institution should permit monitoring and auditing by the sponsor, and inspection by the appropriate regulatory authority(ies).
4.1.5 The investigator should maintain a list of appropriately qualified persons to whom the investigator has delegated significant trial-related duties.

4.2 Adequate Resources

4.2.1 The investigator should be able to demonstrate (e.g., based on retrospective data) a potential for recruiting the required number of suitable subjects within the agreed recruitment period.
4.2.2 The investigator should have sufficient time to properly conduct and complete the trial within the agreed trial period.
4.2.3 The investigator should have available an adequate number of qualified staff and adequate facilities for the foreseen duration of the trial to conduct the trial properly and safely.
4.2.4 The investigator should ensure that all persons assisting with the trial are adequately informed about the protocol, the investigational product(s), and their trial-related duties and functions.

4.3 Medical Care of Trial Subjects

4.3.1 A qualified physician (or dentist, when appropriate), who is an investigator or a sub-investigator for the trial, should be responsible for all trial-related medical (or dental) decisions.
4.3.2 During and following a subject's participation in a trial, the investigator/institution should ensure that adequate medical care is provided to a subject for any adverse events,

including clinically significant laboratory values, related to the trial. The investigator/institution should inform a subject when medical care is needed for intercurrent illness(es) of which the investigator becomes aware.

4.3.3 It is recommended that the investigator inform the subject's primary physician about the subject's participation in the trial if the subject has a primary physician and if the subject agrees to the primary physician being informed.

4.3.4 Although a subject is not obliged to give his/her reason(s) for withdrawing prematurely from a trial, the investigator should make a reasonable effort to ascertain the reason(s), while fully respecting the subject's rights.

4.4 Communication with IRB/IEC

4.4.1 Before initiating a trial, the investigator/institution should have written and dated approval/favorable opinion from the IRB/IEC for the trial protocol, written informed consent form, consent form updates, subject recruitment procedures (e.g., advertisements), and any other written information to be provided to subjects.

4.4.2 As part of the investigator's/institution's written application to the IRB/IEC, the investigator/institution should provide the IRB/IEC with a current copy of the Investigator's Brochure. If the Investigator's Brochure is updated during the trial, the investigator/institution should supply a copy of the updated Investigator's Brochure to the IRB/IEC.

4.4.3 During the trial the investigator/institution should provide to the IRB/IEC all documents subject to review.

4.5 Compliance with Protocol

4.5.1 The investigator/institution should conduct the trial in compliance with the protocol agreed to by the sponsor and, if required, by the regulatory authority(ies) and which was given approval/favorable opinion by the IRB/IEC. The investigator/institution and the sponsor should sign the protocol, or an alternative contract, to confirm agreement.

4.5.2 The investigator should not implement any deviation from, or changes of the protocol without agreement by the sponsor and prior review and documented approval/favorable opinion from the IRB/IEC of an amendment, except where necessary to eliminate an immediate hazard(s) to trial subjects, or when the change(s) involves only logistical or administrative aspects of the trial [e.g., change in monitor(s), change of telephone number(s)].

4.5.3 The investigator, or person designated by the investigator, should document and explain any deviation from the approved protocol.

4.5.4 The investigator may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to trial subjects without prior IRB/IEC approval/favorable opinion. As soon as possible, the implemented deviation or change, the reasons for it, and, if appropriate, the proposed protocol amendment(s) should be submitted:

a. to the IRB/IEC for review and approval/favorable opinion,
b. to the sponsor for agreement and, if required,
c. to the regulatory authority(ies).

4.6 Investigational Product(s)

4.6.1 Responsibility for investigational product(s) accountability at the trial site(s) rests with the investigator/institution.

4.6.2 Where allowed/required, the investigator/institution may/should assign some or all of the investigator's/institution's duties for investigational product(s) accountability at the trial site(s) to an appropriate pharmacist or another appropriate individual who is under the supervision of the investigator/institution.

4.6.3 The investigator/institution and/or a pharmacist or other appropriate individual, who is designated by the investigator/institution, should maintain records of the product's delivery to the trial site, the inventory at the site, the use by each subject, and the return to the sponsor or alternative disposition of unused product(s). These records should include dates, quantities, batch/serial numbers, expiration dates (if applicable), and the unique code numbers assigned to the investigational product(s) and trial subjects. Investigators should maintain records that document adequately that the subjects were provided the doses specified by the protocol and reconcile all investigational product(s) received from the sponsor.

4.6.4 The investigational product(s) should be stored as specified by the sponsor (see 5.13.2 and 5.14.3) and in accordance with applicable regulatory requirement(s).

4.6.5 The investigator should ensure that the investigational product(s) are used only in accordance with the approved protocol.

4.6.6 The investigator, or a person designated by the investigator/institution, should explain the correct use of the investigational product(s) to each subject and should check, at intervals appropriate for the trial, that each subject is following the instructions properly.

4.7 Randomization Procedures and Unblinding

The investigator should follow the trial's randomization procedures, if any, and should ensure that the code is broken only in accordance with the protocol. If the trial is blinded, the investigator should promptly document and explain to the sponsor any premature unblinding (e.g., accidental unblinding, unblinding due to a serious adverse event) of the investigational product(s).

4.8 Informed Consent of Trial Subjects

4.8.1 In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s), and should adhere to GCP and to the ethical principles that have their origin in the Declaration of Helsinki. Prior to the beginning of the trial, the investigator should have the IRB/IEC's written approval/favorable opinion of the written informed consent form and any other written information to be provided to subjects.

4.8.2 The written informed consent form and any other written information to be provided to subjects should be revised whenever important new information becomes available that may be relevant to the subjects' consent. Any revised written informed consent form, and written information should receive the IRB/IEC's approval/favorable opinion in advance of use. The subject or the subjects' legally acceptable representative should be informed in a timely manner if new information becomes available that may be relevant to the subjects' willingness to continue participation in the trial. The communication of this information should be documented.

4.8.3 Neither the investigator, nor the trial staff, should coerce or unduly influence a subject to participate or to continue to participate in a trial.

4.8.4 None of the oral and written information concerning the trial, including the written informed consent form, should contain any language that causes the subject or the subject's legally acceptable representative to waive or to appear to waive any legal rights, or that releases or appears to release the investigator, the institution, the sponsor, or their agents from liability for negligence.

4.8.5 The investigator, or a person designated by the investigator, should fully inform the subject or, if the subject is unable to provide informed consent, the subject's legally acceptable representative, of all pertinent aspects of the trial including the written information and the approval/favorable opinion by the IRB/IEC.

4.8.6 The language used in the oral and written information about the trial, including the written informed consent form, should be as non-technical as practical and should be understandable to the subject or the subject's legally acceptable representative and the impartial witness, where applicable.

4.8.7 Before informed consent may be obtained, the investigator, or a person designated by the investigator, should provide the subject or the subject's legally acceptable representative ample time and opportunity to inquire about details of the trial and to decide whether or not to participate in the trial. All questions about the trial should be answered to the satisfaction of the subject or the subject's legally acceptable representative.

4.8.8 Prior to a subjects' participation in the trial, the written informed consent form should be signed and personally dated by the subject or by the subject's legally acceptable representative, and by the person who conducted the informed consent discussion.

4.8.9 If a subject is unable to read or if a legally acceptable representative is unable to read, an impartial witness should be present during the entire informed consent discussion. After the written informed consent form and any other written information to be provided to subjects, is read and explained to the subject or the subjects' legally acceptable representative, and after the subject or the subjects' legally acceptable representative has orally consented to the subjects' participation in the trial and, if capable of doing so, has signed and personally dated the informed consent form, the witness should sign and personally date the consent form. By signing the consent form, the witness attests that the information in the consent form and any other written information was accurately explained to, and apparently understood by, the subject or the subject's legally acceptable representative, and that informed consent was freely given by the subject or the subjects' legally acceptable representative.

4.8.10 Both the informed consent discussion and the written informed consent form and any other written information to be provided to subjects should include explanations of the following:

a. That the trial involves research.
b. The purpose of the trial.
c. The trial treatment(s) and the probability for random assignment to each treatment.
d. The trial procedures to be followed, including all invasive procedures.
e. The subject's responsibilities.
f. Those aspects of the trial that are experimental.
g. The reasonably foreseeable risks or inconveniences to the subject and, when applicable, to an embryo, fetus, or nursing infant.
h. The reasonably expected benefits. When there is no intended clinical benefit to the subject, the subject should be made aware of this.
i. The alternative procedure(s) or course(s) of treatment that may be available to the subject, and their important potential benefits and risks.
j. The compensation and/or treatment available to the subject in the event of trial-related injury.
k. The anticipated prorated payment, if any, to the subject for participating in the trial.
l. The anticipated expenses, if any, to the subject for participating in the trial.
m. That the subject's participation in the trial is voluntary and that the subject may refuse to participate or withdraw from the trial, at any time, without penalty or loss of benefits to which the subject is otherwise entitled.
n. That the monitor(s), the auditor(s), the IRB/IEC, and the regulatory authority(ies) will be granted direct access to the subject's original medical records for verification of clinical trial procedures and/or data, without violating the confidentiality of the subject, to the extent permitted by the applicable laws and regulations and that, by signing a written informed consent form, the subject or the subject's legally acceptable representative is authorizing such access.
o. That records identifying the subject will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available. If the results of the trial are published, the subjects' identity will remain confidential.
p. That the subject or the subject's legally acceptable representative will be informed in a timely manner if information becomes available that may be relevant to the subject's willingness to continue participation in the trial.

q. The person(s) to contact for further information regarding the trial and the rights of trial subjects, and whom to contact in the event of trial-related injury.
r. The foreseeable circumstances and/or reasons under which the subject's participation in the trial may be terminated.
s. The expected duration of the subject's participation in the trial.
t. The approximate number of subjects involved in the trial.

4.8.11 Prior to participation in the trial, the subject or the subject's legally acceptable representative should receive a copy of the signed and dated written informed consent form and any other written information provided to the subjects. During a subjects' participation in the trial, the subject or the subjects' legally acceptable representative should receive a copy of the signed and dated consent form updates and a copy of any amendments to the written information provided to subjects.

4.8.12 When a clinical trial (therapeutic or non-therapeutic) includes subjects who can only be enroled in the trial with the consent of the subjects' legally acceptable representative (e.g., minors, or patients with severe dementia), the subject should be informed about the trial to the extent compatible with the subjects' understanding and, if capable, the subject should sign and personally date the written informed consent.

4.8.13 Except as described in 4.8.14, a non-therapeutic trial (i.e., a trial in which there is no anticipated direct clinical benefit to the subject), should be conducted in subjects who personally give consent and who sign and date the written informed consent form.

4.8.14 Non-therapeutic trials may be conducted in subjects with consent of a legally acceptable representative provided the following conditions are fulfilled:

a. The objectives of the trial cannot be met by means of a trial in subjects who can give informed consent personally.
b. The foreseeable risks to the subjects are low.
c. The negative impact on the subjects' well-being is minimized and low.
d. The trial is not prohibited by law.
e. The approval/favorable opinion of the IRB/IEC is expressly sought on the inclusion of such subjects, and the written approval/favorable opinion covers this aspect.

Such trials, unless an exception is justified, should be conducted in patients having a disease or condition for which the investigational product is intended. Subjects in these trials should be particularly closely monitored and should be withdrawn if they appear to be unduly distressed.

4.8.15 In emergency situations, when prior consent of the subject is not possible, the consent of the subject's legally acceptable representative, if present, should be requested. When prior consent of the subject is not possible, and the subjects' legally acceptable representative is not available, enrolment of the subject should require measures described in the protocol and/or elsewhere, with documented approval/favorable opinion by the IRB/IEC, to protect the rights, safety and well-being of the subject and to ensure compliance with applicable regulatory requirements. The subject or the subject's legally acceptable representative should be informed about the trial as soon as possible and consent to continue and other consent as appropriate (see 4.8.10) should be requested.

4.9 Records and Reports

4.9.1 The investigator should ensure the accuracy, completeness, legibility, and timeliness of the data reported to the sponsor in the CRFs and in all required reports.

4.9.2 Data reported on the CRF, that are derived from source documents, should be consistent with the source documents or the discrepancies should be explained.

4.9.3 Any change or correction to a CRF should be dated, initialed, and explained (if necessary) and should not obscure the original entry (i.e., an audit trail should be maintained); this applies to both written and electronic changes or corrections (see 5.18.4n). Sponsors

should provide guidance to investigators and/or the investigators' designated representatives on making such corrections. Sponsors should have written procedures to assure that changes or corrections in CRFs made by sponsor's designated representatives are documented, are necessary, and are endorsed by the investigator. The investigator should retain records of the changes and corrections.

4.9.4 The investigator/institution should maintain the trial documents as specified in Essential Documents for the Conduct of a Clinical Trial (see 8) and as required by the applicable regulatory requirement(s). The investigator/institution should take measures to prevent accidental or premature destruction of these documents.

4.9.5 Essential documents should be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by an agreement with the sponsor. It is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained (see 5.5.12).

4.9.6 The financial aspects of the trial should be documented in an agreement between the sponsor and the investigator/institution.

4.9.7 Upon request of the monitor, auditor, IRB/IEC, or regulatory authority, the investigator/institution should make available for direct access all requested trial-related records.

4.10 Progress Reports

4.10.1 The investigator should submit written summaries of the trial status to the IRB/IEC annually, or more frequently, if requested by the IRB/IEC.

4.10.2 The investigator should promptly provide written reports to the sponsor, the IRB/IEC (see 3.3.8) and, where applicable, the institution on any changes significantly affecting the conduct of the trial, and/or increasing the risk to subjects.

4.11 Safety Reporting

4.11.1 All serious adverse events (SAEs) should be reported immediately to the sponsor except for those SAEs that the protocol or other document (e.g., Investigator's Brochure) identifies as not needing immediate reporting. The immediate reports should be followed promptly by detailed written reports. The immediate and follow-up reports should identify subjects by unique code numbers assigned to the trial subjects rather than by the subjects' names, personal identification numbers, and/or addresses. The investigator should also comply with the applicable regulatory requirement(s) related to the reporting of unexpected serious adverse drug reactions to the regulatory authority(ies) and the IRB/IEC.

4.11.2 Adverse events and/or laboratory abnormalities identified in the protocol as critical to safety evaluations should be reported to the sponsor according to the reporting requirements and within the time periods specified by the sponsor in the protocol.

4.11.3 For reported deaths, the investigator should supply the sponsor and the IRB/IEC with any additional requested information (e.g., autopsy reports and terminal medical reports).

4.12 Premature Termination or Suspension of a Trial

If the trial is prematurely terminated or suspended for any reason, the investigator/institution should promptly inform the trial subjects, should assure appropriate therapy and follow-up for the subjects, and, where required by the applicable regulatory requirement(s), should inform the regulatory authority(ies). In addition:

4.12.1 If the investigator terminates or suspends a trial without prior agreement of the sponsor, the investigator should inform the institution where applicable, and the investigator/-institution should promptly inform the sponsor and the IRB/IEC, and should provide the sponsor and the IRB/IEC a detailed written explanation of the termination or suspension.

4.12.2 If the sponsor terminates or suspends a trial (see 5.21), the investigator should promptly inform the institution where applicable and the investigator/institution should promptly inform the IRB/IEC and provide the IRB/IEC a detailed written explanation of the termination or suspension.

4.12.3 If the IRB/IEC terminates or suspends its approval/favorable opinion of a trial (see 3.1.2 and 3.3.9), the investigator should inform the institution where applicable and the investigator/institution should promptly notify the sponsor and provide the sponsor with a detailed written explanation of the termination or suspension.

4.13 Final Report(s) by Investigator

Upon completion of the trial, the investigator, where applicable, should inform the institution; the investigator/institution should provide the IRB/IEC with a summary of the trial's outcome, and the regulatory authority(ies) with any reports required.

5. SPONSOR

5.1 Quality Assurance and Quality Control

5.1.1 The sponsor is responsible for implementing and maintaining quality assurance and quality control systems with written SOPs to ensure that trials are conducted and data are generated, documented (recorded), and reported in compliance with the protocol, GCP, and the applicable regulatory requirement(s).

5.1.2 The sponsor is responsible for securing agreement from all involved parties to ensure direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by domestic and foreign regulatory authorities.

5.1.3 Quality control should be applied to each stage of data handling to ensure that all data are reliable and have been processed correctly.

5.1.4 Agreements, made by the sponsor with the investigator/institution and any other parties involved with the clinical trial, should be in writing, as part of the protocol or in a separate agreement.

5.2 Contract Research Organization (CRO)

5.2.1 A sponsor may transfer any or all of the sponsor's trial-related duties and functions to a CRO, but the ultimate responsibility for the quality and integrity of the trial data always resides with the sponsor. The CRO should implement quality assurance and quality control.

5.2.2 Any trial-related duty and function that is transferred to and assumed by a CRO should be specified in writing.

5.2.3 Any trial-related duties and functions not specifically transferred to and assumed by a CRO are retained by the sponsor.

5.2.4 All references to a sponsor in this guideline also apply to a CRO to the extent that a CRO has assumed the trial related duties and functions of a sponsor.

5.3 Medical Expertise

The sponsor should designate appropriately qualified medical personnel who will be readily available to advise on trial related medical questions or problems. If necessary, outside consultant(s) may be appointed for this purpose.

5.4 Trial Design

5.4.1 The sponsor should utilize qualified individuals (e.g., biostatisticians, clinical pharmacologists and physicians) as appropriate, throughout all stages of the trial process, from designing the protocol and CRFs and planning the analyses to analyzing and preparing interim and final clinical trial reports.

5.4.2 For further guidance: Clinical Trial Protocol and Protocol Amendment(s) (see 6), the ICH Guideline for Structure and Content of Clinical Study Reports, and other appropriate ICH guidance on trial design, protocol and conduct.

5.5 Trial Management, Data Handling, and Record Keeping

5.5.1 The sponsor should utilize appropriately qualified individuals to supervise the overall conduct of the trial, to handle the data, to verify the data, to conduct the statistical analyses, and to prepare the trial reports.

5.5.2 The sponsor may consider establishing an independent data-monitoring committee (IDMC) to assess the progress of a clinical trial, including the safety data and the critical efficacy endpoints at intervals, and to recommend to the sponsor whether to continue, modify, or stop a trial. The IDMC should have written operating procedures and maintain written records of all its meetings.

5.5.3 When using electronic trial data handling and/or remote electronic trial data systems, the sponsor should:

a. Ensure and document that the electronic data processing system(s) conforms to the sponsor's established requirements for completeness, accuracy, reliability, and consistent intended performance (i.e., validation).
b. Maintains SOPs for using these systems.
c. Ensure that the systems are designed to permit data changes in such a way that the data changes are documented and that there is no deletion of entered data (i.e., maintain an audit trail, data trail, edit trail).
d. Maintain a security system that prevents unauthorized access to the data.
e. Maintain a list of the individuals who are authorized to make data changes (see 4.1.5 and 4.9.3).
f. Maintain adequate backup of the data.
g. Safeguard the blinding, if any (e.g., maintain the blinding during data entry and processing).

5.5.4 If data are transformed during processing, it should always be possible to compare the original data and observations with the processed data.

5.5.5 The sponsor should use an unambiguous subject identification code that allows identification of all the data reported for each subject.

5.5.6 The sponsor, or other owners of the data, should retain all of the sponsor-specific essential documents pertaining to the trial.

5.5.7 The sponsor should retain all sponsor-specific essential documents in conformance with the applicable regulatory requirement(s) of the country(ies) where the product is approved, and/or where the sponsor intends to apply for approval(s).

5.5.8 If the sponsor discontinues the clinical development of an investigational product (i.e., for any or all indications, routes of administration, or dosage forms), the sponsor should maintain all sponsor-specific essential documents for at least 2 years after formal discontinuation or in conformance with the applicable regulatory requirement(s).

5.5.9 If the sponsor discontinues the clinical development of an investigational product, the sponsor should notify all the trial investigators/institutions and all the regulatory authorities.

5.5.10 Any transfer of ownership of the data should be reported to the appropriate authority(ies), as required by the applicable regulatory requirement(s).

5.5.11 The sponsor specific essential documents should be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are

no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period, however, if required by the applicable regulatory requirement(s) or if needed by the sponsor.

5.5.12 The sponsor should inform the investigator(s)/institution(s) in writing of the need for record retention and should notify the investigator(s)/institution(s) in writing when the trial related records are no longer needed.

5.6 Investigator Selection

5.6.1 The sponsor is responsible for selecting the investigator(s)/institution(s). Each investigator should be qualified by training and experience and should have adequate resources (see 4.1 and 4.2) to properly conduct the trial for which the investigator is selected. If organization of a coordinating committee and/or selection of coordinating investigator(s) are to be utilized in multicenter trials, their organization and/or selection are the sponsor's responsibility.

5.6.2 Before entering an agreement with an investigator/institution to conduct a trial, the sponsor should provide the investigator(s)/institution(s) with the protocol and an up-to-date Investigator's Brochure, and should provide sufficient time for the investigator/institution to review the protocol and the information provided.

5.6.3 The sponsor should obtain the investigator's/institution's agreement:

a. to conduct the trial in compliance with GCP, with the applicable regulatory requirement(s) (see 4.1.3), and with the protocol agreed to by the sponsor and given approval/favorable opinion by the IRB/IEC (see 4.5.1);
b. to comply with procedures for data recording/reporting;
c. to permit monitoring, auditing and inspection (see 4.1.4); and
d. to retain the trial related essential documents until the sponsor informs the investigator/institution these documents are no longer needed (see 4.9.4 and 5.5.12).

The sponsor and the investigator/institution should sign the protocol, or an alternative document, to confirm this agreement.

5.7 Allocation of Responsibilities

Prior to initiating a trial, the sponsor should define, establish, and allocate all trial-related duties and functions.

5.8 Compensation to Subjects and Investigators

5.8.1 If required by the applicable regulatory requirement(s), the sponsor should provide insurance or should indemnify (legal and financial coverage) the investigator/the institution against claims arising from the trial, except for claims that arise from malpractice and/or negligence.

5.8.2 The sponsor's policies and procedures should address the costs of treatment of trial subjects in the event of trial-related injuries in accordance with the applicable regulatory requirement(s).

5.8.3 When trial subjects receive compensation, the method and manner of compensation should comply with applicable regulatory requirement(s).

5.9 Financing

The financial aspects of the trial should be documented in an agreement between the sponsor and the investigator/institution.

5.10 Notification/Submission to Regulatory Authority(ies)

Before initiating the clinical trial(s), the sponsor [or the sponsor and the investigator, if required by the applicable regulatory requirement(s)] should submit any required application(s) to the appropriate authority(ies) for review, acceptance, and/or permission [as required by the applicable regulatory requirement(s)] to begin the trial(s). Any notification/submission should be dated and contain sufficient information to identify the protocol.

5.11 Confirmation of Review by IRB/IEC

5.11.1 The sponsor should obtain from the investigator/institution:
   a. The name and address of the investigator's/institution's IRB/IEC.
   b. A statement obtained from the IRB/IEC that it is organized and operates according to GCP and the applicable laws and regulations.
   c. Documented IRB/IEC approval/favorable opinion and, if requested by the sponsor, a current copy of protocol, written informed consent form(s) and any other written information to be provided to subjects, subject recruiting procedures, and documents related to payments and compensation available to the subjects, and any other documents that the IRB/IEC may have requested.

5.11.2 If the IRB/IEC conditions its approval/favorable opinion upon change(s) in any aspect of the trial, such as modification(s) of the protocol, written informed consent form and any other written information to be provided to subjects, and/or other procedures, the sponsor should obtain from the investigator/institution a copy of the modification(s) made and the date approval/favorable opinion was given by the IRB/IEC.

5.11.3 The sponsor should obtain from the investigator/institution documentation and dates of any IRB/IEC reapprovals/re-evaluations with favorable opinion, and of any withdrawals or suspensions of approval/favorable opinion.

5.12 Information on Investigational Product(s)

5.12.1 When planning trials, the sponsor should ensure that sufficient safety and efficacy data from nonclinical studies and/or clinical trials are available to support human exposure by the route, at the dosages, for the duration, and in the trial population to be studied.

5.12.2 The sponsor should update the Investigator's Brochure as significant new information becomes available (see 7).

5.13 Manufacturing, Packaging, Labeling, and Coding Investigational Product(s)

5.13.1 The sponsor should ensure that the investigational product(s) (including active comparator(s) and placebo, if applicable) is characterized as appropriate to the stage of development of the product(s), is manufactured in accordance with any applicable GMP, and is coded and labeled in a manner that protects the blinding, if applicable. In addition, the labeling should comply with applicable regulatory requirement(s).

5.13.2 The sponsor should determine, for the investigational product(s), acceptable storage temperatures, storage conditions (e.g., protection from light), storage times, reconstitution fluids and procedures, and devices for product infusion, if any. The sponsor should inform all involved parties (e.g., monitors, investigators, pharmacists, storage managers) of these determinations.

5.13.3 The investigational product(s) should be packaged to prevent contamination and unacceptable deterioration during transport and storage.

5.13.4 In blinded trials, the coding system for the investigational product(s) should include a mechanism that permits rapid identification of the product(s) in case of a medical emergency, but does not permit undetectable breaks of the blinding.

5.13.5 If significant formulation changes are made in the investigational or comparator product(s) during the course of clinical development, the results of any additional studies of the formulated product(s) (e.g., stability, dissolution rate, bioavailability)

needed to assess whether these changes would significantly alter the pharmacokinetic profile of the product should be available prior to the use of the new formulation in clinical trials.

5.14 Supplying and Handling Investigational Product(s)

5.14.1 The sponsor is responsible for supplying the investigator(s)/institution(s) with the investigational product(s).

5.14.2 The sponsor should not supply an investigator/institution with the investigational product(s) until the sponsor obtains all required documentation [e.g., approval/favorable opinion from IRB/IEC and regulatory authority(ies)].

5.14.3 The sponsor should ensure that written procedures include instructions that the investigator/institution should follow for the handling and storage of investigational product(s) for the trial and documentation thereof. The procedures should address adequate and safe receipt, handling, storage, dispensing, retrieval of unused product from subjects, and return of unused investigational product(s) to the sponsor [or alternative disposition if authorized by the sponsor and in compliance with the applicable regulatory requirement(s)].

5.14.4 The sponsor should:

a. Ensure timely delivery of investigational product(s) to the investigator(s).
b. Maintain records that document shipment, receipt, disposition, return, and destruction of the investigational product(s) (see 8).
c. Maintain a system for retrieving investigational products and documenting this retrieval (e.g., for deficient product recall, reclaim after trial completion, expired product reclaim).
d. Maintain a system for the disposition of unused investigational product(s) and for the documentation of this disposition.

5.14.5 The sponsor should:

a. Take steps to ensure that the investigational product(s) are stable over the period of use.
b. Maintain sufficient quantities of the investigational product(s) used in the trials to reconfirm specifications, should this become necessary, and maintain records of batch sample analyses and characteristics. To the extent stability permits, samples should be retained either until the analyses of the trial data are complete or as required by the applicable regulatory requirement(s), whichever represents the longer retention period.

5.15 Record Access

5.15.1 The sponsor should ensure that it is specified in the protocol or other written agreement that the investigator(s)/institution(s) provide direct access to source data/documents for trial-related monitoring, audits, IRB/IEC review, and regulatory inspection.

5.15.2 The sponsor should verify that each subject has consented, in writing, to direct access to his/her original medical records for trial-related monitoring, audit, IRB/IEC review, and regulatory inspection.

5.16 Safety Information

5.16.1 The sponsor is responsible for the ongoing safety evaluation of the investigational product(s).

5.16.2 The sponsor should promptly notify all concerned investigator(s)/institution(s) and the regulatory authority(ies) of findings that could affect adversely the safety of subjects, impact the conduct of the trial, or alter the IRB/IEC's approval/favorable opinion to continue the trial.

5.17 Adverse Drug Reaction Reporting

5.17.1 The sponsor should expedite the reporting to all concerned investigator(s)/institutions(s), to the IRB(s)/IEC(s), where required, and to the regulatory authority(ies) of all adverse drug reactions (ADRs) that are both serious and unexpected.
5.17.2 Such expedited reports should comply with the applicable regulatory requirement(s) and with the ICH Guideline for Clinical Safety Data Management: Definitions and Standards for Expedited Reporting.
5.17.3 The sponsor should submit to the regulatory authority(ies) all safety updates and periodic reports, as required by applicable regulatory requirement(s).

5.18 Monitoring

5.18.1 Purpose

The purposes of trial monitoring are to verify that:

a. The rights and well-being of human subjects are protected.
b. The reported trial data are accurate, complete, and verifiable from source documents.
c. The conduct of the trial is in compliance with the currently approved protocol/amendment(s), with GCP, and with the applicable regulatory requirement(s).

5.18.2 Selection and Qualifications of Monitors

a. Monitors should be appointed by the sponsor.
b. Monitors should be appropriately trained, and should have the scientific and/or clinical knowledge needed to monitor the trial adequately. A monitor's qualifications should be documented.
c. Monitors should be thoroughly familiar with the investigational product(s), the protocol, written informed consent form and any other written information to be provided to subjects, the sponsor's SOPs, GCP, and the applicable regulatory requirement(s).

5.18.3 Extent and Nature of Monitoring

The sponsor should ensure that the trials are adequately monitored. The sponsor should determine the appropriate extent and nature of monitoring. The determination of the extent and nature of monitoring should be based on considerations such as the objective, purpose, design, complexity, blinding, size, and endpoints of the trial. In general there is a need for on-site monitoring, before, during, and after the trial; however, in exceptional circumstances the sponsor may determine that central monitoring in conjunction with procedures such as investigators' training and meetings, and extensive written guidance can assure appropriate conduct of the trial in accordance with GCP. Statistically controlled sampling may be an acceptable method for selecting the data to be verified.

5.18.4 Monitor's Responsibilities

The monitor(s) in accordance with the sponsor's requirements should ensure that the trial is conducted and documented properly by carrying out the following activities when relevant and necessary to the trial and the trial site:

a. Acting as the main line of communication between the sponsor and the investigator.
b. Verifying that the investigator has adequate qualifications and resources (see 4.1, 4.2, and 5.6) and remain adequate throughout the trial period, and that facilities, including laboratories, equipment, and staff, are adequate to safely and properly conduct the trial and remain adequate throughout the trial period.
c. Verifying, for the investigational product(s): (*i*) That storage times and conditions are acceptable, and that supplies are sufficient throughout the trial. (*ii*) That the investigational product(s) are supplied only to subjects who are eligible to receive it and at the protocol specified dose(s). (*iii*) That subjects are provided with necessary instruction on properly using, handling, storing, and returning the investigational product(s). (*iv*) That the receipt, use, and return of the investigational product(s) at

the trial sites are controlled and documented adequately. (*v*) That the disposition of unused investigational product(s) at the trial sites complies with applicable regulatory requirement(s) and is in accordance with the sponsor.

d. Verifying that the investigator follows the approved protocol and all approved amendment(s), if any.
e. Verifying that written informed consent was obtained before each subject's participation in the trial.
f. Ensuring that the investigator receives the current Investigator's Brochure, all documents, and all trial supplies needed to conduct the trial properly and to comply with the applicable regulatory requirement(s).
g. Ensuring that the investigator and the investigator's trial staff are adequately informed about the trial.
h. Verifying that the investigator and the investigator's trial staff are performing the specified trial functions, in accordance with the protocol and any other written agreement between the sponsor and the investigator/institution, and have not delegated these functions to unauthorized individuals.
i. Verifying that the investigator is enrolling only eligible subjects.
j. Reporting the subject recruitment rate.
k. Verifying that source documents and other trial records are accurate, complete, kept up-to-date and maintained.
l. Verifying that the investigator provides all the required reports, notifications, applications, and submissions, and that these documents are accurate, complete, timely, legible, dated, and identify the trial.
m. Checking the accuracy and completeness of the CRF entries, source documents and other trial-related records against each other. The monitor specifically should verify that: (*i*) The data required by the protocol are reported accurately on the CRFs and are consistent with the source documents. (*ii*) Any dose and/or therapy modifications are well documented for each of the trial subjects. (*iii*) Adverse events, concomitant medications and intercurrent illnesses are reported in accordance with the protocol on the CRFs. (*iv*) Visits that the subjects fail to make, tests that are not conducted, and examinations that are not performed are clearly reported as such on the CRFs. (*v*) All withdrawals and dropouts of enrolled subjects from the trial are reported and explained on the CRFs.
n. Informing the investigator of any CRF entry error, omission, or illegibility. The monitor should ensure that appropriate corrections, additions, or deletions are made, dated, explained (if necessary), and initialed by the investigator or by a member of the investigator's trial staff who is authorized to initial CRF changes for the investigator. This authorization should be documented.
o. Determining whether all adverse events (AEs) are appropriately reported within the time periods required by GCP, the protocol, the IRB/IEC, the sponsor, and the applicable regulatory requirement(s).
p. Determining whether the investigator is maintaining the essential documents (see 8).
q. Communicating deviations from the protocol, SOPs, GCP, and the applicable regulatory requirements to the investigator and taking appropriate action designed to prevent recurrence of the detected deviations.

5.18.5 Monitoring Procedures

The monitor(s) should follow the sponsor's established written SOPs as well as those procedures that are specified by the sponsor for monitoring a specific trial.

5.18.6 Monitoring Report

a. The monitor should submit a written report to the sponsor after each trial-site visit or trial-related communication.
b. Reports should include the date, site, name of the monitor, and name of the investigator or other individual(s) contacted.
c. Reports should include a summary of what the monitor reviewed and the monitor's statements concerning the significant findings/facts, deviations and deficiencies,

conclusions, actions taken or to be taken and/or actions recommended to secure compliance.

d. The review and follow-up of the monitoring report with the sponsor should be documented by the sponsor's designated representative.

5.19 Audit

If or when sponsors perform audits, as part of implementing quality assurance, they should consider:

5.19.1 Purpose

The purpose of a sponsor's audit, which is independent of and separate from routine monitoring or quality control functions, should be to evaluate trial conduct and compliance with the protocol, SOPs, GCP, and the applicable regulatory requirements.

5.19.2 Selection and Qualification of Auditors

a. The sponsor should appoint individuals, who are independent of the clinical trials/systems, to conduct audits.
b. The sponsor should ensure that the auditors are qualified by training and experience to conduct audits properly. An auditor's qualifications should be documented.

5.19.3 Auditing Procedures

a. The sponsor should ensure that the auditing of clinical trials/systems is conducted in accordance with the sponsor's written procedures on what to audit, how to audit, the frequency of audits, and the form and content of audit reports.
b. The sponsor's audit plan and procedures for a trial audit should be guided by the importance of the trial to submissions to regulatory authorities, the number of subjects in the trial, the type and complexity of the trial, the level of risks to the trial subjects, and any identified problem(s).
c. The observations and findings of the auditor(s) should be documented.
d. To preserve the independence and value of the audit function, the regulatory authority(ies) should not routinely request the audit reports. Regulatory authority(ies) may seek access to an audit report on a case by case basis when evidence of serious GCP non-compliance exists, or in the course of legal proceedings.
e. When required by applicable law or regulation, the sponsor should provide an audit certificate.

5.20 Noncompliance

5.20.1 Noncompliance with the protocol, SOPs, GCP, and/or applicable regulatory requirement(s) by an investigator/institution, or by member(s) of the sponsor's staff should lead to prompt action by the sponsor to secure compliance.

5.20.2 If the monitoring and/or auditing identifies serious and/or persistent noncompliance on the part of an investigator/institution, the sponsor should terminate the investigator's/institution's participation in the trial. When an investigator's/institution's participation is terminated because of noncompliance, the sponsor should notify promptly the regulatory authority(ies).

5.21 Premature Termination or Suspension of a Trial

If a trial is prematurely terminated or suspended, the sponsor should promptly inform the investigators/institutions, and the regulatory authority(ies) of the termination or suspension and the reason(s) for the termination or suspension. The IRB/IEC should also be informed promptly and provided the reason(s) for the termination or suspension by the sponsor or by the investigator/institution, as specified by the applicable regulatory requirement(s).

5.22 Clinical Trial/Study Reports

Whether the trial is completed or prematurely terminated, the sponsor should ensure that the clinical trial reports are prepared and provided to the regulatory agency(ies) as required by the applicable regulatory requirement(s). The sponsor should also ensure that the clinical trial reports in marketing applications meet the standards of the ICH Guideline for Structure and Content of Clinical Study Reports. (*Note*: The ICH Guideline for Structure and Content of Clinical Study Reports specifies that abbreviated study reports may be acceptable in certain cases.)

5.23 Multicenter Trials

For multicenter trials, the sponsor should ensure that:

5.23.1 All investigators conduct the trial in strict compliance with the protocol agreed to by the sponsor and, if required, by the regulatory authority(ies), and given approval/favorable opinion by the IRB/IEC.
5.23.2 The CRFs are designed to capture the required data at all multicenter trial sites. For those investigators who are collecting additional data, supplemental CRFs should also be provided that are designed to capture the additional data.
5.23.3 The responsibilities of coordinating investigator(s) and the other participating investigators are documented prior to the start of the trial.
5.23.4 All investigators are given instructions on following the protocol, on complying with a uniform set of standards for the assessment of clinical and laboratory findings, and on completing the CRFs.
5.23.5 Communication between investigators is facilitated.

6. CLINICAL TRIAL PROTOCOL AND PROTOCOL AMENDMENT(S)

The contents of a trial protocol should generally include the following topics. However, site specific information may be provided on separate protocol page(s), or addressed in a separate agreement, and some of the information listed below may be contained in other protocol referenced documents, such as an Investigator's Brochure.

6.1 General Information

6.1.1 Protocol title, protocol identifying number, and date. Any amendment(s) should also bear the amendment number(s) and date(s).
6.1.2 Name and address of the sponsor and monitor (if other than the sponsor).
6.1.3 Name and title of the person(s) authorized to sign the protocol and the protocol amendment(s) for the sponsor.
6.1.4 Name, title, address, and telephone number(s) of the sponsor's medical expert (or dentist when appropriate) for the trial.
6.1.5 Name and title of the investigator(s) who is (are) responsible for conducting the trial, and the address and telephone number(s) of the trial site(s).
6.1.6 Name, title, address, and telephone number(s) of the qualified physician (or dentist, if applicable), who is responsible for all trial-site related medical (or dental) decisions (if other than investigator).
6.1.7 Name(s) and address(es) of the clinical laboratory(ies) and other medical and/or technical department(s) and/or institutions involved in the trial.

6.2 Background Information

6.2.1 Name and description of the investigational product(s).
6.2.2 A summary of findings from nonclinical studies that potentially have clinical significance and from clinical trials that are relevant to the trial.
6.2.3 Summary of the known and potential risks and benefits, if any, to human subjects.

6.2.4 Description of and justification for the route of administration, dosage, dosage regimen, and treatment period(s).
6.2.5 A statement that the trial will be conducted in compliance with the protocol, GCP, and the applicable regulatory requirement(s).
6.2.6 Description of the population to be studied.
6.2.7 References to literature and data that are relevant to the trial, and that provide background for the trial.

6.3 Trial Objectives and Purpose

A detailed description of the objectives and the purpose of the trial.

6.4 Trial Design

The scientific integrity of the trial and the credibility of the data from the trial depend substantially on the trial design. A description of the trial design, should include:

6.4.1 A specific statement of the primary endpoints and the secondary endpoints, if any, to be measured during the trial.
6.4.2 A description of the type/design of trial to be conducted (e.g., double-blind, placebo-controlled, parallel design) and a schematic diagram of trial design, procedures and stages.
6.4.3 A description of the measures taken to minimize/avoid bias, including: (*i*) Randomization. (*ii*) Blinding.
6.4.4 A description of the trial treatment(s) and the dosage and dosage regimen of the investigational product(s). Also include a description of the dosage form, packaging, and labeling of the investigational product(s).
6.4.5 The expected duration of subject participation, and a description of the sequence and duration of all trial periods, including follow-up, if any.
6.4.6 A description of the "stopping rules" or "discontinuation criteria" for individual subjects, parts of trial and entire trial.
6.4.7 Accountability procedures for the investigational product(s), including the placebo(s) and comparator(s), if any.
6.4.8 Maintenance of trial treatment randomization codes and procedures for breaking codes.
6.4.9 The identification of any data to be recorded directly on the CRFs (i.e., no prior written or electronic record of data), and to be considered to be source data.

6.5 Selection and Withdrawal of Subjects

6.5.1 Subject inclusion criteria.
6.5.2 Subject exclusion criteria.
6.5.3 Subject withdrawal criteria (i.e., terminating investigational product treatment/trial treatment) and procedures specifying: (*i*) When and how to withdraw subjects from the trial/investigational product treatment. (*ii*) The type and timing of the data to be collected for withdrawn subjects. (*iii*) Whether and how subjects are to be replaced. (*iv*) The follow-up for subjects withdrawn from investigational product treatment/trial treatment.

6.6 Treatment of Subjects

6.6.1 The treatment(s) to be administered, including the name(s) of all the product(s), the dose(s), the dosing schedule(s), the route/mode(s) of administration, and the treatment period(s), including the follow-up period(s) for subjects for each investigational product treatment/trial treatment group/arm of the trial.
6.6.2 Medication(s)/treatment(s) permitted (including rescue medication) and not permitted before and/or during the trial.
6.6.3 Procedures for monitoring subject compliance.

6.7 Assessment of Efficacy

6.7.1 Specification of the efficacy parameters.
6.7.2 Methods and timing for assessing, recording, and analyzing of efficacy parameters.

6.8 Assessment of Safety

6.8.1 Specification of safety parameters.
6.8.2 The methods and timing for assessing, recording, and analyzing safety parameters.
6.8.3 Procedures for eliciting reports of and for recording and reporting adverse event and intercurrent illnesses.
6.8.4 The type and duration of the follow-up of subjects after adverse events.

6.9 Statistics

6.9.1 A description of the statistical methods to be employed, including timing of any planned interim analysis(ses).
6.9.2 The number of subjects planned to be enroled. In multicenter trials, the numbers of enroled subjects projected for each trial site should be specified. Reason for choice of sample size, including reflections on (or calculations of) the power of the trial and clinical justification.
6.9.3 The level of significance to be used.
6.9.4 Criteria for the termination of the trial.
6.9.5 Procedure for accounting for missing, unused, and spurious data.
6.9.6 Procedures for reporting any deviation(s) from the original statistical plan [any deviation(s) from the original statistical plan should be described and justified in protocol and/or in the final report, as appropriate].
6.9.7 The selection of subjects to be included in the analyses (e.g., all randomized subjects, all dosed subjects, all eligible subjects, evaluable subjects).

6.10 Direct Access to Source Data/Documents

The sponsor should ensure that it is specified in the protocol or other written agreement that the investigator(s)/institution(s) will permit trial-related monitoring, audits, IRB/IEC review, and regulatory inspection(s), providing direct access to source data/documents.

6.11 Quality Control and Quality Assurance

6.12 Ethics

Description of ethical considerations relating to the trial.

6.13 Data Handling and Record Keeping

6.14 Financing and Insurance

Financing and insurance if not addressed in a separate agreement.

6.15 Publication Policy

Publication policy, if not addressed in a separate agreement.

6.16 Supplements

(*Note*: Since the protocol and the clinical trial/study report are closely related, further relevant information can be found in the ICH Guideline for Structure and Content of Clinical Study Reports.)

7. INVESTIGATOR'S BROCHURE

7.1 Introduction

The Investigator's Brochure (IB) is a compilation of the clinical and nonclinical data on the investigational product(s) that are relevant to the study of the product(s) in human subjects. Its purpose is to provide the investigators and others involved in the trial with the information to facilitate their understanding of the rationale for, and their compliance with, many key features of the protocol, such as the dose, dose frequency/interval, methods of administration and safety monitoring procedures. The IB also provides insight to support the clinical management of the study subjects during the course of the clinical trial. The information should be presented in a concise, simple, objective, balanced, and non-promotional form that enables a clinician, or potential investigator, to understand it and make his/her own unbiased risk-benefit assessment of the appropriateness of the proposed trial. For this reason, a medically qualified person should generally participate in the editing of an IB, but the contents of the IB should be approved by the disciplines that generated the described data.

This guideline delineates the minimum information that should be included in an IB and provides suggestions for its layout. It is expected that the type and extent of information available will vary with the stage of development of the investigational product. If the investigational product is marketed and its pharmacology is widely understood by medical practitioners, an extensive IB may not be necessary. Where permitted by regulatory authorities, a basic product information brochure, package leaflet, or labeling may be an appropriate alternative, provided that it includes current, comprehensive, and detailed information on all aspects of the investigational product that might be of importance to the investigator. If a marketed product is being studied for a new use (i.e., a new indication), an IB specific to that new use should be prepared. The IB should be reviewed at least annually and revised as necessary in compliance with a sponsor's written procedures. More frequent revision may be appropriate depending on the stage of development and the generation of relevant new information. However, in accordance with Good Clinical Practice, relevant new information may be so important that it should be communicated to the investigators, and possibly to the Institutional Review Boards (IRBs)/Independent Ethics Committees (IECs) and/or regulatory authorities before it is included in a revised IB.

Generally, the sponsor is responsible for ensuring that an up-to-date IB is made available to the investigator(s) and the investigators are responsible for providing the up-to-date IB to the responsible IRBs/IECs. In the case of an investigator sponsored trial, the sponsor-investigator should determine whether a brochure is available from the commercial manufacturer. If the investigational product is provided by the sponsor-investigator, then he or she should provide the necessary information to the trial personnel. In cases where preparation of a formal IB is impractical, the sponsor-investigator should provide, as a substitute, an expanded background information section in the trial protocol that contains the minimum current information described in this guideline.

7.2 General Considerations

The IB should include:

7.2.1 Title Page

This should provide the sponsor's name, the identity of each investigational product [i.e., research number, chemical or approved generic name, and trade name(s) where legally permissible and desired by the sponsor], and the release date. It is also suggested that an edition number, and a reference to the number and date of the edition it supersedes, be provided. An example is given in Appendix 1.

7.2.2 Confidentiality Statement

The sponsor may wish to include a statement instructing the investigator/recipients to treat the IB as a confidential document for the sole information and use of the investigator's team and the IRB/IEC.

7.3 Contents of the Investigator's Brochure

The IB should contain the following sections, each with literature references where appropriate:

7.3.1 Table of Contents

An example of the Table of Contents is given in Appendix 2.

7.3.2 Summary

A brief summary (preferably not exceeding two pages) should be given, highlighting the significant physical, chemical, pharmaceutical, pharmacological, toxicological, pharmacokinetic, metabolic, and clinical information available that is relevant to the stage of clinical development of the investigational product.

7.3.3 Introduction

A brief introductory statement should be provided that contains the chemical name [and generic and trade name(s) when approved] of the investigational product(s), all active ingredients, the investigational product(s) pharmacological class and its expected position within this class (e.g., advantages), the rationale for performing research with the investigational product(s), and the anticipated prophylactic, therapeutic, or diagnostic indication(s). Finally, the introductory statement should provide the general approach to be followed in evaluating the investigational product.

7.3.4 Physical, Chemical, and Pharmaceutical Properties and Formulation

A description should be provided of the investigational product substance(s) [including the chemical and/or structural formula(e)], and a brief summary should be given of the relevant physical, chemical, and pharmaceutical properties.

To permit appropriate safety measures to be taken in the course of the trial, a description of the formulation(s) to be used, including excipients, should be provided and justified if clinically relevant. Instructions for the storage and handling of the dosage form(s) should also be given.

Any structural similarities to other known compounds should be mentioned.

7.3.5 Nonclinical Studies

Introduction

The results of all relevant nonclinical pharmacology, toxicology, pharmacokinetic, and investigational product metabolism studies should be provided in summary form. This summary should address the methodology used, the results, and a discussion of the relevance of the findings to the investigated therapeutic and the possible unfavorable and unintended effects in humans.

The information provided may include the following, as appropriate, if known/available:

a. Species tested
b. Number and sex of animals in each group
c. Unit dose [e.g., milligram/kilogram (mg/kg)]
d. Dose interval
e. Route of administration
f. Duration of dosing
g. Information on systemic distribution
h. Duration of post-exposure follow-up
i. Results, including the following aspects:
    (i) Nature and frequency of pharmacological or toxic effects
    (ii) Severity or intensity of pharmacological or toxic effects
    (iii) Time to onset of effects
    (iv) Reversibility of effects
    (v) Duration of effects
    (vi) Dose response

Tabular format/listings should be used whenever possible to enhance the clarity of the presentation.

The following sections should discuss the most important findings from the studies, including the dose response of observed effects, the relevance to humans, and any

aspects to be studied in humans. If applicable, the effective and nontoxic dose findings in the same animal species should be compared (i.e., the therapeutic index should be discussed). The relevance of this information to the proposed human dosing should be addressed. Whenever possible, comparisons should be made in terms of blood/tissue levels rather than on a mg/kg basis.

a. Nonclinical Pharmacology
   A summary of the pharmacological aspects of the investigational product and, where appropriate, its significant metabolites studied in animals, should be included. Such a summary should incorporate studies that assess potential therapeutic activity (e.g., efficacy models, receptor binding and specificity) as well as those that assess safety [e.g., special studies to assess pharmacological actions other than the intended therapeutic effect(s)].
b. Pharmacokinetics and Product Metabolism in Animals
   A summary of the pharmacokinetics and biological transformation and disposition of the investigational product in all species studied should be given. The discussion of the findings should address the absorption and the local and systemic bioavailability of the investigational product and its metabolites, and their relationship to the pharmacological and toxicological findings in animal species.
c. Toxicology
   A summary of the toxicological effects found in relevant studies conducted in different animal species should be described under the following headings where appropriate:
   – Single dose
   – Repeated dose
   – Carcinogenicity
   – Special studies (e.g., irritancy and sensitisation)
   – Reproductive toxicity
   – Genotoxicity (mutagenicity)

7.3.6 Effects in Humans

Introduction

A thorough discussion of the known effects of the investigational product(s) in humans should be provided, including information on pharmacokinetics, metabolism, pharmacodynamics, dose response, safety, efficacy, and other pharmacological activities. Where possible, a summary of each completed clinical trial should be provided. Information should also be provided regarding results of any use of the investigational product(s) other than from in clinical trials, such as from experience during marketing.

a. Pharmacokinetics and Product Metabolism in Humans—A summary of information on the pharmacokinetics of the investigational product(s) should be presented, including the following, if available:
   – Pharmacokinetics (including metabolism, as appropriate, and absorption, plasma protein binding, distribution, and elimination).
   – Bioavailability of the investigational product (absolute, where possible, and/or relative) using a reference dosage form.
   – Population subgroups (e.g., gender, age, and impaired organ function).
   – Interactions (e.g., product–product interactions and effects of food).
   – Other pharmacokinetic data [e.g., results of population studies performed within clinical trial(s)].
b. Safety and Efficacy
   A summary of information should be provided about the investigational product's/products' (including metabolites, where appropriate) safety, pharmacodynamics, efficacy, and dose response that were obtained from preceding trials in humans (healthy volunteers and/or patients). The implications of this information should be discussed. In cases where a number of clinical trials have been completed, the use of summaries of safety and efficacy across multiple trials by indications in subgroups may provide a clear presentation of the data. Tabular summaries of adverse drug reactions for all the clinical trials (including those for all the studied indications)

would be useful. Important differences in adverse drug reaction patterns/incidences across indications or subgroups should be discussed.

The IB should provide a description of the possible risks and adverse drug reactions to be anticipated on the basis of prior experiences with the product under investigation and with related products. A description should also be provided of the precautions or special monitoring to be done as part of the investigational use of the product(s).

c. Marketing Experience

The IB should identify countries where the investigational product has been marketed or approved. Any significant information arising from the marketed use should be summarized (e.g., formulations, dosages, routes of administration, and adverse product reactions). The IB should also identify all the countries where the investigational product did not receive approval/registration for marketing or was withdrawn from marketing/registration.

7.3.7 Summary of Data and Guidance for the Investigator

This section should provide an overall discussion of the nonclinical and clinical data, and should summarize the information from various sources on different aspects of the investigational product(s), wherever possible. In this way, the investigator can be provided with the most informative interpretation of the available data and with an assessment of the implications of the information for future clinical trials. Where appropriate, the published reports on related products should be discussed. This could help the investigator to anticipate adverse drug reactions or other problems in clinical trials. The overall aim of this section is to provide the investigator with a clear understanding of the possible risks and adverse reactions, and of the specific tests, observations, and precautions that may be needed for a clinical trial. This understanding should be based on the available physical, chemical, pharmaceutical, pharmacological, toxicologic, and clinical information on the investigational product(s). Guidance should also be provided to the clinical investigator on the recognition and treatment of possible overdose and adverse drug reactions that is based on previous human experience and on the pharmacology of the investigational product.

7.4 Appendix 1

Title page (example)

SPONSOR'S NAME
Product:
Research Number:
Name(s): Chemical, Generic (if approved)
Trade Name(s): (if legally permissible and desired by the sponsor)

INVESTIGATOR'S BROCHURE
Edition Number:
Release Date:
Replaces Previous Edition Number:
Date:

7.5 Appendix 2

Table of contents of investigator's brochure (example)

- Confidentiality Statement (optional)
- Signature Page (optional)

1. Table of Contents
2. Summary
3. Introduction
4. Physical, Chemical, and Pharmaceutical Properties and Formulation
5. Nonclinical Studies
   5.1 Nonclinical Pharmacology

5.2 Pharmacokinetics and Product Metabolism in Animals
5.3 Toxicology
6. Effects in Humans
6.1 Pharmacokinetics and Product Metabolism in Humans
6.2 Safety and Efficacy
6.3 Marketing Experience
7. Summary of Data and Guidance for the Investigator

NB: References on

1. Publications
2. Reports

These references should be found at the end of each chapter Appendices (if any).

8. ESSENTIAL DOCUMENTS FOR THE CONDUCT OF A CLINICAL TRIAL

8.1 Introduction

Essential Documents are those documents which individually and collectively permit evaluation of the conduct of a trial and the quality of the data produced. These documents serve to demonstrate the compliance of the investigator, sponsor and monitor with the standards of Good Clinical Practice and with all applicable regulatory requirements.

Essential Documents also serve a number of other important purposes. Filing essential documents at the investigator/institution and sponsor sites in a timely manner can greatly assist in the successful management of a trial by the investigator, sponsor and monitor. These documents are also the ones which are usually audited by the sponsor's independent audit function and inspected by the regulatory authority(ies) as part of the process to confirm the validity of the trial conduct and the integrity of data collected.

The minimum list of essential documents which has been developed follows. The various documents are grouped in three sections according to the stage of the trial during which they will normally be generated: (*i*) before the clinical phase of the trial commences, (*ii*) during the clinical conduct of the trial, and (*iii*) after completion or termination of the trial. A description is given of the purpose of each document, and whether it should be filed in either the investigator/institution or sponsor files, or both. It is acceptable to combine some of the documents, provided the individual elements are readily identifiable.

Trial master files should be established at the beginning of the trial, both at the investigator/institution's site and at the sponsor's office. A final close-out of a trial can only be done when the monitor has reviewed both investigator/institution and sponsor files and confirmed that all necessary documents are in the appropriate files.

Any or all of the documents addressed in this guideline may be subject to, and should be available for, audit by the sponsor's auditor and inspection by the regulatory authority(ies).

8.2 Before the Clinical Phase of the Trial Commences

During this planning stage the following documents should be generated and should be on file before the trial formally starts

8.2.1 Investigator's brochure
8.2.2 Signed protocol and amendments, if any, and sample case report form (CRF)
8.2.3 Information given to trial subject
a. Informed consent form (including all applicable translations)
b. Any other written information
c. Advertisement for subject recruitment (if used).
8.2.4 Financial aspects of the trial
8.2.5 Insurance statement (where required)
8.2.6 Signed agreement between involved parties, e.g.:

a. investigator/institution and sponsor
b. investigator/institution and CRO
c. sponsor and CRO
d. investigator/institution and authority(ies) (where required)

8.2.7 Dated, documented approval/favorable opinion of institutional review board (IRB)/independent ethics committee (IEC) of the following:
a. protocol and any amendments
b. CRF (if applicable)
c. informed consent form(s)
d. any other written information to be provided to the subject(s)
e. advertisement for subject recruitment (if used)
f. subject compensation (if any)
g. any other documents given approval/favorable opinion

8.2.8 Institutional review board/independent ethics committee composition

8.2.9 Regulatory authority(ies) authorization/approval/notification of protocol (where required)

8.2.10 Curriculum vitae and/or other relevant documents evidencing qualifications of investigator(s) and sub-investigator(s)

8.2.11 Normal value(s)/range(s) for medical/laboratory/technical procedure(s) and/or test(s) included in the protocol

8.2.12 Medical/laboratory/technical procedures/tests
a. certification or
b. accreditation or
c. established quality control and/or external quality assessment or
d. other validation (where required)

8.2.13 Sample of label(s) attached to investigational product container(s)

8.2.14 Instructions for handling of investigational product(s) and trial-related materials (if not included in protocol or Investigator's Brochure)

8.2.15 Shipping records for investigational product(s) and trial-related materials

8.2.16 Certificate(s) of analysis of investigational product(s) shipped

8.2.17 Decoding procedures for blinded trials

8.2.18 Master randomization list

8.2.19 Pre-trial monitoring report to document that the site is suitable for the trial

8.2.20 Trial initiation monitoring report

8.3 During the Clinical Conduct of the Trial

In addition to having on file the above documents, the following should be added to the files during the trial as evidence that all new relevant information is documented as it becomes available

8.3.1 Investigator's Brochure updates

8.3.2 Any revision to:
a. protocol/amendment(s) and CRF
b. informed consent form
c. any other written information provided to subjects
d. advertisement for subject recruitment (if used)

8.3.3 Dated, documented approval/favorable opinion of institutional review board (IRB)/independent ethics committee (IEC) of the following:
a. protocol amendment(s)
b. revision(s) of:
c. informed consent form
d. any other written information to be provided to the subject
e. advertisement for subject recruitment (if used)
f. any other documents given approval/favorable opinion
g. continuing review of trial (where required)

8.3.4 Regulatory authority(ies) authorizations/approvals/notifications were required for:
   a. protocol amendment(s) and other documents
8.3.5 Curriculum vitae for new investigator(s) and/or subinvestigator(s)
8.3.6 Updates to normal value(s)/range(s) for medical/laboratory/technical procedure(s)/test(s) included in the protocol
8.3.7 Updates of medical/laboratory/technical procedures/tests
   a. certification or
   b. accreditation or
   c. established quality control and/or external
   d. quality assessment or
   e. other validation (where required)
8.3.8 Documentation of investigational product(s) and trial-related materials shipment
8.3.9 Certificate(s) of analysis for new batches of investigational products
8.3.10 Monitoring visit reports
8.3.11 Relevant communications other than site visits
   a. letters
   b. meeting notes
   c. notes of telephone calls
8.3.12 Signed informed consent forms
8.3.13 Source documents
8.3.14 Signed, dated and completed case report forms (CRF)
8.3.15 Documentation of CRF corrections
8.3.16 Notification by originating investigator to sponsor of serious adverse events and related reports
8.3.17 Notification by sponsor and/or investigator, where applicable, to regulatory authority(ies) and IRB(s)/IEC(s) of unexpected serious adverse drug reactions and of other safety information
8.3.18 Notification by sponsor to investigators of safety information
8.3.19 Interim or annual reports to IRB/IEC and authority(ies)
8.3.20 Subject screening log
8.3.21 Subject identification code list
8.3.22 Subject enrolment log
8.3.23 Investigational products accountability at the site
8.3.24 Signature sheet
8.3.25 Record of retained body fluids/tissue samples (if any)

8.4 After Completion or Termination of the Trial

After completion or termination of the trial, all of the documents identified in sections 8.2 and 8.3 should be in the file together with the following

8.4.1 Investigational product(s) accountability at site
8.4.2 Documentation of investigational product destruction
8.4.3 Completed subject identification code list
8.4.4 Audit certificate (if available)
8.4.5 Final trial close-out monitoring report
8.4.6 Treatment allocation and decoding documentation
8.4.7 Final report by investigator to IRB/IEC where required, and where applicable, to the regulatory authority(ies)
8.4.8 Clinical study report

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11 Good Laboratory Practices for Nonclinical Laboratory Studies

BACKGROUND

The Title 21:58 Code of Federal Regulations (CFR) describes the required practices for nonclinical laboratory testing as it applies to analytical methods development and analysis of biologic samples. Note that this is separate from the good laboratory practices (GLPs) required for clinical trials, which are described elsewhere in the book. Compliance with these GLP guidelines is crucial to meet the audit requirement of Food and Drug Administration (FDA). Given below are the overall principles for GLP compliance and the details of documents required for GLP audits.

The area of GLP compliance is less well understood than the good clinical practices or the analytical method validation; thus, as a preamble to this subject, a few suggestions about GLP compliance are given below.

1. The GLPs do not apply to validation trials conducted to confirm the analytical methods used to determine the concentration of test article in animal tissues and drug dosage forms. However, the GLPs apply to the chemical procedures used to characterize the test article, to determine the stability of the test article and its mixtures, and to determine the homogeneity and concentration of test article mixtures. Likewise, the GLPs apply to the chemical procedures used to analyze specimens (e.g., clinical chemistry and urinalysis). The GLPs do not apply to the work done to develop chemical methods of analysis or to establish the specifications of a test article.
2. GLP compliance applies to all those laboratories that intend to submit data to FDA whether they are located in the United States or anywhere in the world. Photocopies of raw data, which are dated and verified by signature of the copier, are considered to be "exact" copies of the raw data. Records of instrument calibration are considered to be raw data.
3. A computer printout derived from data transferred to computer media from laboratory data sheets is not considered to be raw data.
4. The GLPs do not require a sponsor to approve the study director for a contracted study. It is the responsibility of the testing facility management.
5. If a firm functions as a primary contractor for nonclinical laboratory studies and the actual studies are then subcontracted to nonclinical laboratories, who is considered a "sponsor" will depend on the specific provisions of the contract.
6. The responsibility for test article characterization is not specifically assigned in the GLP, it is a subject of the specific contractual arrangement between the sponsor and the contractor.
7. The contract laboratories do not have to show the sponsor's name on the master schedule sheet, this information can be coded but the code must be revealed to the FDA investigator on request.
8. A contractor need not include in the final report information on test article characterization and stability if such information has been collected by the sponsor. The contractor should identify in its final report which information will be subsequently supplied by the sponsor.
9. The FDA-483 is the written notice of objectionable practices or deviations from the regulations that is prepared by the FDA investigator at the end of the inspection. The items listed on the form serve as the basis for the exit discussion with laboratory

management at which time management can either agree or disagree with the items and can offer possible corrective actions to be taken. Management may also respond to the district office in writing after it has had sufficient time to properly study the FDA-483. The FDA investigator prepares an establishment inspection report (EIR), which summarizes the observations made at the laboratory and which contains exhibits concerning the studies audited [protocols; standard operating procedures, (SOPs); curriculum vitae (CVs), etc.]. The EIR is then reviewed by district personnel and headquarters personnel. This review may reveal additional GLP deviations that should be and are communicated to laboratory management.

10. The GLP investigators should not comment on the scientific merits of a protocol or the scientific interpretation given in the final report. Their function is strictly a noting of observations and verification. Scientific judgments are made by the respective headquarters review units that deal with the test article.
11. A GLP EIR cannot be reviewed by laboratory management prior to its issuance. The GLP EIR is an internal agency document, which reflects the observations and findings of the FDA investigator. It cannot be released to anyone outside the agency until agency action has been completed and the released copy is purged of all trade secret information. Laboratories that disagree with portions of the EIR should write a letter, which contains the areas of disagreement to the local FDA district office. The laboratories can ask that their letters accompany the EIR whenever it is requested under the Freedom of Information Act.
12. The FDA investigators can take photographs of objectionable practices and conditions.
13. Overseas laboratories are scheduled for inspection on the basis of having submitted to FDA the results of significant studies on important products; there is no two-year cycle of inspections.
14. Following background materials are used by agency investigators to prepare for a GLP inspection:
    a. the GLP regulations;
    b. the Management Briefings Post-Conference Report;
    c. assorted memoranda and policy issuances;
    d. the GLP Compliance Program;
    e. the protocol of an ongoing study, if available;
    f. the final report of a completed study, if available;
    g. the inspection report of the most recent inspection.
15. If the results of an inspection reveal that significant deviations from the GLPs exist, the laboratory will be sent a regulatory letter that lists the major deviations and that requests a response within 10 days. The response should describe those actions that the laboratory has taken or plans to take to effect correction. The response should also encompass items that were listed on the FDA-483 and those that were discussed during the exit discussion with laboratory management. A specific timetable should be given for accomplishing the planned actions. The reasonableness of the timetable will be determined by FDA compliance staff, based on the needs of the particular situation. For less significant deviations, the laboratory will be sent a Notice of Adverse Findings letter that also lists the deviations but that requests a response within 30 days. Again, the reasonableness of the response will be determined by FDA staff. The FDA-483 lists observations of violative conditions that have the capability to adversely affect nonclinical laboratory studies. Corrective actions should be instituted as soon as possible. Laboratory management is informed of all routine GLP inspections prior to the inspection, but special compliance or investigative inspections need not be pre-announced.
16. The study director cannot be the chief executive of a nonclinical laboratory. The GLPs require that there be a separation of function between the study director and the quality assurance unit (QAU) director. In the example, the QAU director would be reporting to the study director. The GLPs do permit the designation of an "acting" or "deputy" study director to be responsible for a study when the study director is on leave. Should study records identify the designated "deputy" or "acting" study director?
17. The study director is responsible for adherence to the GLPs. The QAU is not expected to perform a scientific evaluation of a study or to "second-guess" the scientific procedures that

are used. QAU inspections are made to ensure that the GLPs, SOPs, and protocols are being followed and that the data summarized in the final report accurately reflect the results of the study. A variety of procedures can be used to do this but certainly the procedures should include an examination and correlation of the raw data records. The QAU must keep copies of all protocols as currently amended. The only SOPs that the QAU are required to keep are those concerned with the operations and procedures of the QAU.

18. The QAU is not required to monitor compliance with regulations promulgated by other government agencies.
19. An individual who is involved in a nonclinical laboratory study cannot perform QAU functions for portions of the study that the individual is not involved with. However, the individual can perform QAU functions for a study that he/she is not involved with. The QAU does review amendments to the final report.
20. The master schedule sheet should list all nonclinical laboratory studies conducted on FDA regulated products and intended to support an application for a research or marketing permit.
21. The QAU may, in its periodicreports to management and the study director, recommend actions to solve existing problems.
22. The QAU should ensure that the computer-formatted data accurately reflect the raw data. Statistical analyses would comprise a report from participating scientists, which should be checked by QAU and appended to the final report. The QAU is also responsible for maintaining the laboratory archives. The QAU can be constituted as a single person, provided that the workload is not excessive and other duties do not prevent the person from doing an adequate job. It would be prudent to designate an alternate in case of disability, vacations, etc.
23. The GLPs do not isolate responsibility for defining study phases and designating critical study phases; logically, the task should be done by the study director and the participating scientists working in concert with the QAU and laboratory management. It can be covered by an SOP.
24. The agency has not established guidelines for the frequency of calibration of balances used in nonclinical laboratory studies. This would be a large undertaking in part due to the wide variety of equipment that is available and to the differing workloads that would be imposed on the equipment. It is suggested that you work with the equipment manufacturers and your study directors to arrive at a suitable calibration schedule. The key point is that the calibration should be frequent enough to assure data validity. The maintenance and calibration schedules should be part of the SOPs for each instrument.
25. When an equipment manufacturer performs the routine equipment maintenance, do the equipment manufacturer's maintenance procedures do not have to be described in the facilities' SOPs. The facilities' SOPs would have to state that maintenance was being performed by the equipment manufacturer according to their own procedures.
26. The GLPs do not specify the amount of detail to be included in the SOPs. The SOPs are intended to minimize the introduction of systematic error into a study by ensuring that all personnel will be familiar with and use the same procedures. The adequacy of the SOPs is a key responsibility of management. A guideline of adequacy that could be used is to determine whether the SOPs are understood and can be followed by trained laboratory personnel.
27. The study director cannot authorize changes in the SOPs. Each workstation should have access to the SOPs applicable to the work performed at the station. A complete set of the SOPs, including authorized amendments, should be maintained in the archives. The SOPs are approved by the Laboratory Management and not by QAU. The GLPs do not specify the contents of individual SOPs, but the SOP that deals with computerized data acquisition should include the purpose of the program, the specifications, the procedures, the end products, the language, the interactions with other programs, procedures for assuring authorized data entry and access, procedures for making and authorizing changes to the program, the source listing of the program, and perhaps even a flow chart. The laboratory's computer specialists should determine what other characteristics need to be described in the SOP.

28. All reagents used in a nonclinical laboratory have to be labeled to indicate identity, titer or concentration, storage requirements, and expiration date. Purchased reagents usually carry all these items except for the expiration date, so the laboratory should label the reagent containers with an expiration date. The expiration date selected should be inline with laboratory experience and need not require specific stability testing.
29. The procedures for confirming the quality of incoming reagents used in nonclinical laboratory studies are left to laboratory management decision but the SOPs should document the actual procedures used.
30. The GLPs do not require the use of product accountability procedures for reagents and chemicals used in a nonclinical laboratory study.
31. The study director or the QAU is permitted to request analysis of reserved samples; sufficient reserve sample should be retained so that the sample is not exhausted. Physical and chemical tests conducted on test articles are required to be done under the GLPs?
32. An analytical method need not be totally contained in the protocol. The protocol must state the type and frequency of tests to be made. Type can be connoted by reference to literature citations or the SOPs as applicable.
33. Each nonclinical laboratory study requires a sponsor-approved specific protocol; however, the laboratory that conducts the study can also qualify as the sponsor of the study. Unforeseen circumstances, which have only a one-time effect (different date of sample collection) need to be reported only in the raw data and the final report. However, such circumstances, which result in a systematic change, e.g., in the SOPs or in the protocol, should also be made by a protocol amendment. The protocol amendment need not be made in advance but should be made as rapidly as possible.
34. The protocol must list the type and frequency of tests, analyses, and measurements to be made in the study. Where these are covered by SOPs, they should be listed in the protocol.
35. Raw data collected in nonclinical laboratory studies need not be cosigned by a second individual and there is no requirement for maintaining bound copies of data recorded.
36. The GLP requirements that are applicable to computerized data-acquisition systems include the following criteria:
    a. Only authorized individuals can make data entries,
    b. Data entries may not be deleted, but changes may be made in the form of dated amendments which provide the reason for data change,
    c. The data base must be made as tamperproof as possible,
    d. The SOPs should describe the procedures used for ensuring the validity of the data, and
    e. Either the magnetic media or hardcopy printouts are considered to be raw data.
37. It is acceptable to manually transcribe raw data into notebooks if it is verified accurate by signature and date; technically the GLPs do not preclude such an approach. However, it is not a preferred procedure since the chance of transcription errors would exist. Accordingly, such an approach should be used only when necessary and in this event the raw data should also be retained.
38. All circumstances that may have affected the quality of the data have to be described in the final report. The GLPs do not address the issue of approval of the final report. According to the GLPs, the final report is official when it is signed and dated by the study director. If persons reviewing the final report request changes, then such changes must be made by way of a formal amendment. The final report needs to identify only the name of the study director, the names of other participating scientists, and the names of all supervisory personnel.
39. Certain raw data records which are not study specific such as instrument calibration need not be filed in the archives in each study file. These can be filed in a retrievable fashion such as chronological in the archive. At the completion of a study, QAU records and inspection reports should be retained in the archives.
40. At the termination of a nonclinical laboratory study, whether a contractor can send all of the raw data, study records, and specimens to the sponsor of the study is not specifically addressed in GLPs. Section 58.195(g) requires contract laboratories that go out of business to transfer all raw data and records to the sponsor. Likewise, Section 58.190(b) permits raw

data and study records to be stored elsewhere (other than the contract laboratory location) provided that the contract laboratory's archives have reference to the other locations and provided that the final study report identifies the other locations as directed by Section 58.195(a)(13). Consequently, it is permissible for the sponsor to retain all raw data and records from the date of termination of the nonclinical laboratory study. Common sense dictates, however, that the contract laboratory keep copies of the material that has been forwarded to the sponsor.

41. The blood and urine specimens, which are analyzed for both labile and stable constituents, are necessary to retain for the term required by the regulations or for as long as their quality permits meaningful reevaluation, whichever is shorter.
42. The preparation of the conforming amendment statement is the responsibility of the product sponsor and the statement should be submitted as part of the application for a research or marketing permit. The contractor, however, should identify for the sponsor those non-GLP practices, which were used in each nonclinical laboratory study so that a proper conforming amendment statement can be prepared. This can be signed by the same individual in the firm who signs the official application for a research or marketing permit.
43. FDA does not necessarily reject nonclinical laboratory studies that have not been conducted in full compliance with the GLPs. The GLP Compliance Program provides guidance on the issue. For FDA to reject a study, it is necessary to find that there were deviations from the GLPs and that these deviations were of such a nature as to compromise the quality and integrity of the study covered by the agency inspection.
44. The SOPs need not be submitted along with an application for a research or marketing permit.

ORGANIZATION AND PERSONNEL

Personnel

1. Each individual engaged in the conduct of or responsible for the supervision of a nonclinical laboratory study shall have education, training, and experience, or combination thereof, to enable that individual to perform the assigned functions.
2. Each testing facility shall maintain a current summary of training and experience and job description for each individual engaged in or supervising the conduct of a nonclinical laboratory study.
3. There shall be a sufficient number of personnel for the timely and proper conduct of the study according to the protocol.
4. Personnel shall take necessary personal sanitation and health precautions designed to avoid contamination of test and control articles and test systems.
5. Personnel engaged in a nonclinical laboratory study shall wear clothing appropriate for the duties they perform. Such clothing shall be changed as often as necessary to prevent microbiologic, radiologic, or chemical contamination of test systems and test and control articles.
6. Any individual found at any time to have an illness that may adversely affect the quality and integrity of the nonclinical laboratory study shall be excluded from direct contact with test systems, test and control articles, and any other operation or function that may adversely affect the study until the condition is corrected. All personnel shall be instructed to report to their immediate supervisors for any health or medical conditions that may reasonably be considered to have an adverse effect on a nonclinical laboratory study.

Testing Facility Management

For each nonclinical laboratory study, testing facility management shall

1. Designate a study director as described in Section 58.33, before the study is initiated.
2. Replace the study director promptly if it becomes necessary to do so during the conduct of a study.

3. Assure that there is a QAU as described in Section 58.35.
4. Assure that test and control articles or mixtures have been appropriately tested for identity, strength, purity, stability, and uniformity, as applicable.
5. Assure that personnel, resources, facilities, equipment, materials, and methodologies are available as scheduled.
6. Assure that personnel clearly understand the functions they are to perform.
7. Assure that any deviations from these regulations reported by the QAU are communicated to the study director, and corrective actions are taken and documented.

Study Director

For each nonclinical laboratory study, a scientist or other professional of appropriate education, training, and experience, or combination thereof, shall be identified as the study director. The study director has overall responsibility for the technical conduct of the study, as well as for the interpretation, analysis, documentation and reporting of results, and represents the single point of study control. The study director shall assure that

1. The protocol, including any change, is approved as provided by Section 58.120 and is followed.
2. All experimental data, including observations of unanticipated responses of the test system are accurately recorded and verified.
3. Unforeseen circumstances that may affect the quality and integrity of the nonclinical laboratory study are noted when they occur, and corrective action is taken and documented.
4. Test systems are as specified in the protocol.
5. All applicable GLP regulations are followed.
6. All raw data, documentation, protocols, specimens, and final reports are transferred to the archives during or at the close of the study.

The QAU

1. A testing facility shall have a QAU, which shall be responsible for monitoring each study to assure management that the facilities, equipment, personnel, methods, practices, records, and controls are in conformance with the regulations in this part. For any given study, the QAU shall be entirely separate from and independent of the personnel engaged in the direction and conduct of that study.
2. The QAU shall
   a. Maintain a copy of a master schedule sheet of all nonclinical laboratory studies conducted at the testing facility indexed by test article and containing the test system, nature of study, date study was initiated, current status of each study, identity of the sponsor, and name of the study director.
   b. Maintain copies of all protocols pertaining to all nonclinical laboratory studies for which the unit is responsible.
   c. Inspect each nonclinical laboratory study at intervals adequate to assure the integrity of the study, and maintain written and properly signed records of each periodic inspection showing the date of the inspection, the study inspected, the phase or segment of the study inspected, the person performing the inspection, findings and problems, action recommended and taken to resolve existing problems, and any scheduled date for reinspection. Any problems found during the course of an inspection, which are likely to affect study integrity shall be brought to the attention of the study director and management immediately.
   d. Periodically submit to management and the study director written status reports on each study, noting any problems and the corrective actions taken.
   e. Determine that no deviations from approved protocols or SOPs were made without proper authorization and documentation.

f. Review the final study report to assure that such report accurately describes the methods and SOPs, and that the reported results accurately reflect the raw data of the nonclinical laboratory study.
g. Prepare and sign a statement to be included with the final study report, which shall specify the dates of inspections and findings reported to management and to the study director.

3. The responsibilities and procedures applicable to the QAU, the records maintained by the QAU, and the method of indexing such records shall be in writing and shall be maintained. These items, including inspection dates, the study inspected, the phase or segment of the study inspected, and the name of the individual performing the inspection, shall be made available for inspection to authorized employees of the FDA.
4. A designated representative of the FDA shall have access to the written procedures established for the inspection and may request testing facility management to certify that inspections are being implemented, performed, documented, and followed-up in accordance with this paragraph.

FACILITIES

General

Each testing facility shall be of suitable size and construction to facilitate the proper conduct of nonclinical laboratory studies. It shall be designed so that there is a degree of separation that will prevent any function or activity from having an adverse effect on the study.

Facilities for Handling Test and Control Articles

1. As it is necessary to prevent contamination or mix ups, there shall be separate areas for receipt and storage of the test and control articles.

Laboratory Operation Areas

Separate laboratory space shall be provided, as needed, for the performance of the routine and specialized procedures required by nonclinical laboratory studies.

Specimen and Data Storage Facilities

Space shall be provided for archives, limited to access by authorized personnel only, for the storage and retrieval of all raw data and specimens from completed studies.

EQUIPMENT

Equipment Design

Equipment used in the generation, measurement, or assessment of data and for facility environmental control shall be of appropriate design and adequate capacity to function according to the protocol and shall be suitably located for operation, inspection, cleaning, and maintenance.

Maintenance and Calibration of Equipment

1. Equipment shall be adequately inspected, cleaned, and maintained. Equipment used for the generation, measurement, or assessment of data shall be adequately tested, calibrated and/or standardized.
2. The written SOPs required under Section 58.81(b)(11) shall set forth in sufficient detail the methods, materials, and schedules to be used in the routine inspection, cleaning, maintenance, testing, calibration, and/or standardization of equipment, and shall specify, when appropriate, remedial action to be taken in the event of failure or malfunction

of equipment. The written SOPs shall designate the person responsible for the performance of each operation.
3. Written records shall be maintained of all inspection, maintenance, testing, calibrating and/or standardizing operations. These records, containing the date of the operation, shall describe whether the maintenance operations were routine and followed the written SOPs. Written records shall be kept of nonroutine repairs performed on equipment as a result of failure and malfunction. Such records shall document the nature of the defect, how and when the defect was discovered, and any remedial action taken in response to the defect.

TESTING FACILITIES OPERATION

SOPs

1. A testing facility shall have SOPs in writing setting forth nonclinical laboratory study methods that management is satisfied are adequate to ensure the quality and integrity of the data generated during the course of a study. All deviations in a study from SOPs shall be authorized by the study director and shall be documented in the raw data. Significant changes in established SOPs shall be properly authorized in writing by management.
2. SOPs shall be established for, but not limited to, the following:
   a. Receipt, identification, storage, handling, mixing, and method of sampling of the test and control articles
   b. Test system observations
   c. Laboratory tests
   d. Data handling, storage, and retrieval
   e. Maintenance and calibration of equipment
   f. Transfer, proper placement, and identification of animals.
3. Each laboratory area shall have immediately available laboratory manuals and SOPs relative to the laboratory procedures being performed. Published literature may be used as a supplement to SOPs.
4. A historical file of SOPs, and all revisions thereof, including the dates of such revisions, shall be maintained.

Reagents and Solutions

All reagents and solutions in the laboratory areas shall be labeled to indicate or identify titer or concentration, storage requirements, and expiration date. Deteriorated or outdated reagents and solutions shall not be used.

TEST AND CONTROL ARTICLES

Test and Control Article Characterization

1. The identity, strength, purity, and composition or other characteristics which will appropriately define the test or control article shall be determined for each batch and shall be documented. Methods of synthesis, fabrication, or derivation of the test and control articles shall be documented by the sponsor or the testing facility. In those cases where marketed products are used as control articles, such products will be characterized by their labeling.
2. The stability of each test or control article shall be determined by the testing facility or by the sponsor either before study initiation or concomitantly according to written SOPs, which provide for periodic analysis of each batch.
3. Each storage container for a test or control article shall be labeled by name, chemical abstract number or code number, batch number, expiration date, if any, and, where

appropriate, storage conditions are necessary to maintain the identity, strength, purity, and composition of the test or control article. Storage containers shall be assigned to a particular test article for the duration of the study.
4. For studies of more than four weeks' duration, reserve samples from each batch of test and control articles shall be retained for the period of time provided by Section 58.195.

Test and Control Article Handling

Procedures shall be established for a system for the handling of the test and control articles to ensure that

1. There is a proper storage.
2. Distribution is made in a manner designed to preclude the possibility of contamination, deterioration, or damage.
3. Proper identification is maintained throughout the distribution process.
4. The receipt and distribution of each batch is documented. Such documentation shall include the date and quantity of each batch distributed or returned.

PROTOCOL FOR AND CONDUCT OF A NONCLINICAL LABORATORY STUDY

Protocol

1. Each study shall have an approved written protocol that clearly indicates the objectives and all methods for the conduct of the study. The protocol shall contain, as applicable, the following information:
   a. A descriptive title and statement of the purpose of the study.
   b. Identification of the test and control articles by name, chemical abstract number, or code number.
   c. The name of the sponsor, and the name and address of the testing facility at which the study is being conducted.
   d. The procedure for identification of the test system.
   e. A description of the experimental design, including the methods for the control of bias.
   f. The type and frequency of tests, analyses, and measurements to be made.
   g. The records to be maintained.
   h. The date of approval of the protocol by the sponsor and the dated signature of the study director.
   i. A statement of the proposed statistical methods to be used.
2. All changes in or revisions of an approved protocol and the reasons therefor shall be documented, signed by the study director, dated, and maintained with the protocol.

Conduct of a Nonclinical Laboratory Study

1. The nonclinical laboratory study shall be conducted in accordance with the protocol.
2. The test systems shall be monitored in conformity with the protocol.
3. Specimens shall be identified by test system, study, nature, and date of collection. This information shall be located on the specimen container or shall accompany the specimen in a manner that precludes error in the recording and storage of data.
4. All data generated during the conduct of a nonclinical laboratory study, except those that are generated by automated data collection systems, shall be recorded directly, promptly, and legibly in ink. All data entries shall be dated on the date of entry and signed or initialed by the person entering the data. Any change in entries shall be made so as not to obscure the original entry, shall indicate the reason for such change, and shall be dated and signed or

identified at the time of the change. In automated data collection systems, the individual responsible for direct data input shall be identified at the time of data input. Any change in automated data entries shall be made so as not to obscure the original entry, shall indicate the reason for change, shall be dated, and the responsible individual shall be identified.

RECORDS AND REPORTS

Reporting of Nonclinical Laboratory Study Results

1. A final report shall be prepared for each nonclinical laboratory study and shall include, but not necessarily be limited to, the following:
   a. Name and address of the facility performing the study and the dates on which the study was initiated and completed.
   b. Objectives and procedures stated in the approved protocol, including any changes in the original protocol.
   c. Statistical methods employed for analyzing the data.
   d. The test and control articles identified by name, chemical abstract number or code number, strength, purity, and composition, or other appropriate characteristics.
   e. Stability of the test and control articles under the conditions of administration.
   f. A description of the methods used.
   g. A description of the test system used.
   h. A description of all circumstances that may have affected the quality or integrity of the data.
   i. The name of the study director, the names of other scientists or professionals, and the names of all supervisory personnel, involved in the study.
   j. A description of the transformations, calculations, or operations performed on the data, a summary and analysis of the data, and a statement of the conclusions drawn from the analysis.
   k. The signed and dated reports of each of the individual scientists or other professionals involved in the study.
   l. The locations where all specimens, raw data, and the final report are to be stored.
   m. The statement prepared and signed by the QAU
2. The final report shall be signed and dated by the study director.
3. Corrections or additions to a final report shall be in the form of an amendment by the study director. The amendment shall clearly identify that part of the final report that is being added to or corrected and the reasons for the correction or addition, and shall be signed and dated by the person responsible.

Storage and Retrieval of Records and Data

1. All raw data, documentation, protocols, final reports, and specimens generated as a result of a nonclinical laboratory study shall be retained.
2. There shall be archives for orderly storage and expedient retrieval of all raw data, documentation, protocols, specimens, and interim and final reports. Conditions of storage shall minimize deterioration of the documents or specimens in accordance with the requirements for the time period of their retention and the nature of the documents or specimens. A testing facility may contract with commercial archives to provide a repository for all material to be retained. Raw data and specimens may be retained elsewhere provided that the archives have specific reference to those other locations.
3. An individual shall be identified as responsible for the archives.
4. Only authorized personnel shall enter the archives.
5. Materials retained or referred to in the archives shall be indexed to permit expedient retrieval.

Retention of Records

1. Record retention requirements requires a period of at least two years following the date on which an application for a research or marketing permit, in support of which the results of the nonclinical laboratory study were submitted, is approved by the FDA. This requirement does not apply to studies supporting investigational new drug applications (INDs) or applications for investigational device exemptions (IDEs), records of which shall be kept for a period of at least five years following the date on which the results of the nonclinical laboratory study, which are submitted to the FDA in support of an application for a research or marketing permit. This applies to:
   a. The master schedule sheet, copies of protocols, and records of quality assurance inspections, as required shall be maintained by the QAU as an easily accessible system of records.
   b. Summaries of training and experience, and job descriptions may be retained along with all other testing facility employment records.
   c. Records and reports of the maintenance and calibration and inspection of equipment.
   d. Records required by this part may be retained either as original records or as true copies such as photocopies, microfilm, microfiche, or other accurate reproductions of the original records.
2. If a facility conducting nonclinical testing goes out of business, all raw data, documentation, and other material specified in this section shall be transferred to the archives of the sponsor of the study. The FDA shall be notified in writing of such a transfer.

AUDIT OF FACILITIES FOR GLP COMPLIANCE

The FDA routinely conducts audits of facilities submitting data to FDA for approval of applications for marketing authorization. The objective of this audit is to

1. verify the quality and integrity of data submitted in a research or marketing application;
2. inspect (approximately every two years) nonclinical laboratories conducting safety studies that are intended to support applications for research or marketing of regulated products;
3. audit safety studies and determine the degree of compliance with GLP regulations.

The types of inspections include:

1. Surveillance inspections: These are periodic, routine determinations of a laboratory's compliance with GLP regulations. These inspections include a facility inspection and audits of on-going and/or recently completed studies.
2. Directed inspections: These are assigned to achieve a specific purpose, such as
   a. Verifying the reliability, integrity, and compliance of critical safety studies being reviewed in support of pending applications.
   b. Investigating issues involving potentially unreliable safety data and/or violative conditions brought to FDA's attention.
   c. Re-inspecting laboratories previously classified official action indicated (OAI) (usually within six months after the firm responds to a warning letter).
   d. Verifying the results from third party audits or sponsor audits submitted to FDA for consideration in determining whether to accept or reject questionable or suspect studies.

General Instructions to Investigators

1. The investigator will determine the current state of GLP compliance by evaluating the laboratory facilities, operations, and study performance.

2. Organization chart: If the facility maintains an organization chart, obtain a current version of the chart for use during the inspection and submit it in the EIR.
3. Facility floor-plan diagram: Obtain a diagram of the facility. The diagram may identify areas that are not used for GLP activities. If it does not, request that appropriate facility personnel identify any areas that are not used for GLP activities. Use during the inspection and submit it in the EIR.
4. Master schedule sheet: Obtain a copy of the firm's master schedule sheet for all studies listed since the last GLP inspection or last two years and select studies as defined in 21 CFR 58.3(d). If the inspection is the first inspection of the facility, review the entire master schedule. If studies are identified as non-GLP, determine the nature of several studies to verify the accuracy of this designation. See 21 CFR 58.1 and 58.3(d). In contract laboratories determine who decides if a study is a GLP study.
5. Identification of studies
   a. Directed inspections: They will identify studies to be audited.
   b. Surveillance inspections: Inspection assignments may identify one or more studies to be audited. If the assignment does not identify a study for coverage, or if the referenced study is not suitable to assess all portions of current GLP compliance, the investigator will select studies as necessary to evaluate all areas of laboratory operations. When additional studies are selected, first priority should be given to FDA studies for submission to the assigning Center. Note: Studies performed for submission to other government agencies, e.g., Environmental Protection Agency, National Toxicology Program, National Cancer Institute, etc., will not be audited without authorization from the Bioresearch Monitoring Program Coordinator (HFC-230). However, this authorization is not necessary to briefly look at one of these studies to assess the ongoing operations of a portion of the facility.

Establishment Inspections

The facility inspection should be guided by the GLP regulations. The following areas should be evaluated and described as appropriate.

Organization and Personnel (21 CFR 58.29, 58.31, 58.33)

Purpose

To determine whether the organizational structure is appropriate to ensure that studies are conducted in compliance with GLP regulations, and to determine whether management, study directors, and laboratory personnel are fulfilling their responsibilities under the GLPs.

Management Responsibilities (21 CFR 58.31)

Identify the various organizational units, their role in carrying out GLP study activities, and the management responsible for these organizational units. This includes identifying personnel who are performing duties at locations other than the test facility and identifying their line of authority. If the facility has an organization chart, much of this information can be determined from the chart. Determine if management has procedures for assuring that the responsibilities in 58.31 can be carried out. Look for evidence of management involvement, or lack thereof, in the following areas:

1. Assigning and replacing study directors.
2. Control of study director workload (use the master schedule to assess workload).
3. Establishment and support of the QAU, including assuring that deficiencies reported by the QAU are communicated to the study directors and acted upon.
4. Assuring that test and control articles or mixtures are appropriately tested for identity, strength, purity, stability, and uniformity.
5. Assuring that all study personnel are informed of and follow any special test and control article handling and storage procedures.

6. Providing required study personnel, resources, facilities, equipment, and materials.
7. Reviewing and approving protocols and SOPs.
8. Providing GLP or appropriate technical training.

Personnel (21 CFR 58.29)

Identify key laboratory and management personnel, including any consultants or contractors used, and review personnel records, policies, and operations to determine if

1. Summaries of training and position descriptions are maintained and are current for selected employees.
2. Personnel have been adequately trained to carry out the study functions that they perform.
3. Personnel have been trained in GLPs.
4. Practices are in place to ensure that employees take necessary health precautions, wear appropriate clothing, and report illnesses to avoid contamination of the test and control articles and test systems.
5. If the firm has computerized operations, determine the following:
   a. Who was involved in the design, development, and validation of the computer system?
   b. Who is responsible for the operation of the computer system, including inputs, processing, and output of data?
   c. Whether computer system personnel have training commensurate with their responsibilities, including professional training and training in GLPs?
   d. Whether some computer system personnel are contractors who are present on-site full-time, or nearly full-time. The investigation should include these contractors as though they were employees of the firm. Specific inquiry may be needed to identify these contractors, as they may not appear on organization charts.
   e. Interview and observe personnel using the computerized systems to assess their training and performance of assigned duties.

Study Director (21 CFR 58.33)

1. Assess the extent of the study director's actual involvement and participation in the study. In those instances when the study director is located off-site, review any correspondence/records between the testing facility management and QAU and the off-site study director. Determine that the study director is being kept immediately apprised of any problems that may affect the quality and integrity of the study.
2. Assess the procedures by which the study director:
   a. Assures the protocol and any amendments have been properly approved and are followed.
   b. Assures that all data are accurately recorded and verified.
   c. Assures that data are collected according to the protocol and SOPs.
   d. Documents unforeseen circumstances that may affect the quality and integrity of the study and implements corrective action.
   e. Assures that study personnel are familiar with and adhere to the study protocol and SOPs.
   f. Assures that study data are transferred to the archives at the close of the study.

EIR Documentation and Reporting

Collect exhibits to document deficiencies. This may include SOPs, organizational charts, position descriptions, and CVs, as well as study-related memos, records, and reports for the studies selected for review. The use of outside or contract facilities must be noted in the EIR. The assigning Center should be contacted for guidance on inspection of these facilities.

QAU (21 CFR 58.35)

Purpose

To determine if the test facility has an effective, independent QAU that monitors significant study events and facility operations, reviews records and reports, and assures management of GLP compliance.

QAU Operations [21 CFR 58.35(b–d)]

Review QAU SOPs to assure that they cover all methods and procedures for carrying out the required QAU functions, and confirm that they are being followed. Verify that SOPs exist and are being followed for QAU activities including, but not limited to, the following:

1. Maintenance of a master schedule sheet.
2. Maintenance of copies of all protocols and amendments.
3. Scheduling of its in-process inspections and audits.
4. Inspection of each nonclinical laboratory study at intervals adequate to assure the integrity of the study, and maintenance of records of each inspection.
5. Immediately notify the study director and management of any problems that are likely to affect the integrity of the study.
6. Submission of periodic status reports on each study to the study director and management.
7. Review of the final study report.
8. Preparation of a statement to be included in the final report that specifies the dates of inspections and findings reported to management and to the study director.
9. Inspection of computer operations.

Verify that, for any given study, the QAU is entirely separate from and independent of the personnel engaged in the conduct and direction of that study. Evaluate the time QAU personnel spend in performing in-process inspection and final report audits. Determine if the time spent is sufficient to detect problems in critical study phases and if there are adequate personnel to perform the required functions.

Note: The investigator may request the firm's management to certify in writing that inspections are being implemented, performed, documented, and followed-up in accordance with this section [see 58.35(d)].

EIR Documentation and Reporting

Obtain a copy of the master schedule sheet dating from the last routine GLP inspection or covering the past two years. If the master schedule is too voluminous, obtain representative pages to permit headquarters review. When master schedule entries are coded, obtain the code key. Deficiencies should be fully reported and documented in the EIR. Documentation to support deviations may include copies of QAU SOPs, list of QAU personnel, their CVs or position descriptions, study-related records, protocols, and final reports.

Facilities (21 CFR 58.41–51)

Purpose

Assess whether the facilities are of adequate size and design.

Facility Inspection

1. Review environmental controls and monitoring procedures for critical areas (i.e., animal rooms, test article storage areas, laboratory areas, handling of bio-hazardous material, etc.) and determine if they appear adequate and are being followed.
2. Review the SOPs that identify materials used for cleaning critical areas and equipment, and assess the facility's current cleanliness.
3. Determine whether there are appropriate areas for the receipt, storage, mixing, and handling of the test and control articles.

4. Determine whether separation is maintained in rooms where two or more functions requiring separation are performed.
5. Determine that computerized operations and archived computer data are housed under appropriate environmental conditions (e.g., protected from heat, water, and electromagnetic forces).

EIR Documentation and Reporting

Identify which facilities, operations, SOPs, etc., were inspected. Only significant changes in the facility from previous inspections need be described. Facility floor plans may be collected to illustrate problems or changes. Document any conditions that would lead to contamination of test articles or to unusual stress of test systems.

Equipment (21 CFR 58.61–63)

Purpose

To assess whether equipment is appropriately designed and of adequate capacity and is maintained and operated in a manner that ensures valid results.

Equipment Inspection

Assess the following:

1. The general condition, cleanliness, and ease of maintenance of equipment in various parts of the facility.
2. The heating, ventilation, and air conditioning system design and maintenance, including documentation of filter changes and temperature/humidity monitoring in critical areas.
3. Whether equipment is located where it is used and that it is located in a controlled environment, when required.
4. Non-dedicated equipment for preparation of test and control article carrier mixtures is cleaned and decontaminated to prevent cross contamination.
5. For representative pieces of equipment check the availability of the following:
   a. SOPs and/or operating manuals.
   b. Maintenance schedule and log.
   c. Standardization/calibration procedure, schedule, and log.
   d. Standards used for calibration and standardization.
6. For computer systems, assess that the following procedures exist and are documented (see also Attachment A):
   a. Validation study, including validation plan and documentation of the plan's completion.
   b. Maintenance of equipment, including storage capacity and back-up procedures.
   c. Control measures over changes made to the computer system, which include the evaluation of the change, necessary test design, test data, and final acceptance of the change.
   d. Evaluation of test data to ensure that data are accurately transmitted and handled properly when analytical equipment are directly interfaced to the computer.
   e. Procedures for emergency back-up of the computer system (e.g., back-up battery system and data forms for recording data in the event of a computer failure or power outage).

EIR Documentation and Reporting

The EIR should list which equipment, records, and procedures were inspected and the studies to which they are related. Detail any deficiencies that might result in contamination of test articles, uncontrolled stress to test systems, and/or erroneous test results.

Testing Facility Operations (21 CFR 58.81)

Purpose
To determine if the facility has established and follows written SOPs necessary to carry out study operations in a manner designed to ensure the quality and integrity of the data.

SOP Evaluation

1. Review the SOP index and representative samples of SOPs to ensure that written procedures exist to cover at least all of the areas identified in 58.81(b).
2. Verify that only current SOPs are available at the personnel workstations.
3. Review key SOPs in detail and check for proper authorization signatures and dates, and general adequacy with respect to the content (i.e., SOPs are clear, complete, and can be followed by a trained individual).
4. Verify that changes to SOPs are properly authorized and dated and that a historical file of SOPs is maintained.
5. Ensure that there are procedures for familiarizing employees with SOPs.
6. Determine that there are SOPs to ensure the quality and integrity of data, including input (data checking and verification), output (data control), and an audit trail covering all data changes.
7. Verify that a historical file of outdated or modified computer programs is maintained. If the firm does not maintain old programs in digital form, ensure that a hard copy of all programs has been made and stored.
8. Verify that SOPs are periodically reviewed for current applicability and that they are representative of the actual procedures in use.
9. Review selected SOPs and observe employees performing the operation to evaluate SOP adherence and familiarity.

EIR Documentation and Reporting
Submit SOPs, data collection forms, and raw data records as exhibits that are necessary to support and illustrate deficiencies.

Reagents and Solutions (21 CFR 58.83)

Purpose
To determine that the facility ensures the quality of reagents at the time of receipt and subsequent use.

1. Review the procedures used to purchase, receive, label, and determine the acceptability of reagents and solutions for use in the studies.
2. Verify that reagents and solutions are labeled to indicate identity, titer or concentration, storage requirements, and expiration date.
3. Verify that for automated analytical equipment, the profile data accompanying each batch of control reagents are used.
4. Check that storage requirements are being followed.

Test and Control Articles (21 CFR 58.105–113)

Purpose
To determine that procedures exist to assure that test and control articles and mixtures of articles with carriers meet protocol specifications throughout the course of the study, and that accountability is maintained.

Characterization and Stability of Test Articles (21 CFR 58.105)

The responsibility for carrying out appropriate characterization and stability testing may be assumed by the facility performing the study or by the study sponsor. When test article characterization and stability testing is performed by the sponsor, verify that the test facility has received documentation that this testing has been conducted.

1. Verify that procedures are in place to ensure that
   a. The acquisition, receipt, and storage of test articles, and means used to prevent deterioration and contamination are as specified.
   b. The identity, strength, purity, and composition (i.e., characterization) to define the test and control articles are determined for each batch and are documented.
   c. The stability of test and control articles is documented.
   d. The transfer of samples from the point of collection to the analytical laboratory is documented.
   e. Storage containers are appropriately labeled and assigned for the duration of the study.
   f. Reserve samples of test and control articles for each batch are retained for studies lasting more than four weeks.

Test and Control Article Handling (21 CFR 58.107)

1. Determine that there are adequate procedures for
   a. Documentation for receipt and distribution.
   b. Proper identification and storage.
   c. Precluding contamination, deterioration, or damage during distribution.
2. Inspect test and control article storage areas to verify that environmental controls, container labeling, and storage are adequate.
3. Observe test and control articles handling and identification during the distribution and administration to the test system.
4. Review a representative sample of accountability records and, if possible, verify their accuracy by comparing actual amounts in the inventory. For completed studies verify documentation of final test and control articles reconciliation.

Protocol and Conduct of Nonclinical Laboratory Study (21 CFR 58.120–130)

Purpose

To determine if study protocols are properly written and authorized, and that studies are conducted in accordance with the protocol and SOPs.

Study Protocol (21 CFR 58.120)

1. Review SOPs for protocol preparation and approval and verify they are followed.
2. Review the protocol to determine if it contains required elements.
3. Review all changes, revisions, or amendments to the protocol to ensure that they are authorized, signed, and dated by the study director.
4. Verify that all copies of the approved protocol contain all changes, revisions, or amendments.

Conduct of the Nonclinical Laboratory Study (21 CFR 58.130)

Evaluate the following laboratory operations, facilities, and equipment to verify conformity with protocol and SOP requirements:

1. Test system monitoring.
2. Recording of raw data (manual and automated).
3. Corrections to raw data (corrections must not obscure the original entry and must be dated, initialed, and explained).

4. Randomization of test systems.
5. Collection and identification of specimens.
6. Authorized access to data and computerized systems.

EIR Reporting and Documentation

Identify the study(ies) inspected and, if available, the associated FDA research or marketing permit numbers. Report and document any deficiencies observed. Submit, as exhibits, a copy of all protocols and amendments that were reviewed.

Records and Reports (21 CFR 58.185–195)

Purpose

To assess how the test facility stores and retrieves raw data, documentation, protocols, final reports, and specimens.

Reporting of Study Results (21 CFR 58.185)

Determine if the facility prepares a final report for each study conducted. For selected studies, obtain the final report, and verify that it contains the following:

1. The required elements in 21 CFR 58.185(a)(1–14), including the identity (name and address) of any subcontractor facilities and portion of the study contracted, and a description of any computer program changes.
2. Dated signature of the study director [21 CFR 58.185(b)].
3. Corrections or additions to the final report are made in compliance with 21 CFR 58.185(c).

Storage and Retrieval of Records and Data (21 CFR 58.190)

1. Verify that raw data, documentation, protocols, final reports, and specimens have been retained.
2. Identify the individual responsible for the archives. Determine if delegation of duties to other individuals in maintaining the archives has occurred.
3. Verify that archived material retained or referred to in the archives is indexed to permit expedient retrieval. It is not necessary that all data and specimens be in the same archive location. For raw data and specimens retained elsewhere, the archives index must make specific reference to those other locations.
4. Verify that access to the archives is controlled and determine that environmental controls minimize deterioration.
5. Ensure that there are controlled procedures for adding or removing material. Review archive records for the removal and return of data and specimens. Check for unexplained or prolonged removals.
6. Determine how and where computer data and back-up copies are stored, that records are indexed in a way to allow access to data stored on electronic media, and that environmental conditions minimize deterioration.
7. Determine to what electronic media such as tape cassettes or ultra high capacity portable discs the test facility has the capacity of copying records in electronic form. Report names and identifying numbers of both copying equipment type and electronic medium type to enable agency personnel to bring electronic media to future inspections for collecting exhibits.

EIR Documentation and Reporting

Provide a brief summary of the facility's report preparation procedures and their retention and retreival of records, reports, and specimens. If records are archived off-site, obtain a copy of documentation of the records which were transferred and where they are located. Describe and document deficiencies.

Data Audit

In addition to the procedures outlined above for evaluating the overall GLP compliance of a firm, the inspection should include the audit of at least one completed study. Studies for audit may be assigned by the Center or selected by the investigator as described in Part III, A. The audit will include a comparison of the protocol (including amendments to the protocol), raw data, records, and specimens against the final report to substantiate that protocol requirements were met and that findings were fully and accurately reported.

1. For each study audited, the study records should be reviewed for quality to ensure that data are
   a. Attributable: The raw data can be traced, by signature or initials and date to the individual observing and recording the data. Should more than one individual observe or record the data, that fact should be reflected in the data.
   b. Legible: The raw data are readable and recorded in a permanent medium. If changes are made to original entries, the changes
      i. Must not obscure the original entry.
      ii. Indicate the reason for change.
      iii. Must be signed or initialed and dated by the person making the change.
   c. Contemporaneous: The raw data are recorded at the time of the observation.
   d. Original: The first recording of the data.
   e. Accurate: The raw data are true and complete observations. For data entry forms that require the same data to be entered repeatedly, all fields should be completed or a written explanation for any empty fields should be retained with the study records.
2. General
   a. Determine if there were any significant changes in the facilities, operations, and QAU functions other than those previously reported.
   b. Determine whether the equipment used was inspected, standardized, and calibrated prior to, during, and after use in the study. If equipment malfunctioned, review the remedial action, and ensure that the final report addresses whether the malfunction affected the study.
   c. Determine if approved SOPs existed during the conduct of the study.
   d. Compare the content of the protocol with the requirements in 21 CFR.
   e. Review the final report for the study director's dated signature and the QAU statement as required in 21 CFR 58.35(b)(7).
3. Protocol versus final report: Study methods described in the final report should be compared against the protocol and the SOPs to confirm those requirements were met.
4. Final report versus raw data: The audit should include a detailed review of records, memorandum, and other raw data to confirm that the findings in the final report completely and accurately reflect the raw data. Representative samples of raw data should be audited against the final report.
5. Specimens versus final report: The audit should include examination of a representative sample of specimens in the archives for confirmation of the number and identity of specimens in the final report.
6. EIR Documentation and Reporting
7. Full reporting
   A full report will be prepared and submitted in the following situations:
   a. The initial GLP inspection of a facility.
   b. All inspections that may result in an OAI classification.
   c. Any assignment specifically requesting a full report.
8. Abbreviated reporting
   a. Field investigators may use abbreviated reporting for the following types of assignments:
      i. Surveillance inspections (except for initial inspections) of a facility when it is apparent from the findings that the inspection may result in a final classification of NAI or VAI. These reports must include enough documented information to support the final classification.

ii. Directed inspections and data audits provided the report fully covers all aspects of the specific topic of the inspection (i.e., operations, past deficiencies, assigned studies, etc.) and documents significant adverse findings to support the final classification.

Computerized Systems

Computer systems and operations are thoroughly covered during inspection of any facility. No additional reporting is required under this Attachment.

In August 1997, the Agency's regulation on electronic signatures and electronic record keeping became effective. The Regulation, at 21 CFR Part 11, describes the technical and procedural requirements that must be met if a firm chooses to maintain records electronically and/or use electronic signatures. Part 11 works in conjunction with other FDA regulations and laws that require recordkeeping. Those regulations and laws ("predicate rules') establish requirements for record content, signing, and retention.

Certain older electronic systems may not have been in full compliance with Part 11 by August 1997 and modification to these so-called "legacy systems" may take more time. Part 11 does not grandfather legacy systems and FDA expects that firms using legacy systems are taking steps to achieve full compliance with Part 11.

If a firm is keeping electronic records or using electronic signatures, determine if they are in compliance with 21 CFR Part 11. Determine the depth of Part 11 coverage on a case-by-case basis, in light of initial findings and program resources. At a minimum ensure that (1) the firm has prepared a corrective action plan for achieving full compliance with Part 11 requirements, and is making progress toward completing that plan in a timely manner; (2) accurate and complete electronic and human readable copies of electronic records, suitable for review, are made available; and (3) employees are held accountable and responsible for actions taken based on their electronic signatures. If initial findings indicate the firm's electronic records and/or electronic signatures, which may not be trustworthy and reliable, or when electronic recordkeeping systems inhibit meaningful FDA inspection, a more detailed evaluation may be warranted.

Personnel—Part III, C.1.c. (21 CFR 58.29)

Determine the following:

1. Who was involved in the design, development, and validation of the computer system?
2. Who is responsible for the operation of the computer system, including inputs, processing, and output of data?
3. If computer system personnel have training commensurate with their responsibilities, including professional training and training in GLPs.
4. Whether some computer system personnel are contractors who are present on-site full-time, or nearly full-time. The investigation should include these contractors as though they were employees of the firm. Specific inquiry may be needed to identify these contractors, as they may not appear on organization charts.

QAU Operations—Part III, C.2 [21 CFR 58.35(b–d)]

Verify SOPs exist and are being followed for QAU inspections of computer operations.

Facilities—Part III, C.3 (21 CFR 58.41–51)

Determine that computerized operations and archived computer data are housed under appropriate environmental conditions. Equipment—Part III, C.4 (21 CFR 58.61–63).

For computer systems, check that the following procedures exist and are documented:

1. Validation study, including validation plan and documentation of the plan's completion.
2. Maintenance of equipment, including storage capacity and back-up procedures.

3. Control measures over changes made to the computer system, which include the evaluation of the change, necessary test design, test data, and final acceptance of the change.
4. Evaluation of test data to assure that data are accurately transmitted and handled properly when analytical equipment is directly interfaced to the computer and
5. Procedures for emergency back-up of the computer system (e.g., back-up battery system and data forms for recording data in case of a computer failure or power outage).

Testing Facility Operations—Part III, C.5 (21 CFR 58.81)

Verify that a historical file of outdated or modified computer programs is maintained.

Records and Reports (21 CFR 58.185–195) (PART III C.10.b.)

Verify that the final report contains the required elements in 58.185(a)(1–14), including a description of any computer program changes.

Storage and Retrieval of Records and Data—Part III, C.10.c. (21 CFR 58.190)

1. Assess archive facilities for degree of controlled access and adequacy of environmental controls with respect to computer media storage conditions.
2. Determine how and where computer data and back-up copies are stored, that records are indexed in a way to allow access to data stored on electronic media, and that environmental conditions minimize deterioration.
3. Determine how and where original computer data and back-up copies are stored.

12 Computer and Software Validation

BACKGROUND

Over the past 25 years, data collection, recording, analysis, reporting, and regulatory submissions have become greatly dependent on electronic computerized systems. Regulatory agencies worldwide have begun accepting submissions electronically, even allowing these applications being signed off electronically. This change in the traditional paper trail system requires significant changes to data handling and greater emphasis on validating the regulatory submissions. In laboratories conducting bioequivalence (BE) studies, the following instances arise where validation of computer systems is required:

1. Record keeping systems including patient databases.
2. Software controlling operation of analytic equipment.
3. Software used to evaluate data statistics and store data.

Here are some examples of the incidences that lead to greater awareness and the need to validate computer programs. Back in the 1970s, there was reported error in matrix conversion because of numeric overflow; in the 1980s, the erroneous use of "n" instead of "$n-1$" for degrees of freedom threw the automated analysis out; in the 1990s, credibility of a chip maker was questioned when it was shown that the division by 3 does not yield result which is three times the value. All of these software bugs have prompted greater emphasis on the commercial off-the-shelf (COTS) products that are fully validated. There is also greater emphasis today on collaborative research resulting in such projects as Human Genome Project, caBIG™ (Cancer Biomedical Information Grid)—an open source, open access, voluntary information network, and the Gates Group requirement of data sharing for the $287 million funding in AIDS research; the conduct of these projects requires robust hardware and software systems across many platforms. Back in the 1960s, sponsors submitted Fortran code and Food and Drug Administration (FDA) reviewers poured over each line of code that became so onerous that the U.S. Government funded development of the Statistical Analysis System (SAS) software at the University of North Carolina. The regulatory requirement for "validation and verification" has to bias toward the COTS, and the recent Center for Devices and Radiological Health (CDRH) draft guidance on Bayesian mentions that WinBUGS and CDRH has a LINUX cluster. [The Bayesian inference Using Gibbs Sampling (BUGS) project is concerned with flexible software for the Bayesian analysis of complex statistical models using Markov chain Monte Carlo methods. The project began in 1989 in the MRC Biostatistics Unit and led initially to the "Classic" BUGS program, and then onto the WinBUGS software developed jointly with the Imperial College School of Medicine at St Mary's, London. Development now also includes the OpenBUGS project at the University of Helsinki, Finland. There are now a number of versions of BUGS, which might be confusing. WinBUGS 1.4.1 features a graphical user interface and online monitoring and convergence diagnostics. The OpenBUGS project is based at the University of Helsinki. Open source version of the core BUGS code with a variety of interfaces and running under Linux as LinBUGS. OpenBUGS is the main development platform and is currently experimental, but will eventually become the standard version. Just Another Gibbs Sampler (JAGS) by Martyn Plummer is open source software and not really a version of BUGS: JAGS uses essentially the same model description language but it has been completely rewritten. Use of all of this software requires good understanding of Bayesian statistical principles.]

The available software can be classified into three categories: the open source software are programs distributed freely with source code and anyone can modify them and redistribute without any licensing; generally, these programs are technology neutral and include such examples as OpenBUGS and Libraries; there are no regulation prohibiting use of open source software. The General Public License Software Executables include noncommercial "freeware" or "shareware" and examples include the WinBUGS. Finally, there are custom code and open source compilers such as SAS. The CFR Title 21 Sec 11.10 controls for closed systems have the following requirements:

1. Validation to ensure accuracy, reliability, consistent intended performance, and the ability to discern invalid or altered records
2. Accurate and complete copies of records in both human-readable and electronic forms suitable for inspection, review, and copying by the agency
3. Protection of records throughout the records retention period
4. Limiting system access to authorized individuals
5. Use of secure, computer-generated time-stamped audit trails
6. Use of operational systems checks, authority checks, and device checks
7. Education, training, and experience of operators and hold individuals accountable
8. Systems documentation

Software validation principles include:

1. Good software engineering to support final conclusion that the software is validated
2. Approach based on the intended use and the safety risk associated with the software
3. Software validation and verification conducted throughout the entire software life cycle
4. Party with regulatory responsibility needs to establish that the software is validated for the intended use
5. Software validation is a matter of developing a level of confidence

The computerized systems that are used to create, modify, maintain, archive, retrieve, or transmit clinical data are required to be maintained and/or submitted to the FDA regarding the safety and effectiveness of new human and animal drugs, biological products, medical devices, and certain food and color additives are subject to 21 CFR Part 11 requirements for validation and integrity.

The FDA has the authority to inspect all records relating to clinical investigations, which include BE testing, regardless of how they were created or maintained. The FDA established the Bioresearch Monitoring (BIMO) Program of inspections and audits to monitor the conduct and reporting of clinical trials to ensure that supporting data from these trials meet the highest standards of quality and integrity, and conform to the FDA's regulations. The FDA's acceptance of data from clinical trials for decision-making purposes depends on the FDA's ability to verify the quality and integrity of the data during FDA on-site inspections and audits. To be acceptable, the data should meet certain fundamental elements of quality whether collected or recorded electronically or on paper. For example, data should be attributable, legible, contemporaneous, original, and accurate. Individuals using the data from the computerized systems should have confidence that the data are no less reliable than the data in paper form.

The procedures described below may be applicable to data or source documents that are created (*i*) in hardcopy and later entered into a computerized system, (*ii*) by direct entry by a human into a computerized system, and (*iii*) automatically by a computerized system.

DATA HANDLING AND STORAGE PRINCIPLES

The following general principles with regard to the computerized systems that are used to create, modify, maintain, archive, retrieve, or transmit clinical data required to be maintained and/or submitted to the FDA are recommended:

1. Each study protocol identifies at which steps a computerized system will be used to create, modify, maintain, archive, retrieve, or transmit data.
2. For each study, the documentation must identify what software and hardware are to be used in the computerized systems that create, modify, maintain, archive, retrieve, or transmit data. This documentation should be retained as part of the study records.
3. The computerized systems be designed (*i*) so that all requirements assigned to these systems in a study protocol are satisfied (e.g., data are recorded in metric units, the study blinded) and (*ii*) to preclude errors in data creation, modification, maintenance, archiving, retrieval, or transmission.
4. It is important to design a computerized system in such a manner that all applicable regulatory requirements for record keeping and record retention in clinical trials are met with the same degree of confidence as is provided with paper systems.
5. The clinical investigator must retain records required to be maintained for a period of time specified in these regulations. Retaining the original source document or a certified copy of the source document at the site where the investigation was conducted can assist in meeting these regulatory requirements. It can also assist in the reconstruction and evaluation of the trial throughout and after the completion of the trial.
6. When original observations are entered directly into a computerized system, the electronic record is the source document.
7. Records relating to an investigation must be adequate and accurate in the case of investigational new drug applications; complete in the case of new animal drugs for investigational use; and accurate, complete, and current in the case of investigational device exemptions. An audit trail that is electronic or consists of other physical, logical, or procedural security measures to ensure that only authorized additions, deletions, or alterations of information in the electronic record have occurred may be needed to facilitate compliance with applicable records regulations. Firms should determine and document the need for audit trails based on a risk assessment that takes into consideration circumstances surrounding system use, the likelihood that information might be compromised, and any system vulnerabilities. It is recommended that audit trials or other security methods used to capture electronic record activities document who made the changes, when, and why changes were made to the electronic record.
8. It is recommended that data be retrievable in such a fashion that all information regarding each individual subject in a study is attributable to that subject.
9. To ensure the authenticity and integrity of electronic records, it is important that security measures be in place to prevent unauthorized access to the data in the electronic record and to the computerized system.

It is recommended that standard operating procedures (SOPs) pertinent to the use of the computerized system be available on site. It is recommended that SOPs be established for the following:

- System setup/installation
- Data collection and handling
- System maintenance
- Data backup, recovery, and contingency plans
- Security
- Change control
- Alternative recording methods (in the case of system unavailability)

Computer Access Controls

To ensure that individuals have the authority to proceed with data entry, data entry systems must be designed to limit access so that only authorized individuals are able to input data. Examples of methods for controlling access include using combined identification codes/passwords or biometric-based identification at the start of a data entry session. Controls

and procedures must be in place that are designed to ensure the authenticity and integrity of electronic records created, modified, maintained, or transmitted using the data entry system. Therefore, it is recommended that each user of the system have an individual account into which the user logs in at the beginning of a data entry session, inputs information (including changes) on the electronic record, and logs out at the completion of data entry session.

It is recommended that individuals work only with their own password or other access keys and not share these with others. It is recommended that individuals not be allowed to log onto the system to provide another person's access to the system. It is recommended that passwords or other access keys be changed at established intervals.

When someone leaves a workstation, it is recommended that the SOP requires that person to log off the system. Alternatively, an automatic log off may be appropriate for long idle periods. For short periods of inactivity, it is recommended that some kind of automatic protection be installed against unauthorized data entry. An example could be an automatic screen saver that prevents data entry until a password is entered.

Audit Trails or Other Security Measures

Persons who use electronic record systems to maintain an audit trail as one of the procedures to protect the authenticity, integrity, and, when appropriate, the confidentiality of electronic records. As clarified in the *Part 11 Scope and Application* guidance, however, the FDA intends to exercise enforcement discretion regarding specific Part 11 requirements related to computer-generated, time-stamped audit trails, and any corresponding requirements. Persons must still comply with all applicable predicate rule requirements for clinical trials, including, for example, that records related to the conduct of the study must be adequate and accurate. It is therefore important to keep track of all changes made to the information in the electronic records that document activities related to the conduct of the trial. Computer-generated, time-stamped audit trails or information related to the creation, modification, or deletion of electronic records may be useful to ensure compliance with the appropriate predicate rule.

In addition, clinical investigators must, upon request by the FDA, at reasonable times, permit agency employees to have access to, and copy and verify any required records or reports made by the investigator. In order for the FDA to review and copy this information, FDA personnel should be able to review audit trails or other documents that track electronic record activities both at the study site and at any other location where associated electronic study records are maintained. To enable the FDA's review, information about the creation, modification, or deletion of electronic records should be created incrementally in chronological order. To facilitate the FDA's inspection of this information, it is recommended that clinical investigators retain either the original or a certified copy of any documentation created to track electronic records activities.

Even if there are no applicable predicate rule requirements, it may be important to have computer-generated, time-stamped audit trails or other physical, logical, or procedural security measures to ensure the trustworthiness and reliability of electronic records. It is recommended that any decision on whether to apply computer-generated audit trails or other appropriate security measures be based on the need to comply with predicate rule requirements, a justified and documented risk assessment, and a determination of the potential effect on data quality and record integrity. Firms should determine and document the need for audit trails based on a risk assessment that takes into consideration circumstances surrounding system use, the likelihood that information might be compromised, and any system vulnerabilities.

If you determine that audit trails or other appropriate security measures are needed to ensure electronic record integrity, it is recommended that personnel who create, modify, or delete electronic records not be able to modify the documents or security measures used to track electronic record changes. It is recommended that audit trials or other security methods used to capture electronic record activities document who made the changes, when, and why changes were made to the electronic record.

Some methods for tracking changes to electronic records include:

- Computer-generated, time-stamped electronic audit trails.
- Signed and dated printed versions of electronic records that identify what, when, and by whom changes were made to the electronic record. When using this method, it is important that appropriate controls be utilized that ensure the accuracy of these records (e.g., sight verification that the printed version accurately captures all of the changes made to the electronic record).
- Signed and dated printed standard electronic file formatted versions (e.g., pdf, xml, or sgml) of electronic records that identify what, when, and by whom changes were made to the electronic record.
- Procedural controls that preclude unauthorized personnel from creating, modifying, or deleting electronic records or the data contained therein.

Date/Time Stamps

It is recommended that controls be put in place to ensure that the system's date and time are correct. The ability to change the date or time should be limited to authorized personnel and such personnel should be notified if a system date or time discrepancy is detected. It is recommended that someone always document changes to date or time. It is not expected that documentation of time changes that systems make automatically to adjust to daylight savings time conventions be made available.

It is also recommended that dates and times include the year, month, day, hour, and minute. The FDA encourages establishments to synchronize systems to the date and time provided by trusted third parties.

Clinical study computerized systems are likely be used in multicenter trials and may be located in different time zones. For systems that span different time zones, it is better to implement time stamps with a clear understanding of the time zone reference used. It is recommended that system documentation explain time zone references as well as zone acronyms or other naming conventions.

Systems Features

It is recommended that a number of computerized system features be available to facilitate the collection, inspection, review, and retrieval of quality clinical data. Key features are described here.

Systems Used for Direct Entry of Data

It is recommended that prompts, flags, or other help features be incorporated into the computerized system to encourage consistent use of clinical terminology and to alert the user to data that are out of acceptable range. It is recommended against the use of features that automatically enter data into a field when the field is bypassed.

Retrieval of Data and Record Retention

The FDA expects to be able to reconstruct a clinical study submitted to the agency. This means that documentation should fully describe and explain how data were obtained and managed and how electronic records were used to capture data. It is suggested that your decision on how to maintain records be based on predicate rule requirements and that this documented decision be based on a justified risk assessment and a determination of the value of the records over time. The FDA does not object to required records that are archived in electronic format; nonelectronic media such as microfilm, microfiche, and paper; or to a standard electronic file format (such as pdf, xml, or sgml). Persons must still comply with all predicate rule requirements, and the records themselves and any copies of required records should preserve their original content and meaning. Paper and electronic record and signature components can coexist (i.e., as a hybrid system) as long as the predicate requirements are met, and the content and meaning of those records are preserved.

It is not necessary to reprocess data from a study that can be fully reconstructed from available documentation. Therefore, actual application software, operation systems, and software development tools involved in processing of data or records do not need to be retained.

System Security

In addition to internal safeguards built into the computerized system, external safeguards should be put in place to ensure that access to the computerized system and to the data is restricted to authorized personnel. It is recommended that staff be kept thoroughly aware of system security measures and the importance of limiting access to authorized personnel.

SOPs should be developed and implemented for handling and storing the system to prevent unauthorized access. Controlling system access can be accomplished through the following provisions:

- Operational system checks
- Authority checks
- Device (e.g., terminal) checks
- The establishment of and adherence to written policies that hold individuals accountable for actions initiated under their electronic data management programs

It is recommended that access to data be restricted and monitored through the system's software with its required log-on, security procedures, and audit trail (or other selected security measures to track electronic record activities). It is recommended that procedures and controls be implemented to prevent the data from being altered, browsed, queried, or reported via external software applications that do not enter through the protective system software.

It is recommended that a cumulative record be available that indicates, for any point in time, the names of authorized personnel, their titles, and a description of their access privileges. It is recommended that the record be kept in the study documentation, accessible at the site.

If a sponsor supplies computerized systems exclusively for clinical trials, it is recommended that the systems remain dedicated to the purpose for which they were intended and validated. If a computerized system being used for a clinical study is part of a system normally used for other purposes, it is recommended that efforts be made to ensure that the study software be logically and physically isolated as necessary to preclude unintended interaction with nonstudy software. If any of the software programs are changed, it is recommended that the system be evaluated to determine the effect of the changes on logical security.

It is recommended that controls be implemented to prevent, detect, and mitigate effects of computer viruses, worms, or other potentially harmful software code on study data and software.

System Dependability

It is recommended that sponsors ensure and document that all computerized systems conform to their own established requirements for completeness, accuracy, reliability, and consistent intended performance.

It is recommended that systems documentation be readily available at the site where clinical trials are conducted and provide an overall description of the computerized systems and the relationships among hardware, software, and physical environment.

As noted in the *Part 11 Scope and Application* guidance, the FDA intends to exercise enforcement discretion regarding specific Part 11 requirements for validation of the computerized systems. It is suggested that your decision to validate the computerized systems and the extent of the validation take into account the impact the systems have on your ability to meet predicate rule requirements. You should also consider the impact those systems might have on the accuracy, reliability, integrity, availability, and authenticity of required records and signatures. Even if there is no predicate rule requirement to validate a system, it may still be

important to validate the system, based on criticality and risk, to ensure the accuracy, reliability, integrity, availability, and authenticity of required records and signatures.

It is recommended that the sponsors base their approach on a justified and documented risk assessment and determination of the potential of the system to affect data quality and record integrity. For example, in the case where data are directly entered into electronic records and the business practice is to rely on the electronic record, validation of the computerized system is important. However, when a word processor is used to generate SOPs for use at the clinical site, validation would not be important.

If validation is required, the FDA may ask to see the regulated company's documentation that demonstrates software validation. The study sponsor is responsible for making any such documentation available if requested at the time of inspection at the site where software is used. Clinical investigators are not generally responsible for validation unless they originated or modified software.

Legacy Systems

As noted in the *Part 11 Scope and Application* guidance, the FDA intends to exercise enforcement discretion with respect to all Part 11 requirements for systems that otherwise were fully operational prior to August 20, 1997, the effective date of Part 11, under the circumstances described below. These systems are also known as legacy systems. The FDA does not intend to take enforcement action to enforce compliance with any Part 11 requirements if all the following criteria are met for a specific system:

- The system was in operation before the Part 11 effective date.
- The system met all applicable predicate rule requirements prior to the Part 11 effective date.
- The system currently meets all applicable predicate rule requirements.
- There is documented evidence and justification that the system is fit for its intended use.

If a system has changed since August 20, 1997, and if the changes would prevent the system from meeting predicate rule requirements, Part 11 controls should be applied to Part 11 records and signatures pursuant to the enforcement policy expressed in the Part 11 guidance.

Off-the-Shelf Software

While the FDA has announced that it intends to exercise enforcement discretion regarding specific Part 11 requirements for validation of computerized systems, persons must still comply with all predicate rule requirements for validation. It was suggested in the guidance for industry on Part 11 that the impact of computerized systems on the accuracy, reliability, integrity, availability, and authenticity of required records and signatures be considered when you decide whether to validate, and noted that even absent a predicate rule requirement to validate a system, it might still be important to validate in some instances.

For most off-the-shelf (OTS) software, the design-level validation will have already been done by the company that wrote the software. Given the importance of ensuring valid clinical trial data, the FDA suggests that the sponsor or contract research organization (CRO) has documentation (either original validation documents or on-site vendor audit documents) of this design-level validation by the vendor and would itself have performed functional testing (e.g., by use of test datasets) and researched known software limitations, problems, and defect corrections. Detailed documentation of any additional validation efforts performed by the sponsor or CRO will preserve the findings of these efforts.

In the special case of database and spreadsheet software that is (*i*) purchased OTS, (*ii*) designed for and widely used for general purposes, (*iii*) unmodified, and (*iv*) not being used for direct entry of data, the sponsor or CRO may not have documentation of design-level validation. The FDA suggests that the sponsor or CRO performs functional testing (e.g., by use of test datasets) and research known software limitations, problems, and defect corrections.

In the case of OTS software, it is recommended that the following be available to the FDA on request:

- A written design specification that describes what the software is intended to do and how it is intended to do it;
- A written test plan based on the design specification, including both structural and functional analysis; and
- Test results and an evaluation of how these results demonstrate that the predetermined design specification has been met.

Change Control

The FDA recommends that written procedures be put in place to ensure that changes to the computerized system, such as software upgrades, including security and performance patches, equipment, or component replacement, or new instrumentation, will maintain the integrity of the data and the integrity of protocols. It is recommended that the effects of any changes to the system be evaluated and a decision made regarding whether, and if so, what level of validation activities related to those changes would be appropriate. It is recommended that validation be performed for those types of changes that exceed previously established operational limits or design specifications. Finally, it is recommended that all changes to the system be documented.

Systems Control

It is recommended that appropriate system control measures be developed and implemented.

- *Software version control*: It is recommended that measures be put in place to ensure that versions of software used to generate, collect, maintain, and transmit data are the versions that are stated in the systems documentation.
- *Contingency plans*: It is recommended that written procedures describe contingency plans for continuing the study by alternate means in the event of failure of the computerized system.
- *Backup and recovery of electronic records*: When electronic formats are the only ones used to create and preserve electronic records, it is recommended that backup and recovery procedures be outlined clearly in SOPs and be sufficient to protect against data loss. It is recommended that records be backed up regularly in a way that would prevent a catastrophic loss and ensure the quality and integrity of the data. It is recommended that records be stored at a secure location specified in the SOPs. Storage is typically off-site or in a building separate from the original records. It is recommended that backup and recovery logs be maintained to facilitate an assessment of the nature and scope of data loss resulting from a system failure. Firms that rely on electronic and paper systems should determine the extent to which backup and recovery procedures are needed based on the need to meet predicate rule requirements, a justified and documented risk assessment, and a determination of the potential effect on data quality and record integrity.

Training of Personnel

Firms using computerized systems must determine that persons who develop, maintain, or use electronic systems have the education, training, and experience to perform their assigned tasks. It is recommended that training be provided to individuals in the specific operations with regard to computerized systems that they are to perform. It is recommended that training be conducted by qualified individuals on a continuing basis, as needed, to ensure familiarity with the computerized system and with any changes to the system during the course of the study. It is further recommended that employee education, training, and experience be documented.

Copies of Records

The FDA has the authority to inspect all records relating to clinical investigations, regardless of how the records were created or maintained. Therefore, you should provide the FDA investigator with reasonable and useful access to records during an FDA inspection and supply copies of electronic records by

- Producing copies of records held in common portable formats when records are maintained in these formats
- Using established automated conversion or export methods, where available, to make copies available in a more common format (e.g., pdf, xml, or sgml).

Regardless of the method used to produce copies of electronic records, it is important that the copying process used produces copies that preserve the content and meaning of the record. For example, if you have the ability to search, sort, or trend records, copies given to the FDA should provide the same capability if it is reasonable and technically feasible. The FDA expects to inspect, review, and copy records in a human-readable form at your site, using your hardware and following your established procedures and techniques for accessing records.

Electronic Signature Certification

Persons using electronic signatures to meet an FDA signature requirement must, prior to or at the time of such use, certify to the FDA that the electronic signatures in their system, used on or after August 20, 1997, are intended to be the legally binding equivalent of traditional handwritten signatures.

GENERAL PRINCIPLES OF SOFTWARE VALIDATION

Planning, verification, testing, traceability, configuration management, and many other aspects of good software engineering are important activities that together help to support a final conclusion that software is validated. It is recommended to integrate the software life cycle management and risk management activities. Based on the intended use and the safety risk associated with the software to be developed, the software developer should determine the specific approach, the combination of techniques to be used, and the level of effort to be applied. While this guidance does not recommend any specific life cycle model or any specific technique or method, it does recommend that software validation and verification activities be conducted throughout the entire software life cycle.

Since the software operating a device such as HPLC equipment or LC/MS/MS, data storage systems and analysis using statistical software is of utmost importance and are parts of the Quality Assurance (QA) function of the BE testing laboratory. It is unlikely that the user would develop any software on its own to perform the functions incumbent in the operation of a BE laboratory. Since the software is developed by someone other than the user (e.g., OTS software), the software developer may not be directly responsible for compliance with the FDA regulations. In that case, the party with regulatory responsibility (i.e., the user) needs to assess the adequacy of the OTS software developer's activities and determine what additional efforts are needed to establish that the software is validated for the device manufacturer's intended use. The FDA believes in reducing burden and encourages firms to suggest an alternative approach that would be less burdensome.

Quality System Regulations

While the quality system regulation states that design input requirements must be documented, and that specified requirements must be verified, the regulation does not further clarify the distinction between the terms "requirement" and "specification." A requirement can be any need or expectation for a system or its software. Requirements reflect the stated or implied needs of the customer, and may be market-based, contractual, or statutory, as well as an organization's internal requirements. There can be many different kinds of requirements

(e.g., design, functional, implementation, interface, performance, or physical requirements). Software requirements are typically derived from the system requirements for those aspects of system functionality that have been allocated to software. Software requirements are typically stated in functional terms and are defined, refined, and updated as a development project progresses. Success in accurately and completely documenting software requirements is a crucial factor in successful validation of the resulting software.

A specification is defined as "a document that states requirements." It may refer to or include drawings, patterns, or other relevant documents and usually indicates the means and the criteria whereby conformity with the requirement can be checked. There are many different kinds of written specifications, e.g., system requirements specification, software requirements specification, software design specification, software test specification, and software integration specification. All of these documents establish "specified requirements" and are design outputs for which various forms of verification are necessary.

Verification and Validation

The quality system regulation is harmonized with *ISO 8402*:1994, which treats "verification" and "validation" as separate and distinct terms. On the other hand, many software engineering journal articles and textbooks use the terms verification and validation interchangeably, or in some cases refer to software "verification, validation, and testing" as if it is a single concept, with no distinction among the three terms.

Software verification provides objective evidence that the design outputs of a particular phase of the software development life cycle meet all of the specified requirements for that phase. Software verification looks for consistency, completeness, and correctness of the software and its supporting documentation, as it is being developed, and provides support for a subsequent conclusion that software is validated. Software testing is one of many verification activities intended to confirm that software development output meets its input requirements. Other verification activities include various static and dynamic analyses, code and document inspections, walk-throughs, and other techniques.

Software validation is a part of the design validation for a finished device, but is not separately defined in the quality system regulation. For purposes of this guidance, the FDA considers software validation to be "confirmation by examination and provision of objective evidence that software specifications conform to user needs and intended uses, and that the particular requirements implemented through software can be consistently fulfilled." In practice, software validation activities may occur both during and at the end of the software development life cycle to ensure that all requirements have been fulfilled. Since software is usually part of a larger hardware system, the validation of software typically includes evidence that all software requirements have been implemented correctly and completely and are traceable to system requirements. A conclusion that software is validated is highly dependent upon comprehensive software testing, inspections, analyses, and other verification tasks performed at each stage of the software development life cycle.

Software verification and validation are difficult because a developer cannot test forever, and it is hard to know how much evidence is enough. In large measure, software validation is a matter of developing a "level of confidence" that the device meets all requirements and user expectations for the software-automated functions and features of the device. Measures such as defects found in specifications documents, estimates of defects remaining, testing coverage, and other techniques are all used to develop an acceptable level of confidence before shipping the product. The level of confidence, and therefore the level of software validation, verification, and testing effort needed, will vary depending upon the safety risk (hazard) posed by the automated functions of the device.

IQ/OQ/PQ

For many years, both the FDA and regulated industry have attempted to understand and define software validation within the context of process validation terminology. For example, industry documents and other FDA validation guidance sometimes describe user site software validation in terms of installation qualification (IQ), operational qualification (OQ), and performance qualification (PQ). While IQ/OQ/PQ terminology has served its purpose well

and is one of many legitimate ways to organize software validation tasks at the user site, this terminology may not be well understood among many software professionals, and it is not used elsewhere in this document.

While software shares many of the same engineering tasks as hardware, it has some very important differences. For example

- The vast majority of software problems are traceable to errors made during the design and development process. While the quality of a hardware product is highly dependent on design, development, and manufacture, the quality of a software product is dependent primarily on design and development with a minimum concern for software manufacture. Software manufacturing consists of reproduction that can be easily verified. It is not difficult to manufacture thousands of program copies that function exactly the same as the original; the difficulty comes in obtaining the original program to meet all specifications.
- One of the most significant features of software is branching, i.e., the ability to execute alternative series of commands, based on differing inputs. This feature is a major contributing factor for another characteristic of software—its complexity. Even short programs can be very complex and difficult to fully understand.
- Typically, testing alone cannot fully verify that software is complete and correct. In addition to testing, other verification techniques and a structured and documented development process should be combined to ensure a comprehensive validation approach.
- Unlike hardware, software is not a physical entity and does not wear out. In fact, software may improve with age, as latent defects are discovered and removed. However, as software is constantly updated and changed, such improvements are sometimes countered by new defects introduced into the software during the change.
- Unlike some hardware failures, software failures occur without advanced warning. The software's branching that allows it to follow differing paths during execution may hide some latent defects until long after a software product has been introduced into the marketplace.
- Another related characteristic of software is the speed and ease with which it can be changed. This factor can cause both software and non-software professionals to believe that software problems can be corrected easily. Combined with a lack of understanding of software, it can lead managers to believe that tightly controlled engineering is not needed as much for software as it is for hardware. In fact, the opposite is true. Because of its complexity, the development process for software should be even more tightly controlled than for hardware, in order to prevent problems that cannot be easily detected later in the development process.
- Seemingly insignificant changes in software code can create unexpected and very significant problems elsewhere in the software program. The software development process should be sufficiently well planned, controlled, and documented to detect and correct unexpected results from software changes.
- Given the high demand for software professionals and the highly mobile workforce, the software personnel who make maintenance changes to software may not have been involved in the original software development. Therefore, accurate and thorough documentation is essential.
- Historically, software components have not been as frequently standardized and interchangeable as hardware components. However, medical device software developers are beginning to use component-based development tools and techniques. Object-oriented methodologies and the use of OTS software components hold promise for faster and less expensive software development. However, component-based approaches require very careful attention during integration. Prior to integration, time is needed to fully define and develop reusable software code and to fully understand the behavior of OTS components.

For these and other reasons, software engineering needs an even greater level of managerial scrutiny and control than does hardware engineering.

Software validation is a critical tool used to assure the quality of control and output; software validation can increase the usability and reliability and increased robustness of the data obtained. Software validation can also reduce long-term costs by making it easier and less costly to reliably modify software and revalidate software changes. Software maintenance can represent a very large percentage of the total cost of software over its entire life cycle. An established comprehensive software validation process helps reduce the long-term cost of software by reducing the cost of validation for each subsequent release of the software.

Principles of Software Validation

Whereas it is unlikely that a BE laboratory personnel will be involved in the validation of software more than testing it at the IQ/OQ and PQ levels, it is important to understand how software is validated when requiring this certification from the vendors.

Software quality assurance needs to focus on preventing the introduction of defects into the software development process and not on trying to "test quality into" the software code after it is written. Software testing is very limited in its ability to surface all latent defects in software code. For example, the complexity of most software prevents it from being exhaustively tested. Software testing is a necessary activity. However, in most cases, software testing by itself is not sufficient to establish confidence that the software is fit for its intended use. In order to establish that confidence, software developers should use a mixture of methods and techniques to prevent software errors and to detect software errors that do occur. The "best mix" of methods depends on many factors including the development environment, application, size of project, language, and risk.

To build a case that the software is validated requires time and effort. Preparation for software validation should begin early, i.e., during design and development planning and design input. The final conclusion that the software is validated should be based on evidence collected from planned efforts conducted throughout the software life cycle.

Software validation takes place within the environment of an established software life cycle. The software life cycle contains software engineering tasks and documentation necessary to support the software validation effort. In addition, the software life cycle contains specific verification and validation tasks that are appropriate for the intended use of the software. This guidance does not recommend any particular life cycle models—only that they should be selected and used for a software development project.

The software validation process is defined and controlled through the use of a plan. The software validation plan defines "what" is to be accomplished through the software validation effort. Software validation plans are a significant quality system tool. Software validation plans specify areas such as scope; approach; resources; schedules; and the types and extent of activities, tasks, and work items.

The software validation process is executed through the use of procedures. These procedures establish "how" to conduct the software validation effort. The procedures should identify the specific actions or sequence of actions that must be taken to complete individual validation activities, tasks, and work items.

Due to the complexity of software, a seemingly small local change may have a significant global system impact. When any change (even a small change) is made to the software, the validation status of the software needs to be reestablished. Whenever software is changed, a validation analysis should be conducted not just for validation of the individual change, but also to determine the extent and impact of that change on the entire software system. Based on this analysis, the software developer should then conduct an appropriate level of software regression testing to show that unchanged but vulnerable portions of the system have not been adversely affected. Design controls and appropriate regression testing provide the confidence that the software is validated after a software change.

Validation coverage should be based on the software's complexity and safety risk—not on firm size or resource constraints. The selection of validation activities, tasks, and work items should be commensurate with the complexity of the software design and the risk associated with the use of the software for the specified intended use. For lower risk devices, only baseline validation activities may be conducted. As the risk increases, additional

validation activities should be added to cover the additional risk. Validation documentation should be sufficient to demonstrate that all software validation plans and procedures have been completed successfully.

Validation activities should be conducted using the basic quality assurance precept of "independence of review." Self-validation is extremely difficult. When possible, an independent evaluation is always better, especially for higher risk applications. Some firms contract out for a third-party independent verification and validation, but this solution may not always be feasible. Another approach is to assign internal staff members that are not involved in a particular design or its implementation, but who have sufficient knowledge to evaluate the project and conduct the verification and validation activities. Smaller firms may need to be creative in how tasks are organized and assigned in order to maintain internal independence of review.

Specific implementation of these software validation principles may be quite different from one application to another. The user has flexibility in choosing how to apply these validation principles, but retains ultimate responsibility for demonstrating that the software has been validated.

Software is designed, developed, validated, and regulated in a wide spectrum of environments, and for a wide variety of devices with varying levels of risk. The FDA-regulated medical device applications include software that

- is a component, part, or accessory of a medical device;
- is itself a medical device; or
- is used in manufacturing, design and development, or other parts of the quality system.

In each environment, software components from many sources may be used to create the application (e.g., in-house developed software, OTS software, contract software, shareware). In addition, software components come in many different forms (e.g., application software, operating systems, compilers, debuggers, configuration management tools, and many more). The validation of software in these environments can be a complex undertaking; therefore, it is appropriate that all of these software validation principles be considered when designing the software validation process. The resultant software validation process should be commensurate with the safety risk associated with the system, device, or process.

Software validation activities and tasks may be dispersed, occurring at different locations and being conducted by different organizations. However, regardless of the distribution of tasks, contractual relations, source of components, or the development environment, the user or specification developer retains ultimate responsibility for ensuring that the software is validated.

Software validation is accomplished through a series of activities and tasks that are planned and executed at various stages of the software development life cycle. These tasks may be one time occurrences or may be iterated many times, depending on the life cycle model used and the scope of changes made as the software project progresses.

For each of the software life cycle activities, there are certain "typical" tasks that support a conclusion that the software is validated. However, the specific tasks to be performed, their order of performance, and the iteration and timing of their performance will be dictated by the specific software life cycle model that is selected and the safety risk associated with the software application. For very low-risk applications, certain tasks may not be needed at all. However, the software developer should at least consider each of these tasks and should define and document which tasks are or are not appropriate for their specific application. The following discussion is generic and is not intended to prescribe any particular software life cycle model or any particular order in which tasks are to be performed.

Typical Tasks

Quality Planning

- Risk (hazard) management plan
- Configuration management plan

- Software quality assurance plan
- Software verification and validation plan
  - Verification and validation tasks, and acceptance criteria
  - Schedule and resource allocation (for software verification and validation activities)
  - Reporting requirements
- Formal design review requirements
- Other technical review requirements
- Problem reporting and resolution procedures
- Other support activities

Requirements

- Preliminary risk analysis
- Traceability analysis
  - Software requirements to system requirements (and vice versa)
  - Software requirements to risk analysis
- Description of user characteristics
- Listing of characteristics and limitations of primary and secondary memory
- Software requirements evaluation
- Software user interface requirements analysis
- System test plan generation
- Acceptance test plan generation
- Ambiguity review or analysis

Design

- Updated software risk analysis
- Traceability analysis—design specification to software requirements (and vice versa)
- Software design evaluation
- Design communication link analysis
- Module test plan generation
- Integration test plan generation
- Test design generation (module, integration, system, and acceptance)

Construction or Coding

- Traceability analyses
  - Source code to design specification (and vice versa)
  - Test cases to source code and to design specification
- Source code and source code documentation evaluation
- Source code interface analysis
- Test procedure and test case generation (module, integration, system, and acceptance)

Testing by the Software Developer

- Test planning
- Structural test case identification
- Functional test case identification
- Traceability Analysis—Testing
  - Unit (module) tests to detailed design
  - Integration tests to high-level design
  - System tests to software requirements
- Unit (module) test execution

- Integration test execution
- Functional test execution
- System test execution
- Acceptance test execution
- Test results evaluation
- Error evaluation/resolution
- Final test report

User Site Testing

- Acceptance test execution
- Test results evaluation
- Error evaluation/resolution
- Final test report

VALIDATION OF AUTOMATED PROCESS EQUIPMENT AND QUALITY SYSTEM SOFTWARE

The quality system regulation requires that "when computers or automated data processing systems are used as part of production or the quality system, the [device] manufacturer shall validate computer software for its intended use according to an established protocol." [See 21 CFR §820.70(i).] This has been a regulatory requirement of FDA's medical device good manufacturing practice regulations since 1978.

In addition to the above validation requirement, computer systems that implement part of a device manufacturer's production processes or quality system (or that are used to create and maintain records required by any other FDA regulation) are subject to the electronic records (Electronic Signatures regulation; see 21 CFR Part 11). This regulation establishes additional security, data integrity, and validation requirements when records are created or maintained electronically. These additional Part 11 requirements should be carefully considered and included in system requirements and software requirements for any automated record keeping systems. System validation and software validation should demonstrate that all Part 11 requirements have been met.

Computers and automated equipment are used extensively throughout all aspects of medical device design, laboratory testing and analysis, product inspection and acceptance, production and process control, environmental controls, packaging, labeling, traceability, document control, complaint management, and many other aspects of the quality system. Increasingly, automated plant floor operations can involve extensive use of embedded systems in

- programmable logic controllers;
- digital function controllers;
- statistical process control;
- supervisory control and data acquisition;
- robotics;
- human–machine interfaces;
- input/output devices; and
- computer operating systems.

Software tools are frequently used to design, build, and test the software that goes into an automated medical device. Many other commercial software applications, such as word processors, spreadsheets, databases, and flowcharting software are used to implement the quality system. All of these applications are subject to the requirement for software validation, but the validation approach used for each application can vary widely.

Whether production or quality system software is developed in-house by the device manufacturer, developed by a contractor, or purchased OTS, it should be developed using the basic principles outlined above. The user has latitude and flexibility in defining how validation of that software will be accomplished, but validation should be a key consideration in deciding how and by whom the software will be developed or from whom it will be purchased. The software developer defines a life cycle model. Validation is typically supported by

- verifications of the outputs from each stage of that software development life cycle and
- checking for proper operation of the finished software in the device manufacturer's intended use environment.

The level of validation effort should be commensurate with the risk posed by the automated operation. In addition to risk other factors, such as the complexity of the process software and the degree to which the user is dependent upon that automated process to produce a safe and effective device, determine the nature and extent of testing needed as part of the validation effort. Documented requirements and risk analysis of the automated process help define the scope of the evidence needed to show that the software is validated for its intended use. For example, an automated milling machine may require very little testing if the user can show that the output of the operation is subsequently fully verified against the specification before release. On the other hand, extensive testing may be needed for

- a plant-wide electronic record and electronic signature system;
- an automated controller for a sterilization cycle; or
- automated test equipment used for inspection and acceptance of finished circuit boards in a life-sustaining/life-supporting device.

Numerous commercial software applications may be used as part of the quality system (e.g., a spreadsheet or statistical package used for quality system calculations, a graphics package used for trend analysis, or a commercial database used for recording device history records or for complaint management). The extent of validation evidence needed for such software depends on the device manufacturer's documented intended use of that software. For example, a user who chooses not to use all the vendor-supplied capabilities of the software only needs to validate those functions that will be used and for which the user is dependent upon the software results as part of production or the quality system. However, high-risk applications should not be running in the same operating environment with nonvalidated software functions, even if those software functions are not used. Risk mitigation techniques such as memory partitioning or other approaches to resource protection may need to be considered when high-risk applications and lower risk applications are to be used in the same operating environment. When software is upgraded or any changes are made to the software, the user should consider how those changes may impact the "used portions" of the software and must reconfirm the validation of those portions of the software that are used [see 21 CFR §820.70(i)].

User Requirements

A very important key to software validation is a documented user requirements specification that defines

- the "intended use" of the software or automated equipment and
- the extent to which the user is dependent upon that software or equipment for production of a quality medical device.

The user needs to define the expected operating environment including any required hardware and software configurations, software versions, utilities, etc. The user also needs to

- document requirements for system performance, quality, error handling, start-up, shutdown, security, etc.;

- identify any safety-related functions or features, such as sensors, alarms, interlocks, logical processing steps, or command sequences; and
- define objective criteria for determining acceptable performance.

The validation must be conducted in accordance with a documented protocol, and the validation results must also be documented [see 21 CFR §820.70(i)]. Test cases should be documented which will exercise the system to challenge its performance against the predetermined criteria, especially for its most critical parameters. Test cases should address error and alarm conditions, start-up, shutdown, all applicable user functions and operator controls, potential operator errors, maximum and minimum ranges of allowed values, and stress conditions applicable to the intended use of the equipment. The test cases should be executed and the results should be recorded and evaluated to determine whether the results support a conclusion that the software is validated for its intended use.

A user may conduct a validation using their own personnel or may depend on a third party such as the equipment/software vendor or a consultant. In any case, the user retains the ultimate responsibility for ensuring that the production and quality system software

- is validated according to a written procedure for the particular intended use and
- will perform as intended in the chosen application.

The user should have documentation including

- defined user requirements;
- validation protocol used;
- acceptance criteria;
- test cases and results; and
- a validation summary

which objectively confirms that the software is validated for its intended use.

VALIDATION OF OTS SOFTWARE AND AUTOMATED EQUIPMENT

Most of the automated equipment and systems used by device manufacturers are supplied by third-party vendors and are purchased OTS. The user is responsible for ensuring that the product development methodologies used by the OTS software developer are appropriate and sufficient for the device manufacturer's intended use of that OTS software. For OTS software and equipment, the user may or may not have access to the vendor's software validation documentation. If the vendor can provide information about their system requirements, software requirements, validation process, and the results of their validation, the medical user can use that information as a beginning point for their required validation documentation. The vendor's life cycle documentation, such as testing protocols and results, source code, design specification, and requirements specification, can be useful in establishing that the software has been validated. However, such documentation is frequently not available from commercial equipment vendors, or the vendor may refuse to share their proprietary information.

Where possible and depending upon the risk involved, the user should consider auditing the vendor's design and development methodologies used in the construction of the OTS software and should assess the development and validation documentation generated for the OTS software. Such audits can be conducted by the user or by a qualified third party. The audit should demonstrate that the vendor's procedures for and results of the verification and validation activities performed by the OTS software are appropriate and sufficient for the safety and effectiveness requirements of the medical device to be produced using that software.

Some vendors who are not accustomed to operating in a regulated environment may not have a documented life cycle process that can support the device manufacturer's validation

requirement. Other vendors may not permit an audit. Where necessary validation information is not available from the vendor, the user will need to perform sufficient system-level "black box" testing to establish that the software meets their "user needs and intended uses." For many applications, black box testing alone is not sufficient. Depending upon the risk of the device produced, the role of the OTS software in the process, the ability to audit the vendor, and the sufficiency of vendor-supplied information, the use of OTS software or equipment may or may not be appropriate, especially if there are suitable alternatives available. The user should also consider the implications (if any) for continued maintenance and support of the OTS software should the vendor terminate their support.

For some OTS software development tools, such as software compilers, linkers, editors, and operating systems, exhaustive black box testing by the user may be impractical. Without such testing—a key element of the validation effort—it may not be possible to validate these software tools. However, their proper operation may be satisfactorily inferred by other means. For example, compilers are frequently certified by independent third-party testing, and commercial software products may have "bug lists", system requirements and other operational information available from the vendor which can be compared to the device manufacturer's intended use to help focus the black box testing effort. OTS operating systems need not be validated as a separate program. However, system-level validation testing of the application software should address all the operating system services used, including maximum loading conditions, file operations, handling of system error conditions, and memory constraints that may be applicable to the intended use of the application program.

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13 Bioequivalence Reports

BACKGROUND

Sponsors would do well if they require their contract research organizations (CROs) to produce these reports in a clear, comprehensive format that allows easy access to all the information submitted. Although the FDA does not require any specific format for the submission of the report, the layout described here has been successful in achieving fast approval of bioequivalence studies, and it is recommended that sponsors check their CRO's standard format against the recommendations made here for the purpose of assuring completeness, accuracy, ready accessibility, and compliance.

Document type	Description	Control system
Cover page	Single page listing the document number (describe in the GDP SOP), study number, full title of the study including the sponsor's name, generic name of drug, the brand names of sponsor as well as RLD (including lot numbers, date of manufacture, and date of expiry), dosage strength (and all the strengths the application supports), basic statistical design used, single or multiple dosing, fed or unfed study, route of administration, duration of study, place where study was completed, and date of completion. This page will also bear signatures of at least the following: 1. Written by 2. Reviewed by 3. Approved by (CRO) 4. Approval by sponsor 5. Certification of compliance with GDP, GLP, GCP by the Head of QA at CRO 6. Certification of compliance with GDP 7. Certification of compliance with GLP 8. Certificate of compliance with GCP (IRB) 9. Certificate of compliance with 21 CFR various parts on use of computers and software 10. Certification of current calibration of instruments by QC Head	Good document practice SOP describing the convention used for naming and numbering the documents, numbering of studies that can be traced to location where the original documents are stored.
Table of contents	Extensive listing of the entire document including all tables, figures, charts, references, and enclosures is required. Preparation of this table of content is simplified if a predetermined convention of naming the categories is used; the preferred formatting form of the tabulation is numerical such as 1.2.3. While quoting references do not use footnote function, instead use a superscript; this allows	

(Continued)

Document type	Description	Control system
Table of contents (*continued*)	separation of documents at the regulatory submission without losing control of document. There should be a single document file compiled and one table of content prepared; however, it is possible that the various sections of the study may be separated and reviewed by different reviewers; to expedite that separate each section by a divider and provide a complete table of contents before each section (a redundant function)	Good document practice
Study summary	A tabulated document summarizing all aspects of the study, the results, and conclusions reached including composite plasma level curves; the purpose of this section is to prepare the reviewer for specific exploration of the report	See example in the appendix to this chapter
Abbreviations and glossary of terms	Whereas sponsors and CROs may create their own abbreviations, a glossary of all terms used should be provided making use of standard terms only; all references quoted are provided here	See example in the appendix to this chapter
Section I: bioequivalence report	This is the actual report of bioequivalence testing and includes reference to any deviations made from the approved protocol; full description of the drug used and its pharmacokinetic and other disposition properties that might affect the time course of drug in the body. More specifically this report will include:	The bioequivalence protocol vis-á-vis the report includes the history of how the document was developed, the source documents used (e.g., information from published sources; protocols submitted to FDA; and available through FOI, RLD's promotional and label material used, guidelines of FDA, ICH, WHO, and others used in preparing the protocol); all correspondence (final forms) with the regulatory authorities and a justification of the dosage form, strength, and the manner of sourcing used. This protocol should be all inclusive of acceptance and rejection criteria, study design, analytical methods (to be developed or where developed their sensitivity), statistical analysis, certification for GLP, and GCP compliance. The purpose of this section to lay out the road map that was supposed to be followed in conducting the study; later in the study report, any deviations from the agreed and approved protocol will be highlighted and reasons for deviation described and justified
	1. Ethics	The bioanalytical methodology and its validation is generally the proprietary property of the CRO and is not allowed publication and is mostly the portion redacted from the FOI-based documentation; also redacted are some critical inclusion and exclusion criteria in the study

(Continued)

Document type	Description	Control system
Section I: bioequivalence report (*continued*)	1.1. Institutional Review Board 1.2. Ethical Conduct of the Study 1.3. Informed Consent 1.4. Justification of the Study 2. Introduction 2.1. History and Source of Drug 2.2. Chemistry 2.3. Pharmacology 2.4. Pharmacokinetics 2.4.1. Absorption and Bioavailability 2.4.2. Distribution 2.4.3. Metabolism and Elimination 2.5. Therapeutic Uses 2.6. Adverse Events 3. Investigation 3.1. Investigators and Study Administrative Structure 3.2. Study Objectives 3.3. Investigational Plan 3.4. Rationale Of Study Design 3.5. Selection of Study Population 3.5.1. Study Subjects Demography 3.5.2. Inclusion Criteria 3.5.3. Exclusion Criteria 3.6. Subjects Identification 3.7. Case Report Form Note 3.8. Confinement 3.9. Removal of Subjects from Study 3.10. Dietary Restrictions, Standardized Diet, and Fluid Intake 3.11. Study Drug Administration 3.12. Identity of Study Medications 3.13. Assignment of Study's Subjects and Randomization 3.14. Times of Dosing 3.15. Treatment Compliance 3.16. Physical Activities After Drug Intake 3.17. Prior and Concurrent Medication 3.18. Clinical Laboratory 3.19. Description of Study Facilities 3.19.1. Room for Prestudy Examinations 3.19.2. Clinical Investigator's Office 3.19.3. Room for the Administration of the Study Drugs, Vital Signs, and Blood Collection 3.19.4. Pharmacy 3.19.5. Emergency Room 3.19.6. Subject's Rooms 3.19.7. Kitchen 3.19.8. Dormitories 3.19.9. Dining rooms 3.19.10. Bathrooms/Toilets 3.20. Collection and Handling of Blood Samples for Analysis 3.21. Bioanalytical Drug Determination Methodology 3.22. Data Quality Assurance 3.23. Pharmacokinetic Calculations 3.24. Statistical Analysis 3.24.1. Confidence Intervals	

(Continued)

Document type	Description	Control system
Section I: bioequivalence report (*continued*)	3.24.2. ANOVA 3.24.3. Sample Size Determination 3.25. Data Tabulation, Descriptive Statistics, and Diagrammatic Data Presentation 4. Study Subjects 4.1. Disposition of Subjects 4.2. Withdrawals and Exclusions 4.3. Demographic Characteristics 4.4. Variations from the Study Protocol 5. Safety Evaluation 5.1. Benefit-to-Risk Ratio 5.2. Extent of Exposure5.2. Extent of Exposure 5.3. Adverse Events 5.3.1. Brief Summary Of Adverse Events 5.3.2. Display of Adverse Events 5.4. Clinical Laboratory Evaluation 5.5. Vital Signs, Physical Assessment, and Other Clinical Observations 5.6. Safety and Tolerance 6. Results and Bioequivalence Evaluation 6.1. Datasets from Study Subjects 6.2. Adjustment due to Anomalies 6.2.1. Adjustment due to Collection Anomalies 6.2.2. Adjustment due to Analytical Anomalies 6.2.3. Adjustment due to Pharmacokinetic Anomalies 6.2.4. Nonzero Predose Concentrations 6.3. Handling of Withdrawals and Missing Data 6.4. Pharmacokinetic Parameters 6.5. Statistical Inferences 6.5.1. Bioequivalence Conclusion 6.5.2. ANOVA 7. Discussion and Conclusions References Appendix A A.1. Comparative Bioavailability Study Protocol A.2. Approval of the Institutional Review Board A.3. Sample CRF A.4. Sample ICF A.5. Curriculum Vitae of Investigators and Coinvestigators A.6. Randomization Plan A.7. Pharmacokinetics and Statistical Output A.8. Bioanalytical Report A.9. Bioequivalence Report Appendix B (not included for brevity) Appendix C C.1 Bioequivalence Evaluation of Lansoprazole 30 MG Capsules (Lanfast® and Lanzor®) in Healthy Volunteers	

The reports included here were provided by the courtesy of Gulf Pharmaceutical Industries, an FDA-certified manufacturing facility located in the United Arab Emirates and remain the property of the company. Not all portions of the reports are reproduced here for brevity purpose.

COMPARATIVE RANDOMIZED, SINGLE DOSE, TWO-WAYCROSSOVER OPEN-LABEL STUDY TO DETERMINE THE BIOEQUIVALENCE OF METFORMIN HCL FROM DIALON®1000 MG TABLET[GULF PHARMACEUTICAL INDUSTRIES (JULPHAR)] AND GLUCOPHAGE ®1000 MG TABLET (MERCK), AFTER ORAL ADMINISTRATION OF1000 MG TO HEALTHY ADULT MALES UNDER FASTING CONDITIONS

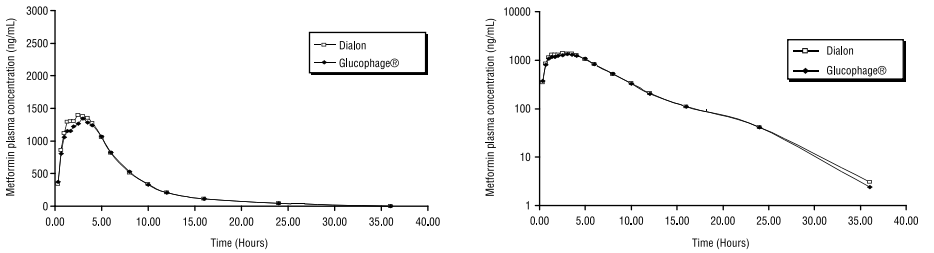
Study Synopsis

SPONSOR: ***GULF PHARMACEUTICAL INDUSTRIES (JULPHAR)***	
GENERIC NAME ***Metformin***	TABLE REFERENCE PART(S)
TEST PRODUCT ***Dialon®***	1. ETHICS 2. INTRODUCTION 3. INVESTIGATION
REFERENCE PRODUCT ***Glucophage ®***	
STUDY TITLE	***Comparative Randomized, Single Dose, Two-Way Crossover Open-Label Study to Determine the Bioequivalence of Metformin HCl from Dialon® 1000 mg Tablet [Gulf Pharmaceutical Industries (Julphar)] and Glucophage ®1000 mg Tablet (Merck), After Oral Administration of 1000 mg to Healthy Adult Males Under Fasting Conditions***
IPRC PROTOCOL CODE	***METF-T009***
IPRC STUDY CODE	***METF-GUL-T0904/339***
INSTITUTIONAL REVIEW BOARD	***Institutional Review Board of IPRC, Jordan***
OBJECTIVES	***To investigate the single-dose bioequivalence of Gulf Pharmaceutical Industries (Julphar). (TEST product, Dialon® Tablet) and Merck (REFERENCE product, Glucophage ® Tablet) 1000 mg metformin hydrochloride per tablet in healthy adult males under fasting conditions.***
INVESTIGATORS	Principal Investigator: ***Naji M. Najib, B.Sc. Pharm., Ph.D., International Pharmaceutical Research Center, Jordan*** Clinical Investigator: ***Usama Harb, M.D., International Pharmaceutical Research Center, Jordan***
DOSAGE REGIMEN	*Treatment A (TEST Product): Single-Dose,1000 mg of Dialon®* *(1000 mg metformin hydrochloride per tablet). Batch No. 0022, Exp. Date 08/06* ***Treatment B (REFERENCE Product): Single-Dose, 1000 mg of Glucophage ® (1000 mg metformin hydrochloride per tablet). Batch No.108940, Exp. Date 01/06***

CLINICAL LABORATORY	IPRC Clinical Site
SPONSOR: ***Gulf Pharmaceutical Industries (Julphar)***	
GENERIC NAME *Metformin* ®	TABLE REFERENCE PART(S)
TEST PRODUCT *Dialon* ®	3. INVESTIGATION (CONTINUED) 4. STUDY SUBJECTS
REFERENCE PRODUCT *Glucophage* ®	
STUDY SUBJECTS	***24 subjects plus 14 alternates, selected randomly from the Jordan population, were enrolled in the study, to investigate the bioequivalence in 24 subjects.***
DEMOGRAPHIC DATA (N=24)	*Age: 26 ± 4.96 year* *Height: 172 ± 4 41 cm* ***Weight: 70 ± 8.84 kg***
ADMISSION AND CONFINEMENT	***Subjects were admitted the night before Study Drug Administration, supervised for at least 10 hours of overnight fasting, and confined until collecting the 24-hour sample.***
DRUG ADMINISTRATION	***Each subject received an oral dose of the assigned formulation, according to a randomization scheme, administered with 240 mL of water.***
STUDY PERIODS	*Screening: 17/11/2004* *Enrollment: 22/11/2004* *Period I: 23/11/2004* ***Period II: 30/11/2004*** *First Sample: 23/11/2004* ***Last sample: 01/12/2004***
WASHOUT PERIOD	***Seven days from the first study drug administration.***
ANCILLARY ASSESSMENT	***Safety/adverse events, laboratory tests, physical examination, vital signs***
BLOOD SAMPLING SCHEDULE	***Twenty blood samples were drawn at 0.00 (two pre-dose samples) and 0.33, 0.66, 1.00, 1.33, 1.66, 2.00, 2.50, 3.00, 3.50, 4.00, 5.00, 6.00, 8.00, 10.00, 12.00, 16.00, 24.00 and 36.00 hours (post-dose). The total volume of blood drawn did not exceed 330 mL.***
BLOOD SAMPLES HANDLING	***The blood samples for metformin were placed in lithium heparin tubes, centrifuged, and the resulting plasma samples were immediately stored at –20˚C until analyzed.***

CLINICAL SAMPLES STORAGE	***Metformin plasma samples were stored under a nominal temperature of –20˚C until analyzed.***
BIOANALYTICAL METHODOLOGY	***HPLC, with LLOQ = 50 ng/mL***
SPONSOR: ***Gulf Pharmaceutical Industries (Julphar)***	
GENERIC NAME *Metformin* ®	TABLE REFERENCE PART(S)
TEST PRODUCT *Dialon* ®	
REFERENCE PRODUCT *Glucophage* ®	5. SAFETY EVALUATION 6. RESULTS AND BIOEQUIVALENCE EVALUATION 7. DISCUSSION AND CONCLUSION
TOLERANCE	***Both treatments were well tolerated***
SURROGATE PARAMETERS	***Drug plasma levels to indicate clinical activity***
PRIMARY PHARMACOKINETIC PARAMETERS	C_{max}, $AUC_{0\rightarrow t}$ and $AUC_{0\rightarrow\infty}$
SECONDARY PHARMACOKINETIC PARAMETERS	K_e, t_{max}, $t_{½e}$, and ($AUC_{0\rightarrow t}$/ $AUC_{0\rightarrow\infty}$)%
CONFIDENCE INTERVALS (PARAMETRIC METHOD)	*Confidence Intervals for the log-transformed Test/Reference Ratios of:* C_{max} 108.07 (100.61–116.09) % $AUC_{0\rightarrow t}$ 101.57 (96.00–107.46) % $AUC_{0\rightarrow\infty}$ **101.66 (96.39–107.21) %**
CONCLUSIONS	*Point estimates and the 90% Confidence Intervals for the log transformed ratios (TEST/REFERENCE) for $AUC_{0\rightarrow t}$ and $AUC_{0\rightarrow\infty}$ were within the 80.00–125.00%. And for C_{max} to be within 75.00–133.00 %. Therefore, the bioequivalence of Gulf Pharmaceutical Industries (Julphar) (Dialon® Tablet) and Merck (Glucophage® Tablet) 1000 mg metformin hydrochloride per tablet, can be concluded.*

Metformin means after single dose administration of one tablet (1000 mg metformin hydrochloride per tablet) for both treatments. Dialon® Tablet (TEST Product) and Glucophage® Tablet (REFERENCE Product).



Final Report Issuance Date	*19 April 2007*

THIS STUDY WAS CONDUCTED IN ACCORDANCE WITH INTERNATIONAL CONFERENCE OF HARMONIZATION (ICH) GOOD CLINICAL PRACTICE (GCP) GUIDELINES ADOPTED BY THE EUROPEAN AGENCY FOR THE EVALUATION OF MEDICINAL PRODUCTS (EMEA).

ESSENTIAL DOCUMENTS AND RECORDS WERE ALL ARCHIVED ACCORDING TO INTERNATIONAL PHARMACEUTICAL RESEARCH CENTER (IPRC) INTERNAL PROCEDURES FOR AUTHORIZED DIRECT ACCESS

THIS FINAL REPORT WAS GENERATED IN REFERENCE TO INTERNATIONAL CONFERENCE OF HARMONIZATION (ICH) GUIDELINES ADOPTED BY THE EUROPEAN AGENCY FOR THE EVALUATION OF MEDICINAL PRODUCTS (EMEA) FOR STRUCTURE AND CONTENT OF CLINICAL STUDY REPORTS

LIST OF ABBREVIATIONS

ADR	Adverse drug reaction
AE	Adverse event
ANOVA	Analysis of variance
AUC	Area under the plasma concentration–time curve
$AUC_{0\rightarrow t}$	Area under the plasma concentration–time curve from zero hours to time t
$AUC_{0\rightarrow \infty}$	Area under the plasma concentration–time curve from zero hours to infinity
BUN	Blood urea nitrogen
C_{last}	Last quantifiable concentration
C_{max}	Maximal plasma concentration
CRF	Case report form
EMEA	The European Agency for the evaluation of medicines for human use
FDA	Food and Drug Administration
GCP	Good clinical practice
GLP	Good laboratory practice
HPLC	High-performance liquid chromatography
ICF	Informed consent form
ICH	International Conference on Harmonization
IPRC	International Pharmaceutical Research Center
IRB	Institutional Review Board
K_e	Elimination rate constant
LLOQ	Lower limit of quantitation
Log	Logarithm
OECD	Organization for Economic Cooperation and Development
QAU	Quality assurance unit
SAE	Serious adverse events
Serious ADR	Serious adverse drug reactions
SOP	Standard operating procedure
SGOT	Serum glutamic oxalate transaminase
SGPT	Serum glutamic pyruvate transaminase
t_{max}	Time point of maximal plasma concentration
$t_{(1/2)e}$	Elimination half-life
USP-NF	The United States Pharmacopeia—The National Formulary United States Pharmacopial Convention
V/F	Apparent volume of distribution

LIST OF DEFINITIONS

Adverse Drug Reaction (ADR)

In the preapproval clinical experience with a new medicinal product or its new usages, particularly as the therapeutic dose(s) may not be established: all noxious and unintended responses to a medicinal product related to any dose should be considered ADRs. The phrase responses to a medicinal product means that a causal relationship between a medicinal product and an adverse event is at least a reasonable possibility, i.e., the relationship cannot be ruled out.

Regarding marketed medicinal products: a response to a drug which is noxious and unintended and which occurs at doses normally used in man for prophylaxis, diagnosis, or therapy of diseases or for modification of physiological function.

Adverse Event (AE)

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

Approval (in Relation to Institutional Review Boards)

The affirmative decision of the Institutional Review Board (IRB) that the clinical trial has been reviewed and may be conducted at the institution site within the constraints set forth by the IRB, the institution, Good Clinical Practice (GCP), and the applicable regulatory requirements.

Bioavailability

It is the rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action.

Bioequivalence

It is the absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar conditions in an appropriately designed study.

Case Report Form (CRF)

A printed, optical, or electronic document designed to record all of the protocol required information to be reported to the sponsor on each trial subject.

Clinical Trial/Study Report

A written description of a trial/study of any therapeutic, prophylactic, or diagnostic agent conducted in human subjects, in which the clinical and statistical description, presentations, and analyses are fully integrated into a single report.

Confidentiality

Prevention of disclosure, to other than authorized individuals, of a sponsor's proprietary information or of a subject's identity.

Direct Access

Permission to examine, analyze, verify, and reproduce any records and reports that are important to the evaluation of a clinical trial. Any party (e.g., domestic and foreign regulatory authorities, sponsor's monitors, and auditors) with direct access should take all reasonable precautions within the constraints of the applicable regulatory requirement(s) to maintain the confidentiality of subjects' identities and sponsor's proprietary information.

Essential Documents

Documents that individually and collectively permit evaluation of the conduct of a study and the quality of the data produced.

Good Clinical Practice (GCP)

A standard for the design, conduct, performance, monitoring, auditing, recording, analyses, and reporting of clinical trials that provides assurance that the data and reported results are credible and accurate, and that the rights, integrity, and confidentiality of trial subjects are protected.

Informed Consent

A process by which a subject voluntarily confirms his or her willingness to participate in a particular trial, after having been informed of all aspects of the trial that are relevant to the subject's decision to participate. Informed consent is documented by means of a written, signed, and dated informed consent form.

Institutional Review Board (IRB)

An independent body constituted of medical, scientific, and nonscientific members, whose responsibility is to ensure the protection of the rights, safety, and well-being of human subjects involved in a trial by, among other things, reviewing, approving, and providing continuing review of trial protocol and amendments and of the methods and material to be used in obtaining and documenting informed consent of the trial subjects.

Pharmaceutical Equivalents

Defined as drug products that contain identical amounts of the identical active drug ingredient, i.e., the same salt or ester of the same therapeutic moiety, in identical dosage forms, but not necessarily containing the same inactive ingredients, and that meet the identical compendial or other applicable standard of identity, strength, quality, and purity, including potency and, where applicable, content uniformity, disintegration times and/or dissolution rates.

Protocol

A document that describes the objective(s), design, methodology, statistical considerations, and organization of a trial. The protocol usually also gives the background and rationale for the trial, but these could be provided in other protocol referenced documents. Throughout the report guideline, the term protocol refers to protocol and protocol amendments.

Protocol Amendment

A written description of a change(s) to or formal clarification of a protocol.

Serious Adverse Event (SAE) or Serious Adverse Drug Reaction (Serious ADR)

Any untoward medical occurrence that at any dose results in death, is life-threatening—requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, or is a congenital anomaly/birth defect.

Source Data

All information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents (original records or certified copies).

Source Documents

Original documents, data, and records (e.g., hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate copies, microfiches, photographic negatives, microfilm or magnetic media, x rays, subject files, and records kept at the pharmacy, at the laboratoires and at medico-technical departments involved in the clinical trial).

Standard Operating Procedures (SOPs)

Detailed, written instructions to achieve uniformity of the performance of a specific function.

Subject Identification Code

A unique identifier assigned by the investigator to each trial subject to protect the subject's identity and used in lieu of the subject's name when the investigator reports adverse events and/or other trial related data.

1. ETHICS

1.1. Institutional Review Board

The Institutional Review Board (IRB) of International Pharmaceutical Research Center (IPRC), Amman, Jordan was dedicated to endorse the ethical conduct of the study and to approve the protocol. The board is constituted and operates in accordance with the principles and requirements described in the Guidelines on Research Involving Human Subjects. The study protocol was reviewed by the IRB of IPRC. The approval for the study protocol was given on 26/10/2004 as demonstrated in the section "Approval of the IRB" (Appendix A.2).

1.2. Ethical Conduct of the Study

This research was carried out in accordance with conditions stipulated by the International Clinical Research guidelines, enunciated in the Declaration of Helsinki resolved in Helsinki in 1964 and amended in Scotland, 2000; and the ICH harmonized tripartite guideline regarding good clinical practice (GCP) adopted by the European Agency for the Evaluation of Medicinal Products (EMEA). In addition, all local regulatory requirements were adhered to, in particular, those that afford greater protection to the safety of the study participants.

1.3. Informed Consent

Before screening procedures, the IPRC staff informed the subjects, in nontechnical terms, of the objectives, dates, drugs, diet, potential risks, and general activities during the clinical part of the study. The informed consent forms (ICFs) were carefully read before signing. Any questions were discussed in detail with the IPRC staff. Special emphasis was placed on the adherence of subjects to the study protocol and on the possible adverse event (AEs). At the end of consent procedures, each subject received a copy of the ICF, a sample of which is enclosed in the section "Sample ICF" (Appendix A.4).

1.4. Justification of the Study

Since drug formulation plays a key role in drug absorption, variations are expected from one formula to another for the same particular drug. Moreover, drug pharmacodynamics can be affected by its pharmacokinetics, which is invariably influenced by drug product formulation. All these necessitate the need for a biometric tool to prove the drug pharmaceutical equivalence or bioequivalency. Accordingly, the interchangeable use of bioequivalent products is justified and should afford the same therapeutic efficacy.

2. INTRODUCTION

This study was performed to investigate the bioequivalence of metformin between a generic test product Dialon [1000 mg metformin hydrochloride per tablet; Gulf Pharmaceutical Industries (Julphar), U.A.E.], and reference product Glucophage® (1000 mg metformin hydrochloride per tablet; Merck, France). The study protocol called for 24 healthy volunteers. The subjects received one tablet of each product, Dialon and Glucophage (1000 mg metformin hydrochloride per tablet), in a randomized fashion with a washout period of one week. Twenty-four healthy male volunteers plus four alternates completed the crossover. The bioanalysis of clinical plasma samples was accomplished by validated chromatographic method, which was developed and validated in accordance with the international guidelines at IPRC. Pharmacokinetic parameters, determined by standard noncompartmental methods, and analysis of variance (ANOVA) statistics were calculated using Kinetica™ 2000 statistical software. The significance of a sequence effect was tested using the subjects nested in sequence as the error term. The 90% confidence intervals for the ratio (or difference) between the test and reference product pharmacokinetic parameters of $AUC_{0\to t}$ and $AUC_{0\to\infty}$ were calculated and found to be within the 80.00% to 125.00% and for C_{max} to be within 75.00% to 133.00% confidence limits.

TABLE 1 Bioequivalence Confidence Intervals of Metformin (Dialon Tablet, the Test Product vs. Glucophage Tablet, the Reference Product)

Pharmacokinetic parameter	90% Confidence intervals of parametric means		
	Point estimate (%)	Lower limit (%)	Upper limit (%)
C_{max}	108.07	100.61	116.09
$AUC_{0\rightarrow t}$	101.57	96.00	107.46
$AUC_{0\rightarrow \infty}$	101.66	96.39	107.21

TABLE 2 Pharmacokinetics Parameters of Metformin (Dialon Tablet, the Test Product vs. Glucophage Tablet, the Reference Product)

Pharmacokinetic parameter	Treatment (mean $\pm$ SD)	
	Test product	Reference product
C_{max} (ng/mL)	1575 $\pm$ 420.11	1435 $\pm$ 299.57
t_{max} (hr)	2.44 $\pm$ 0.89	2.73 $\pm$ 1.13
$AUC_{0\rightarrow t}$ (ng hr/mL)	10561.8 $\pm$ 3057.45	10268.2 $\pm$ 2448.62
$AUC_{0\rightarrow \infty}$ (ng hr/mL)	10980.0 $\pm$ 3089.05	10681.6 $\pm$ 2479.11
K_e (1/hr)	0.1660 $\pm$ 0.03	0.1732 $\pm$ 0.04
$t_{(1/2)e}$ (hr)	4.29 $\pm$ 0.75	4.22 $\pm$ 1.02
$(AUC_{0\rightarrow t}/AUC_{0\rightarrow \infty})\%$	95.93 $\pm$ 1.49	96.01 $\pm$ 0.93

In conclusion, the study demonstrated that the test product, Dialon tablet [Gulf Pharmaceutical Industries (Julphar), U.A.E.], 1000 mg metformin hydrochloride per tablet, is bioequivalent to the reference product, Glucophage tablet (Merck, France) 1000 mg metformin hydrochloride per tablet, as summarized by Tables 1 and 2.

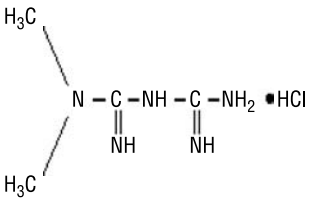
The report was issued in consensus with the ICH guidelines concerning the structure and content of the clinical study reports adopted by EMEA.

2.1. History and Source of Metformin

Metformin is an oral antihyperglycemic drug used in the management of type II diabetes. Metformin hydrochloride (*N*,*N*-dimethylimidodicarbonimidic diamide hydrochloride) is not chemically or pharmacologically related to any other classes of oral antihyperglycemic agents.

2.2. Chemistry

The structural formula of metformin is shown below:



Metformin hydrochloride is a white to off-white crystalline compound with the molecular formula of $C_4H_{11}N_5 \cdot HCl$ and a molecular weight of 165.63. Metformin hydrochloride is freely soluble in water and is practically insoluble in acetone, ether, and chloroform. The pK_a of metformin is 12.4. The pH of a 1% aqueous solution of metformin hydrochloride is 6.68.

2.3. Pharmacology

Metformin is an antihyperglycemic agent that improves glucose tolerance in patients with type II diabetes, lowering both basal and postprandial plasma glucose. Its pharmacologic mechanisms of action are different from other classes of oral antihyperglycemic agents. Metformin decreases hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization. Unlike sulfonylureas, metformin does not produce hypoglycemia in either patients with type II diabetes or normal subjects (except in special circumstances) and does not cause hyperinsulinemia. With metformin therapy, insulin secretion remains unchanged while fasting insulin levels and daylong plasma insulin response may actually decrease.

2.4. Pharmacokinetics

2.4.1. Absorption and Bioavailability

The absolute bioavailability of a metformin 500-mg tablet given under fasting conditions is approximately 50% to 60%. Studies using single oral doses of metformin 500 to 1500 and 850 to 2550 mg indicate that there is a lack of dose proportionality with increasing doses, which is due to decreased absorption rather than an alteration in elimination. Food decreases the extent of and slightly delays the absorption of metformin, as shown by approximately a 40% lower mean peak plasma concentration (C_{max}), a 25% lower area under the plasma concentration versus time curve (AUC), and a 35-minute prolongation of time to peak plasma concentration (t_{max}) following administration of a single 850-mg tablet of metformin with food, compared to the same tablet strength administered fasting. The clinical relevance of these decreases is unknown. Peak plasma levels are approximately 0.6, 1.1, 1.4, and 1.8 μg/mL for 500, 1000, 1500, and 2000 mg once-daily doses, respectively.

2.4.2. Distribution

The apparent volume of distribution (V/F) of metformin following single oral doses of 850 mg averaged 654 ± 358 L. Metformin is negligibly bound to plasma proteins, in contrast to sulfonylureas, which are more than 90% protein bound. Metformin partitions into erythrocytes, most likely as a function of time. At usual clinical doses and dosing schedules, steady state plasma concentrations of metformin are reached within 24 to 48 hours and are generally $<$ 1 mg/mL. During controlled clinical trials, maximum metformin plasma levels did not exceed 5 mg/mL, even at maximum doses.

2.4.3. Metabolism and Elimination

Intravenous single-dose studies in normal subjects demonstrate that metformin is excreted unchanged in the urine and does not undergo hepatic metabolism (no metabolites have been identified in humans) nor biliary excretion. Renal clearance is approximately 3.5 times greater than creatinine clearance, which indicates that tubular secretion is the major route of metformin elimination. Following oral administration, approximately 90% of the absorbed drug is eliminated via the renal route within the first 24 hours, with a plasma elimination half-life ($t_{(1/2)e}$) of approximately 6.2 hours. In blood, $t_{(1/2)e}$ is approximately 17.6 hours, suggesting that the erythrocyte mass may be a compartment of distribution.

2.5. Therapeutic Uses

Metformin is indicated as an adjunct to diet and exercise to improve glycemic control in patients with type II diabetes. Metformin may be used concomitantly with a sulfonylurea or insulin to improve glycemic control in adults (17 years of age and older).

2.6. Adverse Events

Most commonly reported adverse effects for metformin are diarrhea, nausea/vomiting, flatulence, asthenia, indigestion, abdominal discomfort, and headache. And less common

adverse reactions were reported as abnormal stools, hypoglycemia, myalgia, light-headedness, dyspnea, nail disorder, rash, sweating increased, taste disorder, chest discomfort, chills, flu syndrome, and palpitation.

3. INVESTIGATION

3.1. Investigators and Study Administrative Structure

The clinical part of the study was performed in IPRC (Amman, Jordan) under the supervision of Naji Najib, the Principal Investigator; and Usam Harb, M.D., Clinical Investigator. The calculations of the pharmacokinetics and statistical evaluation of data were performed at IPRC. Data entry was performed by Lara AL-Zaghari (B.Sc. Pharm.) and the results were authorized by Prof. Naji Najib. Bioanalysis was performed at IPRC using the in-house developed and validated method under the supervision of Mohammad Bader (B.Sc. Chem.), HPLC Manager. The final report of the study was authored by Lara AL-Zaghari (B.Sc. Pharm.). The quality assurance unit (QAU) was entirely involved in auditing and checking, throughout the study conduction and completion.The curriculum vita of each investigator and coinvestigator is enclosed in the section "Curriculum Vitae of the Investigators and Coinvestigators" (Appendix A.5), "Curriculum Vitae." In addition to IPRC's clinical staff, nurses were present in both periods I and II, and were assigned their responsibilities under the supervision of IPRC's Clinical Investigator.

3.2. Study Objectives

In this study, the bioavailability of a single dose of one tablet (1000 mg) of Gulf Pharmaceutical Industries (Julphar) (Dialon) and Merck (Glucophage), 1000 mg metformin hydrochloride per tablet, were compared under fasting conditions. Bioequivalence was investigated by determining the 90% confidence limits for the log-transformed ratio (test product/reference product) for the bioequivalence parameters (C_{max}, $AUC_{0\to t}$, and $AUC_{0\to\infty}$), while other pharmacokinetic parameters of K_e, $t_{(1/2)e}$, t_{max}, and $(AUC_{0\to t}/AUC_{0\to\infty})\%$ were reported. The influence of sequence, product, and period effect was tested by ANOVA.

3.3. Investigational Plan

This study was a single-center, open-label, randomized, single-dose study with two-way crossover design to compare the bioavailability of metformin between two products, in 24 healthy (one to four) alternates adult, male volunteers.

The study was conducted according to ICH GCP guidelines adopted by EMEA. For all the steps carried out in this study, IPRC has written standard operating procedures (SOPs), of which IPRC personnel have control of the training on and the use of the SOPs. The IRB of IPRC, Amman, Jordan, reviewed the study protocol and approval was given on 26/10/2004 (see section "Approval of the IRB" in Appendix A.2). The clinical part of the study was initiated at IPRC, by the first screening examination on 17/11/2004. After the screening examination, subjects were sequenced according to a preassigned randomization plan. The first administration of the study drug, as well as, the first blood collection for drug analysis took place on 23/11/2004. A washout period of one week between the two study drug administrations was allowed. The last study drug administration took place on 30/11/2004, while the last blood collection for drug analysis took place on 01/12/2004. Blood sampling per each study period was carried out as per sampling schedule (Fig. 1).

The clinical study site facilities were designed and equipped appropriately to accommodate all running activities of the study. A detailed description of the study site facilities is mentioned under "Description of Study Facilities."

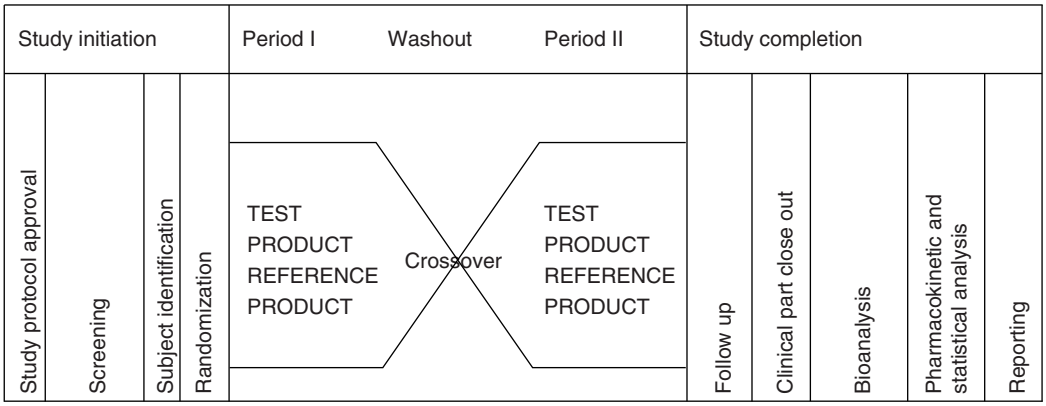


FIGURE 1 Study design and plan.

3.4. Rationale of Study Design

Bioequivalence evaluation is usually carried out by comparing the in vivo rate and extent of drug absorption of a test and reference formulation in healthy subjects. In a standard in vivo bioequivalence study design, study participants received test and reference products on separate occasions, in single dose, with random assignment to the two possible sequences of product administration. Samples of plasma were analyzed for drug concentrations, and pharmacokinetic parameters were obtained from the resulting concentration–time curves. These pharmacokinetic parameters were then analyzed statistically to determine whether the test and reference products yielded comparable values. Standard statistical methodology based on the two one-sided tests procedure to determine whether average values for pharmacokinetic parameters measured after administration of the test and reference products are comparable. This procedure involves the calculation of a 90% confidence interval for the ratio (or difference) between the test and reference product pharmacokinetic variable averages. The limits of the observed confidence intervals were within a predetermined range for the ratio (or difference) of the product averages. The determination of the confidence interval range and the statistical level of significance based on parametric (normal theory) standard noncompartmental procedures was employed for the analysis of pharmacokinetic data derived from in vivo bioequivalence studies. An ANOVA was performed on the pharmacokinetic parameters to assess the effect of variables [subject (sequence), subject, period, and formulation] on the study outcome. On the basis of these considerations, a single-dose, two-treatment, two-period, two-sequence crossover bioequivalence study on healthy normal subjects was adopted. The study was conducted in preplanned scheme, as depicted in Table 3 below.

3.5. Selection of Study Population

For participation in the study, subjects had to meet the selection criteria outlined in the study protocol. Volunteers were informed, by IPRC representative, about the aim of the study and any potential risk associated with the study. Volunteers signed a written informed consent statement after which they were run in the study, and they were free to withdraw at any time during the course of the study.

3.5.1. Study Subjects Demography

The following demographic data for each subject were obtained:

- Volunteer name, age, height, weight, date of birth, race, medical history, and vital signs.
- Complete physical examination and neurological assessment.
- Urine analysis and blood (hematology, biochemistry, and serology).

TABLE 3 Study Schematic

	Study period[a]			
Procedure	**Screening[b]**	**Period I**	**Period II**	**Follow-up[c]**
Subject identification	☐	☐	☐	☐
Informed consent[d]	☐			
Demographic data	☐			
Selection criteria[e]	☐			
Study drug administration		☐	☐	
Medical history	☐			
Physical examination	☐			☐
Vital signs	☐	☐	☐	☐
Hepatitis B	☐			
Hematology	☐			
Biochemistry	☐			
Urinalysis	☐			
Check for other medication	☐	☐	☐	
Blood sampling for pharmacokinetics		☐	☐	
Check for adverse effect		☐	☐	☐

[a] There is a washout period of seven days between two administrations of study drugs.
[b] Between 30 days and 24 hours, before first study drug administration in study period I.
[c] Follow-up is to be done within at least 24 hours of last blood sample.
[d] Before screening examination, the subject has to sign the informed consent form.
[e] To be eligible for participation in the study, subjects must meet all selection criteria before the first study drug administration in study period I is established.

3.5.2. Inclusion criteria

In summary, to be eligible for participation in the study, subjects should have met the following criteria before their enrollment in the study:

1. Age 18 to 45 years, inclusive.
2. Body weight within 15% of ideal weights for height and weight.
3. Medical history, vital signs, physical examination (including neurological assessment), without evidence of clinically significant deviation from normal medical condition, performed not longer than two weeks before the initiation of the clinical study.
4. Results of laboratory tests are within the normal range or deviation is not considered clinically significant by the Clinical Investigator and the Principal Investigator. (Laboratory tests are performed not longer than one month before the initiation of the clinical study.)
5. Subject does not have allergy to the drugs under investigation.

3.5.3. Exclusion Criteria

1. Medical history and/or physical examination with evidence of clinically significant deviation from normal medical condition.
2. Results of laboratory tests, which are clinically significant.
3. Acute infection within one week preceding first study drug administration.
4. History of drug or alcohol abuse.
5. Subject is a heavy smoker (more than 10 cigarettes per day).
6. Subject does not agree not to take any prescription or nonprescription drugs within two weeks before first study drug administration until the end of the study.
7. Subject does not agree not to take any vitamins taken for nutritional purposes within two days before first study drug administration until the end of the study.

8. Subject is on a special diet (e.g., subject is vegetarian).
9. Subject consumes large quantities of alcohol or beverages containing methylxanthines, e.g., caffeine, tea, cola, chocolate.
10. Subject does not agree not to consume any beverages or foods containing alcohol 48 hours prior to study drug administration until donating the last sample in each respective period.
11. Subject does not agree not to consume any beverages or foods containing methylxanthines, e.g., caffeine (coffee, tea, cola, chocolate, etc.) 48 hours prior to the study drug administration of either study period until donating the last sample in each respective period.
12. Subject does not agree not to consume any beverages or foods containing grapefruit seven days prior to first study drug administration until donating the last sample in each respective period.
13. Subject has a history of severe diseases that have direct impact on the study.
14. Participation in a bioequivalence study or in a clinical study within the last three months before first study drug administration.
15. Subject intends to be hospitalized within three months after the first study drug administration.
16. Subjects who, through completion of this study, would have donated more than 500 mL of blood in 14 days, 750 mL in 30 days, 1000 mL in 90 days, 1250 mL in 120 days, 1500 mL in 180 days, 2000 mL in 270 days, and 2500 mL in 1 year.

3.6. Subjects Identification

During screening subjects were identified solely by their initials. During admission for period I, participating subjects were assigned numbers in sequential order. The subjects retained their numbers for the duration of the study. For subsequent data processing and reporting, subjects were identified only using their numbers and initials.

3.7. Case Report Form Note

All data of the clinical part of the study were documented in case report forms (CRFs) by the staff of the IPRC. The Principal Investigator checked correct completion of the CRFs. A sample CRF is enclosed in the section "Sample CRF" (Appendix A.3). IPRC performed quality assurance of CRFs' data entry by comparison with source records.

3.8. Confinement

According to the study protocol in each study period, the subjects were admitted to the study site in the evening before study drug administration on study day 1 of each study period and confined until the 24-hour blood sample was collected. Subject returned to donate the last samples.

3.9. Removal of Subjects from Study

Each subject had the right to withdraw from the study at any time without jeopardy or prejudice. The Principal Investigator and the Clinical Investigator have the right to discontinue the subjects' participation if they felt it is necessary, for any reason including AEs or failure to comply with the study protocol.

When a subject withdrew from the study, the reasons were stated on the CRF and a final evaluation of the subject was performed.

Subjects' withdrawal may be divided into three groups as follows:

Withdrawal group 1: Withdrawal after screening procedures have been performed but before study drug administration in study period I.

Withdrawal group 2: Withdrawal after study drug administration in study period I but before study drug administration in study period II.

Withdrawal group 3: Withdrawal after study drug administration in study period II but before last sample collection in study period II.

3.10. Dietary Restrictions, Standardized Diet, and Fluid Intake

No consumption of alcohol was permitted for the subjects 48 hours prior to the study's drugs administration until the collection of the last sample of the respective study period. No consumption of any beverages or foods containing methylxanthines, e.g., caffeine (coffee, tea, cola, cocoa, chocolate, etc.) was permitted for the subjects 48 hours prior to the study's drugs administration until the collection of last blood sample of the respective study period. In addition, the consumption of any beverages or foods containing grapefruit was prohibited one week before the first study's drugs administration and through out the entire study.

Food and fluid intake were identical in both study periods, starting from the dinner served 10 hours before study's drugs administration on study day 1 until the end of confinement. Meals were standardized in composition and amount in both periods. The subjects were not allowed to consume any additional beverages or foodstuffs other than those provided through out the period of confinement. The subjects received their standardized meals as shown in Table 4.

Details of the diet's composition are provided in Appendix B.6 (not included for brevity).

No excessive fluid intake (>120 mL of water per hour) was allowed from 1 to 10 hours prior to dosing. From one hour before study's drugs administration to two hours after, no fluid intake was allowed apart from the 240 mL of water used for the administration. Following the study's drugs administration, the subjects were served 100 mL of 10% glucose solution at 0.50, 1.50, 2.00, 2.50, 3.00, 5.00 hours. Following the four hours, subjects were allowed to drink water but not exceeding 120 mL per hour.

3.11. Study Drug Administration

On study day 1 of each study period, the study drugs were administered according to a randomization plan (see section "Randomization Plan" in Appendix A.6). The administration of the study drugs was documented in the drug administration form.

Study drugs were administered by the clinical staff of IPRC as follows:

Treatment A: One tablet of Dialon, test product, 1000 mg metformin hydrochloride per tablet, was given with 240 mL which was at room temperature and was measured with a 250 mL cylinder.

Treatment B: One tablet of Glucophage, reference product, 1000 mg metformin hydrochloride per tablet, was given with 240 mL which was at room temperature and was measured with a 250 mL cylinder.

3.12. Identity of Study Medications

Identification	Test product, treatment A	Reference product, treatment B
Brand name	Dialon®	Glucophage®
Dosage form	Tablet	Tablet
Strength	1000 mg metformin hydrochloride per tablet	1000 mg metformin hydrochloride per tablet
Manufacturer	Gulf Pharmaceutical Industries (Julphar), U.A.E.	Merck, France
Batch number	0022	108940
Expiry date	08/06	01/06

TABLE 4 Standardized Diets Served During the Study

Study day	Standardized diet	Time received
−1	Dinner	Finished at least 10 hr before the scheduled time of study drug administration in the morning of study day 1
1	Lunch	4 hr after study drug administration
1	Dinner	12 hr after study drug administration

3.13. Assignment of Study's Subjects and Randomization

The study was randomized as a two-way two-sequence crossover design. Administration was done according to a plan of randomization (see section "Randomization Plan" in Appendix A.6).

3.14. Times of Dosing

The first administration of the study drugs took place on 23/11/2004. And the last study's drugs administration took place on 30/11/2004. The study's drugs administration took place between 8.00 and 8.54 hours for both periods in the morning of study day 1 of each study period. For detailed information about date and time of study drug administration, see Appendix B.5.

3.15. Treatment Compliance

Visual inspection of the subject's hands and mouth was immediately done after the study's drugs administration to ensure that the subject did swallow the drug.

3.16. Physical Activities After Drug Intake

After the study's drugs administration, the subjects remained ambulatory. Their activity was restricted to talking, watching television, or reading until the four-hour blood collection (except for the scheduled time of blood sampling and going to the toilet). The blood collection rooms and the toilets were on the same floor. The study personnel took great care to ensure that the physical activity of the subjects was identical in study day 1 of each study period.

3.17. Prior and Concurrent Medication

According to the study's protocol, no prescription medication or nonprescription medication was to be taken starting two weeks before the first study's drugs administration until the end of the study (collection of the last sample of period II).

3.18. Clinical Laboratory

IPRC clinical laboratory, Amman, Jordan, performed the safety laboratory investigations (hematology, biochemistry, urinalysis, and serology).

3.19. Description of Study Facilities

The clinical site of IPRC is set in two floors of the IPRC building. Each floor is designed to withstand a full-scale study completely sealed from the remaining running activities in the IPRC building. The following rooms were used for this study:

3.19.1. Room for Prestudy Examinations

All prestudy examination took place in a separate room. This room was equipped with a blood pressure monitor, stethoscope, scale, etc. and all the necessary materials. All equipment necessary for handling of the blood and urine samples were available.

3.19.2. Clinical Investigator's Office

Here the Clinical Investigator carries out all the physical examinations of the study's subjects either by screening or follow-up.

3.19.3. Room for the Administration of the Study Drugs, Vital Signs, and Blood Collection

In this room, cannula insertion, study's drug administration, as well as blood collection was carried out. For each sampling time, a nurse was delegated for the responsibility of blood collection at that time. The volunteers were called into the room at the assigned collection time and had their blood drawn. Similarly, a nurse was delegated for taking the vital signs at the

assigned time and the volunteers were called in to have their vital signs taken. A clock and all equipment necessary for blood collection from the volunteers were available.

3.19.4. Pharmacy

The study's drug was stored in sealed containers in a closed cabinet in the pharmacy located on the first floor of the IPRC building. All necessary storage conditions were taken into account in handling and dispensing of the study's drug. Temperature and humidity monitoring were carried out as appropriate. A data logger placed in the drug cabinet records the temperature and humidity level in the cabinet.

3.19.5. Emergency Room

Located on the first floor and equipped with all the necessary equipment needed in case of an emergency and drugs necessary for treatment of serious adverse events were available. In addition to IPRC's emergency room, IPRC has signed an agreement with the Arab Medical Centre for the use of its available emergency unit to handle emergencies that may occur during the study. The Arab Medical Centre emergency unit supervisor has been well informed of the study's nature, including the study's drug (strength and dose to be used), number of involved subjects, and dates of admission to each study period.

3.19.6. Subject's Rooms

Here subjects spend most of the study's time, watching TV, or reading except when they are called for blood sampling or vital signs measurements.

3.19.7. Kitchen

Food was prepared and stored in the kitchen situated in the basement. The meals were always stored in locked meal trolleys. The kitchen was equipped with a cupboard for the dishes, an oven, fridge, freezer, and a washbasin with running hot and cold water for cleaning up.

3.19.8. Dormitories

Each of the two floors of the clinical site has one room furnished with all the necessary beds, sheets, linen, and pillows. The study's subjects slept in these rooms.

3.19.9. Dining Rooms

One room was used for dining. This room is on the same floor of the study.

3.19.10. Bathrooms/Toilets

Subjects could use any of the four bathrooms present (but preferably the one in their respective room for good control on study subjects). These contained a washbasin, toilet, and a shower facility.

3.20. Collection and Handling of Blood Samples for Analysis

In the morning of study day 1 of each study period and before study's drugs administration, a cannula was inserted into the subject's forearm vein and it remained there until the 24-hour blood sample was collected.

The volume of blood taken for the determination of metformin in plasma was 8 mL per sample. The following blood samples for the analysis of metformin in plasma were collected:

Immediately two before [(1×8 mL)+(1×8 mL)] and at 0.33, 0.66, 1.00, 1.33, 1.66, 2.00, 2.50, 3.00, 3.50, 4.00, 5.00, 6.00, 8.00, 10.00, 12.00, 16.00, 24.00, and 36.00 hours (1×8 mL) after administration of study's drugs. The number of blood collections for drug analysis was 20 samples in each study period.

Blood samples were collected into tubes containing lithium heparin as an anticoagulant (Dispo®, AFMA, Jordan), slightly shaken, and centrifuged at approximately 3500 r.p.m. for 10 minutes. After centrifugation, plasma samples were transferred directly into a 5 mL-plastic tubes (Dispo, AFMA, Jordan). These samples were immediately stored at the study site in a freezer at a nominal temperature of −20°C. The label of the collecting tubes had the study's

code number, subject number, study period, and the designated sample number. It did not contain information that would allow identifying the given treatment. This assured that the analysts at IPRC analyzed the samples blindly. The total amount of blood loss during the whole study (including blood for laboratory tests) did not exceed 330 mL.

[(8 mL×18 blood collection times)+(8 mL×1)+(8 mL×1) for predose samples]×2 study periods and 1×10 mL for laboratory tests.

3.21. Bioanalytical Drug Determination Methodology

An high-performance liquid chromatography (HPLC) assay was developed at the IPRC for the determination of metformin in human plasma. Samples from the first 24 subjects (who completed both periods of the study) were analyzed. The bioanalytical method was validated according to the international guidelines. Details of the validation of the assay procedure are given in the section "Bioanalytical Report" (Appendix A.8).

3.22. Data Quality Assurance

The IPRC's quality assurance procedures were implemented to assure the built-in quality system. All data entry was done by the trained staff of IPRC and checked by the QAU personnel. All procedures were performed according to the internal IPRC-approved SOPs with the results being documented and reported.

Deliberately, all in-use manuals were archived by the QAU. All sheets used to document results were issued and approved by the QAU serially, and ultimately reserved in the QAU. Logbooks were audited internally by the IPRC QAU personnel during the internal audit of both the clinical part and the analytical part of the study. All laboratory (clinical and analytical) results were checked and their source documents retained by the QAU. Source document verification was done by the QAU after each data entry. Instrumental outputs after calculations were checked by the QAU personnel. Necessary actions were taken and corrective and/or preventive measures were recommended. A report after each audit period was delivered to the IPRC management. Report of audits were followed up and reserved by the QAU. The QAU implements an internal quality system to keep all essential records related to the study guaranteeing the appropriate authorized direct access and traceability of data with utmost confidentiality. All audit trails were enabled within the operated software.

After the study report preparation, the QAU audited the report and released its quality assurance statement, which evidenced each audit task.

3.23. Pharmacokinetic Calculations

Under the direction of Prof. Naji Najib, the pharmacokinetic parameters of metformin were estimated using standard noncompartmental methods. The maximal plasma concentration (C_{max}) and the time to peak plasma concentration (t_{max}) of metformin were taken directly from the measured data.

The area under the plasma concentration–time curve ($AUC_{0\to t}$) was calculated from measured data points from the time of administration to the time of last quantifiable concentration (C_{last}) by the linear trapezoidal rule.

The area under the plasma concentration–time curve extrapolated to infinity ($AUC_{0\to\infty}$) was calculated according to the following formula:

$$AUC_{0\to\infty} = AUC_{0\to t} + C_{last}/[\ln(2)t_{(1/2)e}],$$

where C_{last} is the last quantifiable concentration.

The ratio $AUC_{0\to t}/AUC_{0\to\infty}$ as a percent was determined as an indicator for the adequacy of sampling time.

The $t_{(1/2)e}$ was calculated as $t_{(1/2)e} = \ln(2)/(-b)$, where b was obtained as the slope of the linear regression of the ln-transformed plasma concentrations versus time in the terminal period of the plasma curve.

The pharmacokinetic calculations were performed on a Pentium MMX MHz computer using the computer program Kinetica 2000.

3.24. Statistical Analysis

Statistical analysis was performed by the Kinetica 2000 program, with the aid of Microsoft® Excel (2002).

3.24.1. Confidence Intervals

The extent of absorption is determined by $AUC_{0\to t}$ and $AUC_{0\to\infty}$. The rate of absorption is determined by C_{max}. For the parametric analysis of bioequivalence for log-transformed data, the acceptance boundaries were set at 80.00% to 125.00% for $AUC_{0\to t}$ and $AUC_{0\to\infty}$ and for C_{max} to be within 75.00% to 133.00%.

A multiplicative model with respect to the untransformed bioequivalence parameters was selected. A logarithmic transformation of the original data was used. Under the assumption of a logarithmic normal distribution, a parametric approach recommended by Steinijans and Diletti based on the inclusion of the shortest 90% confidence interval in the bioequivalence range was adopted.

3.24.2. ANOVA

An ANOVA tested for sequence, period, subject (sequence), and treatment effects were used. ANOVA was performed on $AUC_{0\to t}$, $AUC_{0\to\infty}$, C_{max}, t_{max}, $t_{(1/2)e}$, K_e, $\log AUC_{0\to t}$, $\log AUC_{0\to\infty}$, and $\log C_{max}$.

A multiplicative linear model was used for the two-way crossover design.

$$Y_{ijk} = \log(X_{ijk}) = \mu + G_k S_{ik} + P_j + F(j,k) + e_{ijk}$$

where Y_{ijk}, a pharmacokinetic parameter of the ith subject ($i=1,2,\ldots n_k$) in the sequence (1,2,…n) for the jth period ($j=1,2,\ldots p$); μ, the overall mean; G_k, the fixed effect of the kth sequence; S_{ik}, the random effect of the ith subject in the kth sequence; P_j, the fixed effect of the jth period; F (j,k), the fixed effect of the formulation in the kth sequence, which is administered at the jth period; e_{ijk}, the (within-subject) random error in observing Y_{ijk}.

It was assumed that $\{S_{ik}\}$ and $\{e_{ijk}\}$ are mutually independent and normally distributed with mean zero and variances σ_S^2 and σ_e^2.

3.24.3. Sample Size Determination

Confidence interval approach and Schuirmann's two one-sided tests procedure for interval hypotheses were used. Sample size calculation based on the power of Schuirmann's two one-sided t-tests procedure for interval hypotheses using the ± 20 rule for the assessment of average bioequivalence was reported.

3.25. Data Tabulation, Descriptive Statistics, and Diagrammatic Data Presentation

All results and diagrammatic data presentation are depicted in the section labeled, Tables and Figures. (For conservation of space, these tables and figures are not included in the book.)

4. STUDY SUBJECTS

4.1. Disposition of Subjects

Twenty-four healthy male subjects plus four alternates were recruited according to the selection criteria described in the study protocol and volunteered for participation in the study. All participating subjects were treated as a single group:

Group	Number of subjects	Study day 1 of study period I	Study day 1 of study period II
1	1–28	23/11/2004	30/11/2004

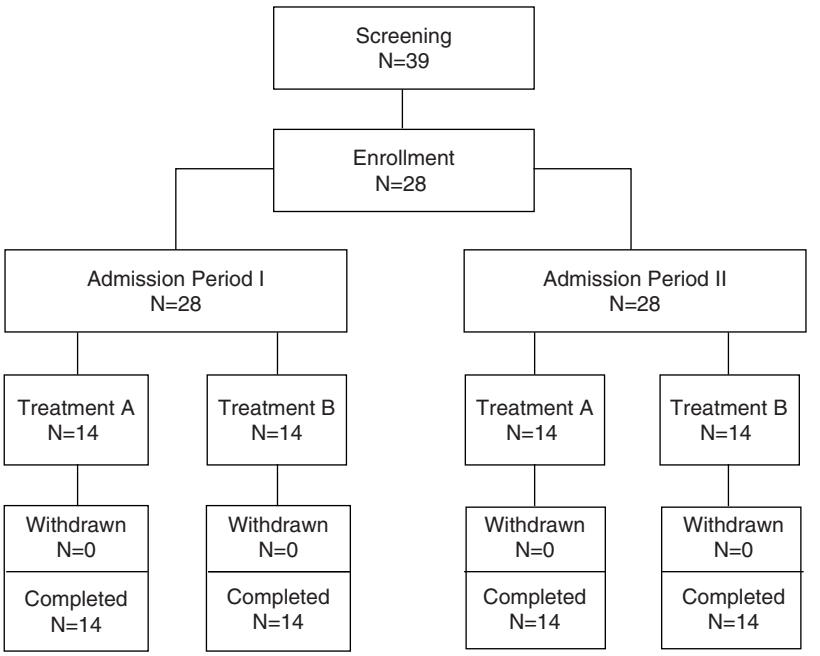


FIGURE 2 Disposition of subjects.

Each subject was examined thoroughly during screening procedures as described in the study protocol (the screening time being set to be between 1 month and 24 hours prior to the first study drug administration of study period I). Twenty-four subjects plus four alternates were enrolled and completed the study period II (Fig. 2).

4.2. Withdrawals and Exclusions

During this study, 39 subjects were screened. Two subjects withdrew for significant variation of their lab results, 11 subjects withdrew for personal reasons and two subjects withdrew for protocol violation. A total of 28 subjects were enrolled in period I and have completed both periods of the study. For details on withdrawals, see Appendix B.1.

4.3. Demographic Characteristics

The demographic characteristics of the 24 subjects, who completed the study and included in the pharmacokinetic analysis, were as follows:

- Age ranging between 18 and 36 years (26 ± 4.96 years).
- Weight at screening examination between 56 and 87 kg (70 ± 8.84 kg).
- Height between 160 and 179 cm (172 ± 4.41 cm).

For detailed information about the demographic data obtained for the 28 volunteers at screening examination, see Appendix B.3.

4.4. Variations from the Study Protocol

Slight variations from the study protocol concerning the clinical laboratory tests were observed (a list of these variations is found in Appendix B.2. "Impact of the Variations from the Study Protocol on the Study Outcome"). These variations were judged by the Principal Investigator to be insignificant.

5. SAFETY EVALUATION

The study was performed according to ICH GCP guidelines under the direction of the Clinical Investigator. There were no significant deviations from the study protocol that could have affected the outcome of this study. All subjects met the inclusion criteria described in the study protocol. Foreseeable risks were weighed before study initiation. Rights, safety, and well-being of the study subject were considered the most important issues, prevailing over interests of science and society. All medical care and medical decisions were given on behalf of the subjects under the full supervision of the Principal Investigator. All the subjects were in good health before the initiation of the study. The clinical results of the screened laboratory examinations (biochemistry, hematology, serology, and urine analysis) were, occasionally, outside their respective normal ranges but not to an extent to be considered clinically significant by both the Clinical Investigator and the Principal Investigator.

5.1. Benefit-to-Risk Ratio

Adverse effects encountered during the study were very minimal. The study outcome will help ensure safe and clinically reliable management of diabetes mellitus therefore benefiting society by lowering treatment costs. The drug is a prescribed medication; we therefore conclude that in view of the small risks involved, it was significant to perform this study.

5.2. Extent of Exposure

During this study, 24 subjects plus 4 alternates volunteered. The study is designed as a single-dose two-way crossover. Thus, the risk to a healthy volunteer taking two oral doses each containing 1000 mg metformin hydrochloride from the two products (Dialon and Glucophage) with a seven-day interval is minimal.

5.3. Adverse Events

5.3.1. Brief Summary of Adverse Events

The study's subjects were asked to inform the clinical staff of occurrence of any AEs immediately once experienced. Furthermore, the clinical staff was instructed to check on the subjects for the occurrence of any AE at specified time intervals (before dosing, 1.00, 2.00, 4.00, 7.00, 9.00, and 12.00 hours from study's drugs administration) and to notify immediately the Clinical Investigator.

The Clinical Investigator monitored closely the subjects for AE and took all necessary actions that he saw best in the subject's interest. None of the subjects dropped out from the study because of AEs. Appendix B.4 summarizes all AEs that occurred during the study.

5.3.2. Display of Adverse Events

During the study six subjects were reported to manifest AEs. The outcomes of all AEs were complete recovery.

5.4. Clinical Laboratory Evaluation

Medical histories and laboratory tests of hematology, hepatic and renal functions, and serology were all performed for each subject on screening examination. Only medically healthy subjects with clinically normal laboratory profiles were enrolled in the study. Physical examination was performed after completion of period II of the study.

5.5. Vital Signs, Physical Assessment, and Other Clinical Observations

Each subject received a thorough physical assessment, and vital signs evaluation (blood pressure, pulse, respiratory rate, and temperature) on screening examination. The subjects received the same physical assessment as well as the vital signs evaluation on follow-up examination, which was within 24 hours from collecting the last sample in period II.

5.6. Safety and Tolerance

Having completed the study, subjects underwent a thorough physical assessment on follow-up examination to assure their safety.

Clinical assessment for all subjects was carried out to evaluate their tolerability to the study's medications. Study subjects demonstrated good tolerance to the two study's drugs. See Appendix B.8. "Clinical Assessment for All Subjects."

6. RESULTS AND BIOEQUIVALENCE EVALUATION

6.1. Datasets from Study Subjects

Demographic data and all clinical assessment along with laboratory evaluation were performed for all enrolled subjects. However, for pharmacokinetic evaluations, the data from the first 24 subjects, who were crossed over and completed the balance design, were involved in the calculation.

6.2. Adjustment Due to Anomalies

6.2.1. Adjustment Due to Collection Anomalies

There were no collection anomalies reported for which adjustments to the datasets were deemed necessary.

6.2.2. Adjustment Due to Analytical Anomalies

Where necessary samples of concentrations above the upper limit of quantitation (ULOQ) of calibration curves were diluted.

6.2.3. Adjustment Due to Pharmacokinetic Anomalies

There were no pharmacokinetic anomalies for which adjustments to the datasets were considered necessary.

6.2.4. Nonzero Pre-dose Concentrations

There were no instances of nonzero pre-dose concentrations of the drug.

6.3. Handling of Withdrawals and Missing Data

No missing data were reported.

6.4. Pharmacokinetic Parameters

Drug plasma levels were designated as surrogate parameters to indicate clinical activity. Primary pharmacokinetic parameters were set to be C_{max}, $AUC_{0\to t}$, and $AUC_{0\to\infty}$, and were also considered to be the bioequivalence determinants. Finally, K_e, t_{max}, $t_{(1/2)e}$, and $(AUC_{0\to t}/AUC_{0\to\infty})\%$ were set as the secondary pharmacokinetic parameters.

6.5. Statistical Inferences

6.5.1. Bioequivalence Conclusion

The details of metformin results of this bioequivalence study are shown in Tables 6–8 in the Tables and Figures section (these tables and figures are not included in the book for brevity). Bioequivalence could be demonstrated for metformin within the prescribed 90% confidence interval of 80.00% to 125.00% for $AUC_{0\to t}$ and $AUC_{0\to\infty}$ and for C_{max} to be within 75.00% to 133.00% with respect to the parametric method on log-transformed data.

The test product, Dialon tablet [Gulf Pharmaceutical Industries (Julphar), U.A.E.; 1000 mg metformin hydrochloride per tablet], investigated in this study was shown to be bioequivalent with the reference product; Glucophage tablet (Merck, France; 1000 mg metformin hydrochloride per tablet). Plasma levels may be used as surrogate parameters for clinical activity. Therefore, the data obtained in this study prove, by appropriate statistical

methods, the essential similarity of plasma levels of metformin from the test product Dialon tablet [Gulf Pharmaceutical Industries (Julphar), U.A.E.] and from the reference product Glucophage tablet (Merck, France) suggesting equal clinical efficacy of these two products. The product, Dialon tablet developed by Gulf Pharmaceutical Industries (Julphar), U.A.E., may be used interchangeably with the reference product Glucophage tablet (Merck, France) that was shown to have an acceptable therapeutic efficacy.

6.5.2. ANOVA

ANOVA of log-transformed data for C_{max}, $AUC_{0\rightarrow t}$, and $AUC_{0\rightarrow\infty}$, and of the untransformed data for C_{max}, $AUC_{0\rightarrow t}$, $AUC_{0\rightarrow\infty}$, $t_{(1/2)e}$, K_e, and t_{max} demonstrated that sequence effect, product effect, and period effect for all bioequivalence metrics did not significantly influence the outcome of the study. ANOVA results obtained for each bioequivalence metric are located in the Tables and Figures section (for brevity, these tables and figures are not reproduced in the book). Further details may be found in the section "Pharmacokinetics and Statistical Outputs" (Appendix A.7).

7. DISCUSSION AND CONCLUSIONS

This study was a single-center, open-label, randomized, single-dose study with two-way crossover design to compare the bioavailability of metformin between two products, in 24 healthy, adult, male volunteers.

The results of this bioequivalence study showed the equivalence of the two studied products in terms of the rate of absorption as indicated by C_{max} and in terms of the extent of absorption as indicated by $AUC_{0\rightarrow t}$ and $AUC_{0\rightarrow\infty}$. The parametric 90% confidence intervals of the mean values for the test/reference ratio were in each case well within the bioequivalence acceptable boundaries of 80.00% to 125.00% for the pharmacokinetic parameters $AUC_{0\rightarrow t}$ and $AUC_{0\rightarrow\infty}$ and for C_{max}, to be within 75.00% to 133.00%.

ANOVA analysis on the log-transformed data, C_{max}, $AUC_{0\rightarrow t}$, and $AUC_{0\rightarrow\infty}$, and untransformed data for C_{max}, $AUC_{0\rightarrow t}$, $AUC_{0\rightarrow\infty}$, K_e, $t_{(1/2)e}$, and t_{max} showed that sequence effect, product, or period effect for all these parameters did not significantly influence the outcome of the study. The mean plasma curves of both products are almost superimposable, suggesting that not only C_{max} and AUC but also the time course of plasma levels over the whole sampling period are identical.

Since plasma levels are a meaningful surrogate for pharmacodynamic action and AEs, this demonstrates that an equivalent therapeutic activity and tolerance is to be expected from Dialon tablet [Gulf Pharmaceutical Industries (Julphar), U.A.E.] generic product when compared with Glucophage tablet (Merck, France), the reference product.

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APPENDIX A

A.1. COMPARATIVE BIOAVAILABILITY STUDY PROTOCOL

A.2. APPROVAL OF THE IRB

A.3. SAMPLE CRF

Annex VI
Exhibit 1
To be used in conjunction with SOP: CLP-016 (Revision C)

Sugar Administration Form

(Applicable for hypoglycemic drugs only)
Study Name: 1000 mg metformin tablets

IPRC study code	**Subject number**	**Subject initials**

Study Period: I

A. Sugar Administration Schedule: (Orally)

Concentration of glucose: 10% **Volume: 100 mL**

	Theoretical time	**Actual time**	**Adminis-tered by**	**Date**
(0.00) hour after study drug administration	__:__ (hh:mm)	__:__ (hh:mm)		
(0.50) hour after study drug administration	__:__ (hh:mm)	__:__ (hh:mm)		
(1.50) hour after study drug administration	__:__ (hh:mm)	__:__ (hh:mm)		
(2.00) hour after study drug administration	__:__ (hh:mm)	__:__ (hh:mm)		
(2.50) hour after study drug administration	__:__ (hh:mm)	__:__ (hh:mm)		
(3.00) hour after study drug administration	__:__ (hh:mm)	__:__ (hh:mm)		
(5.00) hour after study drug administration	__:__ (hh:mm)	__:__ (hh:mm)		

II. Unscheduled Sugar Administration: (Orally)

Concentration of glucose: 20%

	Actual time	**Administered by**	**Date**
( ) hour after study drug administration	__:__ (hh:mm)		
( ) hour after study drug administration	__:__ (hh:mm)		
( ) hour after study drug administration	__:__ (hh:mm)		

III. Unscheduled Sugar Administration: (Intravenously)

Concentration of glucose: 20%

	Actual time	**Administered by**	**Date**
( ) hour after study drug administration	__:__ (hh:mm)		
( ) hour after study drug administration	__:__ (hh:mm)		

Clinical Investigator Signature: _____ Date: _____

Exhibit 1

To be used in conjunction with SOP: CLP-016 (Revision C)

Sugar Administration Form

(Applicable for hypoglycemic drugs only)

Study Name: 1000 mg metformin tablets

IPRC study code	**Subject number**	**Subject initials**

Study Period: II

B. Sugar Administration Schedule: (Orally)

Concentration of glucose: 10% **Volume: 100 mL**

	Theoretical time	**Actual time**	**Administered by**	**Date**
(0.00) hour after study drug administration	__:__ (hh:mm)	__:__ (hh:mm)		
(0.50) hour after study drug administration	__:__ (hh:mm)	__:__ (hh:mm)		
(1.50) hour after study drug administration	__:__ (hh:mm)	__:__ (hh:mm)		
(2.00) hour after study drug administration	__:__ (hh:mm)	__:__ (hh:mm)		
(2.50) hour after study drug administration	__:__ (hh:mm)	__:__ (hh:mm)		
(3.00) hour after study drug administration	__:__ (hh:mm)	__:__ (hh:mm)		
(5.00) hour after study drug administration	__:__ (hh:mm)	__:__ (hh:mm)		

II. Unscheduled Sugar Administration: (Orally)

Concentration of glucose: 20%

	Actual time	**Administered by**	**Date**
( ) hour after study drug administration	__:__ (hh:mm)		
( ) hour after study drug administration	__:__ (hh:mm)		
( ) hour after study drug administration	__:__ (hh:mm)		

III. Unscheduled Sugar Administration: (Intravenously)

Concentration of glucose: 20%

	Actual time	**Administered by**	**Date**
( ) hour after study drug administration	__:__ (hh:mm)		
( ) hour after study drug administration	__:__ (hh:mm)		

Clinical Investigator Signature: _____ Date: _____

A.4. SAMPLE ICF

A.5. CURRICULUM VITAE OF THE INVESTIGATORS AND COINVESTIGATORS

A.6. RANDOMIZATION PLAN

Subject number	Treatment	
	Study period I	Study period II
1	B	A
2	A	B
3	A	B
4	B	A
5	A	B
6	A	B
7	A	B
8	B	A
9	B	A
10	B	A
11	B	A
12	A	B
13	B	A
14	A	B
15	A	B
16	B	A
17	B	A
18	B	A
19	A	B
20	A	B
21	B	A
22	A	B
23	A	B
24	B	A
25	B	A
26	A	B
27	A	B
28	B	A

A, One tablet of the test product, Dialon, 1000 mg metformin hydrochloride per tablet; B, One tablet of the reference product, Glucophage®, 1000 mg metformin hydrochloride per tablet.

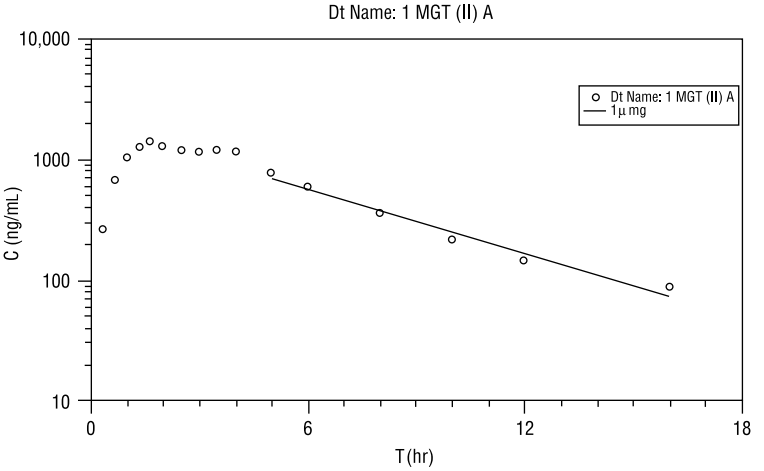
A.7. PHARMACOKINETICS AND STATISTICAL OUTPUTS

[NOTE: DATA FOR ONLY ONE SUBJECT IS DESCRIBED HERE]
Kinetica Report
H:\PK Analysis\Metformin\METF-GUL-T0904-339\MET-339.kdb
Kinetica Version 4.2
Saturday, December 18, 16 h 02 m 55 s 2004
Dataset name: 1 MGT (II) A
AUC * calculation
AUC0 option set to (c=0 when t=0)
c0 = 0
t0 = 0
The AUC extrapolation rule selected for C0 was:
AUC0 = (C0+Cmax)*Tmax/2 = (0+261)*0.33/2 = 43.065
AUMC0 = (Tmax*Cmax)*Tmax/2 = (0.33*261)*0.33/2 = 14.2115

With:
C0 = 0
Cmax = 261
Tmax = 0.33
The AUC was computed using Trapezoidal Method
AUCtot = AUClast+ CLast/Lz
From 5 to infinity (user defined interval),
curve may be approximated by : (with R = −0.988358)
C(T) = 1832.62 * exp(−0.201773*T)
giving Lz = −B = 0.201773
and thalf = log(2)/Lz = 3.43528
and AUCextra = CLast/Lz = 85 / 0.201773 = 421.265
and AUMCextra = (CLast*TLast)/Lz + CLast/(Lz²) = 8828.05

Table of Results

T hr	C ng/mL	AUC ng/mL*hr	AUCcum ng/mL*hr	AUMC ng/mL*(hr)^2	AUMCcum ng/mL*(hr)^2
0.33	261	43.065	43.065	14.2115	14.2115
0.66	665	152.79	195.855	86.63	100.841
1	1008	284.41	480.265	245.973	346.814
1.33	1242	371.25	851.515	438.877	785.691
1.66	1366	430.32	1281.84	646.704	1432.4
2	1236	442.34	1724.18	805.725	2238.12
2.5	1167	600.75	2324.93	1347.38	3585.5
3	1148	578.75	2903.68	1590.38	5175.87
3.5	1150	574.5	3478.18	1867.25	7043.12
4	1131	570.25	4048.43	2137.25	9180.37
5	757	944	4992.43	4154.5	13334.9
6	566	661.5	5653.93	3590.5	16925.4
8	348	914	6567.93	6180	23105.4
10	213	561	7128.93	4914	28019.4
12	142	355	7483.93	3834	31853.4
16	85	454	7937.92	6128	37981.4



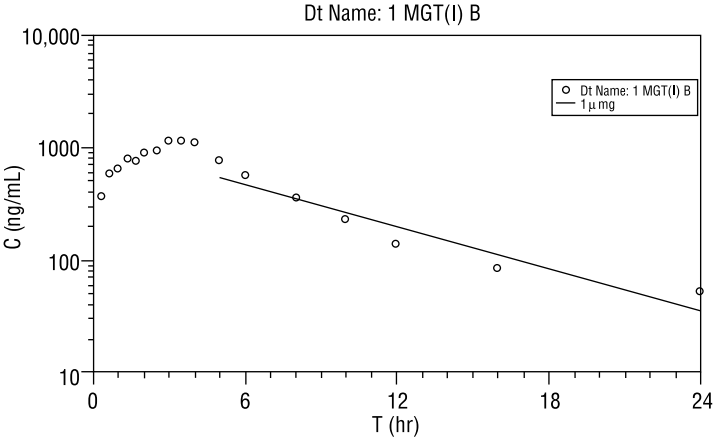
Text field name	Data field
ConcUnit	ng/mL
Subject No	1
Sequence	BA
Treatment	A

Numerical field name	Units	Data field
Dose	mg	
Cmax	ng/mL	1366
Tmax	hr	1.66
AUClast	ng/mL*hr	7937.92
AUCextra	ng/mL*hr	421.265
AUCtot	ng/mL*hr	8359.19
%AUCextra		5.03954
Lz	1/hr	0.201773
AUMClast	ng/mL*$(hr)^2$	37981.4
AUMCextra	ng/mL*$(hr)^2$	8828.05
AUMCtot	ng/mL*$(hr)^2$	46809.4
R		−0.988358
G		0.971066
Rstart	hr	5
Rend	hr	16
Rnbpoint		6
Rsmooth		0.764461
Raccurate		−0.0115898
thalf	hr	3.43528
MRT	hr	5.59976
Clearance	/(nmol/l*hr)	
Vz	*hr/(nmol/l*hr)	
Vss	*hr/(nmol/l*hr)	
C0	ng/mL	0
ComputedCLast	ng/mL	72.6119
TLast	hr	16
A		7.5135
B	1/hr	−0.201773
R^2		0.976853
AUCall(CPred)	ng/mL*hr	7937.92
AUCall(CObs)	ng/mL*hr	7937.92

Dataset name: 1 MGT (I) B
AUC * calculation
AUC0 option set to (c=0 when t=0)
c0 = 0
t0 = 0
The AUC extrapolation rule selected for C0 was:
AUC0 = (C0+Cmax)*Tmax/2 = (0+358)*0.33/2 = 59.07
AUMC0 = (Tmax*Cmax)*Tmax/2 = (0.33*358)*0.33/2 = 19.4931
With:
C0 = 0
Cmax = 358
Tmax = 0.33
The AUC was computed usingTrapezoidal Method
AUCtot = AUClast+ CLast/Lz
From 5 to infinity (user defined interval),
curve may be approximated by : (with R = −0.952747)
C(T) = 1093.61 * exp(−0.141751* T)
giving Lz = −B = 0.141751
and thalf = log(2)/Lz = 4.88988
and AUCextra = CLast/Lz = 52 / 0.141751 = 366.84
and AUMCextra = (CLast*TLast)/Lz + CLast/(Lz^2) = 11392.1

Table of Results

T hr	C ng/mL	AUC ng/mL*hr	AUCcum ng/mL*hr	AUMC ng/mL*(hr)^2	AUMCcum ng/mL*(hr)^2
0.33	358	59.07	59.07	19.4931	19.4931
0.66	585	155.595	214.665	83.1996	102.693
1	645	209.1	423.765	175.287	277.98
1.33	792	237.105	660.87	280.229	558.209
1.66	761	256.245	917.115	382.242	940.451
2	882	279.31	1196.43	514.634	1455.09
2.5	926	452	1648.43	1019.75	2474.84
3	1114	510	2158.43	1414.25	3889.09
3.5	1111	556.25	2714.68	1807.63	5696.71
4	1070	545.25	3259.93	2042.13	7738.84
5	752	911	4170.93	4020	11758.8
6	557	654.5	4825.43	3551	15309.8
8	351	908	5733.43	6150	21459.8
10	222	573	6306.43	5028	26487.8
12	137	359	6665.43	3864	30351.8
16	83	440	7105.43	5944	36295.8
24	52	540	7645.43	10304	46599.8



Text field name	Data field
ConcUnit	ng/mL
Subject No	1
Sequence	BA
Treatment	B

Numerical field name	Units	Data field
Dose	mg	
Cmax	ng/mL	1114
Tmax	hr	3
AUClast	ng/mL*hr	7645.43
AUCextra	ng/mL*hr	366.84
AUCtot	ng/mL*hr	8012.26
%AUCextra		4.57848
Lz	1/hr	0.141751
AUMClast	ng/mL*(hr)^2	46599.8
AUMCextra	ng/mL*(hr)^2	11392.1
AUMCtot	ng/mL*(hr)^2	57991.9

(Continued)

(Continued from previous page)

Numerical field name	Units	Data field
R		−0.952747
G		0.889273
Rstart	hr	5
Rend	hr	24
Rnbpoint		7
Rsmooth		0.751752
Raccurate		−0.0202996
thalf	hr	4.88988
MRT	hr	7.23789
Clearance	/(nmol/l*hr)	
Vz	*h/(nmol/l*hr)	
Vss	*h/(nmol/l*hr)	
C0	ng/mL	0
ComputedCLast	ng/mL	36.4234
TLast	hr	24
A		6.99724
B	1/h	−0.141751
R^2		0.907727
AUCall(CPred)	ng/mL*h	7645.43
AUCall(CObs)	ng/mL*h	7645.43

LATIN SQUARE DESIGN : ANOVA TABLE for AUClast
LATIN SQUARE with Log (neperian) option

Source	D.F	SS	MS	F	p
Period	1	0.000285821	0.000285821	0.0221118	0.8831 NS
Subject(Seq)	22	3.21816	0.14628	11.3166	1.648e-007 ***
Formulation	1	0.00292384	0.00292384	0.226196	0.639 NS
Sequence	1	0.00245387	0.00245387	0.0167752	0.8981 NS
Error	22	0.284376	0.0129262		
Total	47	3.5082			

	N	Mean	SD	SEM
Formulation:num = A	24	9.2235	0.299361	0.0611067
Formulation:num = B	24	9.20789	0.250572	0.0511478

Root Mean Square Error = 0.113693 ; CV = 0.0123369
phi = 0.3363
Power of the test = 0.074054
1 – (Power of the test) = 0.925946
Minimum detectable difference = 0.0156094

BIOEQUIVALENCE TESTS FOR
Level B and level A
Reference Confidence Interval: [0.8, 1.25]
90% standard confidence interval
(around the ratio:[test form]/[ref form])=[0.96007, 1.0746]
t(0.05 – 22df) = 1.7171
Can conclude equivalence.

LATIN SQUARE DESIGN: ANOVA TABLE for AUCtot
LATIN SQUARE with Log (neperian) option

Source	D.F	SS	MS	F	p
Period	1	5.92869e-005	5.92869e-005	0.00515788	0.9434 NS
Subject(Seq)	22	3.03529	0.137968	12.003	9.468e-008 ***
Formulation	1	0.00324792	0.00324792	0.282565	0.6004 NS
Sequence	1	0.00384096	0.00384096	0.0278395	0.869 NS
Error	22	0.252877	0.0114944		
Total	47	3.29532			

	N	Mean	SD	SEM
Formulation:num = A	24	9.26515	0.288205	0.0588297
Formulation:num = B	24	9.2487	0.245094	0.0500297

Root Mean Square Error = 0.107212 ; CV = 0.0115818
phi = 0.375876
Power of the test = 0.0801378
1 – (Power of the test) = 0.919862
Minimum detectable difference = 0.0164518

BIOEQUIVALENCE TESTS FOR
Level B and level A
Reference Confidence Interval: [0.8, 1.25]
90% standard confidence interval
(around the ratio: [test form]/[ref form])=[0.96397, 1.0721]
t(0.05 – 22df) = 1.7171
Can conclude equivalence.

LATIN SQUARE DESIGN: ANOVA TABLE for Cmax
LATIN SQUARE with Log (neperian) option

Source	D.F	SS	MS	F	p
Period	1	0.0376657	0.0376657	1.80695	0.1926 NS
Subject(Seq)	22	2.31168	0.105076	5.04087	0.0001759 ***
Formulation	1	0.072282	0.072282	3.46761	0.07599 NS
Sequence	1	0.0245228	0.0245228	0.233381	0.6338 NS
Error	22	0.458587	0.0208449		
Total	47	2.90474			

	N	Mean	SD	SEM
Formulation:num = A	24	7.3258	0.281666	0.0574948
Formulation:num = B	24	7.24819	0.209319	0.042727

Root Mean Square Error = 0.144378 ; CV = 0.019813
phi = 1.31674
Power of the test = 0.428999
1 – (Power of the test) = 0.571001
Minimum detectable difference = 0.0776112

BIOEQUIVALENCE TESTS FOR
Level B and level A
Reference Confidence Interval: [0.8,1.25]
90% standard confidence interval
(around the ratio:[test form]/[ref form])=[1.0061, 1.1609]
t(0.05 – 22df) = 1.7171
Can conclude equivalence.

LATIN SQUARE DESIGN: ANOVA TABLE for AUClast

Source	D.F	SS	MS	F	p
Period	1	15281	15281	0.00887628	0.9258 NS
Subject(Seq)	22	3.14912e+008	1.43142e+007	8.31466	2.744e-006 ***
Formulation	1	1.03429e+006	1.03429e+006	0.600786	0.4465 NS
Sequence	1	102379	102379	0.00715229	0.9334 NS
Error	22	3.78743e+007	1.72156e+006		
Total	47	3.53938e+008			

	N	Mean	SD	SEM
Formulation:num = A	24	10561.8	3057.44	624.098
Formulation:num = B	24	10268.2	2448.61	499.82

Root Mean Square Error = 1312.08 ; CV = 0.12598
phi = 0.548081
Power of the test = 0.115016
1 – (Power of the test) = 0.884984
Minimum detectable difference = 293.582

LATIN SQUARE DESIGN: ANOVA TABLE for AUCtot

Source	D.F	SS	MS	F	p
Period	1	5045.52	5045.52	0.00292774	0.9573 NS
Subject(Seq)	22	3.22742e+008	1.46701e+007	8.51255	2.227e-006 ***
Formulation	1	1.07024e+006	1.07024e+006	0.621025	0.4391 NS
Sequence	1	166464	166464	0.0113472	0.9161 NS
Error	22	3.79137e+007	1.72335e+006		
Total	47	3.61898e+008			

	N	Mean	SD	SEM
Formulation:num=A	24	10980.3	3089.04	630.547
Formulation:num=B	24	10681.6	2479.11	506.046

Root Mean Square Error = 1312.76 ; CV = 0.121205
phi = 0.557236
Power of the test = 0.11726
1 – (Power of the test) = 0.88274
Minimum detectable difference = 298.642

LATIN SQUARE DESIGN: ANOVA TABLE for Cmax

Source	D.F	SS	MS	F	p
Period	1	47502.1	47502.1	1.0872	0.3084 NS
Subject(Seq)	22	5.07836e+006	230835	5.28322	0.0001221 ***
Formulation	1	234361	234361	5.36392	0.03026 ***
Sequence	1	36190.1	36190.1	0.156779	0.696 NS
Error	22	961225	43692.1		
Total	47	6.35764e+006			

	N	Mean	SD	SEM
Formulation:num=A	24	1575	420.107	85.754
Formulation:num=B	24	1435.25	299.565	61.1485

Root Mean Square Error = 209.026 ; CV = 0.138876
phi = 1.63767
Power of the test = 0.600356
1 – (Power of the test) = 0.399644
Minimum detectable difference = 139.75

LATIN SQUARE DESIGN: ANOVA TABLE for Lz

Source	D.F	SS	MS	F	p
Period	1	0.000971632	0.000971632	1.0862	0.3086 NS
Subject(Seq)	22	0.0311575	0.00141625	1.58324	0.1444 NS
Formulation	1	0.000607752	0.000607752	0.679413	0.4186 NS
Sequence	1	0.0026651	0.0026651	1.8818	0.184 NS
Error	22	0.0196796	0.000894526		
Total	47	0.0550816			

	N	Mean	SD	SEM
Formulation:num=A	24	0.166042	0.0278732	0.00568959
Formulation:num=B	24	0.173159	0.0398938	0.00814328

Root Mean Square Error = 0.0299086 ; CV = 0.176348
phi = 0.582843
Power of the test = 0.123748
1 – (Power of the test) = 0.876252
Minimum detectable difference = 0.0071166

LATIN SQUARE DESIGN: ANOVA TABLE for thalf

Source	D.F	SS	MS	F	p
Period	1	0.159144	0.159144	0.237661	0.6307 NS
Subject(Seq)	22	20.3658	0.925719	1.38245	0.2268 NS
Formulation	1	0.0700608	0.0700608	0.104627	0.7494 NS
Sequence	1	1.79725	1.79725	1.94147	0.1774 NS
Error	22	14.7317	0.669623		
Total	47	37.124			

	N	Mean	SD	SEM
Formulation:num = A	24	4.29225	0.749939	0.153081
Formulation:num = B	24	4.21584	1.02403	0.209029

Root Mean Square Error = 0.818305 ; CV = 0.192359
phi = 0.228722
Power of the test = 0.0610502
1 – (Power of the test) = 0.93895
Minimum detectable difference = 0.0764094

LATIN SQUARE DESIGN: ANOVA TABLE for Tmax

Source	D.F	SS	MS	F	p
Period	1	0	0	0	1 NS
Subject(Seq)	22	25.236	1.14709	1.19882	0.3372 NS
Formulation	1	1.02667	1.02667	1.07298	0.3115 NS
Sequence	1	1.22241	1.22241	1.06566	0.3131 NS
Error	22	21.0506	0.956847		
Total	47	48.5357			

	N	Mean	SD	SEM
Formulation:num = A	24	2.43625	0.88853	0.18137
Formulation:num = B	24	2.72875	1.12966	0.23059

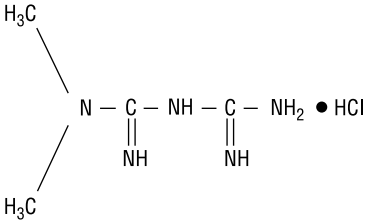
Root Mean Square Error = 0.978185 ; CV = 0.378775
phi = 0.732454
Power of the test = 0.16789
1 – (Power of the test) = 0.83211
Minimum detectable difference = 0.2925

A.8. BIOANALYTICAL REPORT: SPECIFIC INFORMATION REDACTED

A.8.1. Introduction

Metformin hydrochloride: *N,N*-Dimethylimidodicarbonimidic diamide hydrochloride. Metformin hydrochloride is an oral antihyperglycemic drug used in the management of non-insulin–dependent diabetes mellitus. Metformin hydrochloride is not chemically or pharmacologically related to the oral sulfonylureas.

The structural formula of metformin hydrochloride is shown below:



Metformin hydrochloride is a white to off-white crystalline compound with a molecular formula of $C_4H_{11}N_5 \cdot HCl$ and a molecular weight of 165.63. Metformin hydrochloride is freely soluble in water and is practically insoluble in acetone, ether, and chloroform. The pK_a of metformin is 12.4. The pH of a 1% aqueous solution of metformin hydrochloride is 6.68.

The current study was devised to develop and validate a liquid chromatographic (HPLC) method for metformin determination in human plasma. To support clinical studies, the method was approved as a feasible analytical tool to quantitate metformin for pharmacokinetic purposes in bioequivalence and/or bioavailability studies. Furthermore, the attempted method was to provide a reliable lower limit of quantitation (LLOQ) deemed 50 ng/mL with appreciable accuracy and reproducibility.

A.8.2. Methodology

A.8.2.1. Compound's Chemistry

A.8.2.1.1. Metformin Hydrochloride

Chemical name: *N,N*-Dimethylimidodicarbonimidic diamide hydrochloride
Molecular formula: $C_4H_{11}N_5 \cdot HCl$
Molecular weight: 165.63

A.8.2.1.2. Phenformin Hydrochloride (Internal Standard)

Chemical name: 1-Phenethyl-biguanide hydrochloride
Molecular formula: $C_{10}H_{15}N_5 \cdot HCl$
Molecular weight: 241.56

A.8.2.2. Experimental

An HPLC-UV The method has been developed for the quantitation of metformin in human plasma.

A.8.2.2.1. Instrumentation

A.8.2.3. Standards and Reagents

A.8.2.3.1. Standards

A.8.2.3.2. Reagents

A.8.2.4. Standard and Reagents Solutions Preparation

A.8.2.4.1. Metformin Stock Standard Solution

A.8.2.4.1.1. Metformin Stock Standard Solution.

A.8.2.4.1.2. Metformin Purity Check Solution.

A.8.2.4.2. Internal Standard Solutions

A.8.2.4.2.1. Is Stock Solution.

A.8.2.4.2.2. Is Working Solution.

A.8.2.4.2.3. Phenformin Purity Check Solution.

A.8.2.4.3. Identification Solution

A.8.2.4.4. Preparation of Standard Calibration Curve Samples

A.8.2.4.5. Preparation of Quality Control Samples

A.8.2.4.5.1. Quality Control Samples For Regular Run.

A.8.2.4.5.2. Quality Control Samples for Dilution Integrity.

A.8.2.5. Description of Method

A.8.2.5.1. Sample Preparation

A.8.2.5.2. Chromatographic Conditions

A.8.2.5.3. Standardization and Calculation

A.8.2.6. Method Development

A.8.2.7. Method Validation

A.8.2.7.1. Specificity/Selectivity

A.8.2.7.2. Linearity

A.8.2.7.3. Accuracy and Precision

A.8.2.7.3.1. Intra-Day Accuracy and Precision.

A.8.2.7.3.2. Inter-Day Accuracy and Precision.

A.8.2.7.4. Recovery

A.8.2.7.4.1. Absolute Analytical Recovery.

A.8.2.7.4.2. Relative Analytical Recovery.

A.8.2.7.5. Sensitivity

A.8.2.7.6. Stability

A.8.2.7.6.1. Stability in Biological Plasma Samples.
A.8.2.7.6.1.1. Short- Term Stability.
A.8.2.7.6.1.2. Freeze and Thaw Stability.
A.8.2.7.6.1.3. Long- Term Stability.
A.8.2.7.6.2. Post-Preparative Stability.
A.8.2.7.6.2.1. Autosampler Stability.
A.8.2.7.6.2.2. Dry Extract Stability.
A.8.2.7.6.3. Stock Solution Stability.

A.8.2.7.7. Dilution Integrity

A.8.3. Data and Results

A.8.3.1. Specificity/Selectivity

A.8.3.2. Linearity

A.8.3.3. Accuracy and Precision

A.8.3.3.1. Intra-Day Accuracy and Precision
A.8.3.3.2. Inter-Day Accuracy and Precision

A.8.3.4. Recovery

A.8.3.4.1. Absolute Analytical Recovery
A.8.3.4.2. Relative Analytical Recovery

A.8.3.5. Sensitivity

A.8.3.6. Stability

A.8.3.6.1. Stability in Biological Plasma Samples

A.8.3.6.1.1. Short-Term Stability.

A.8.3.6.1.2. Freeze and Thaw Stability.

A.8.3.6.1.3. Long-Term Stability.

A.8.3.6.2. Post-Preparative Stability

A.8.3.6.3. Stock Solution Stability

A.8.3.7. Dilution Integrity

A.8.4. Conclusions

The developed method of analysis provided a sensitive and specific assay for metformin in human plasma. It was shown that this method is suitable for the analysis of metformin in the biological plasma samples. For the bioequivalence study, the following recommendations were implemented:

- Clinical samples collection, handling, processing, and running should take into consideration the stability conditions furnished by the stability tests in this validation study.
- A standard calibration curve including blank sample and standard zero sample should be generated for each analytical run and should be used to determine the sample concentrations in the unknown clinical samples.
- For each run, QC samples at each of the low, medium, and high concentrations should be included.
- QC samples should be analyzed together with the unknown clinical samples and should be allocated judiciously taking into consideration the estimated drug level throughout the batch, in order to detect any analytical drift.
- Clinical plasma samples of volunteers in all periods should be analyzed with their own calibration curve and QC samples as one batch in a single analytical run.
- No determinations should be done below the LLOQ or above the ULOQ of the standard calibration curve. Alternatively, appropriate dilution should be intended for samples of concentration above the ULOQ (Figs. A1–A7).

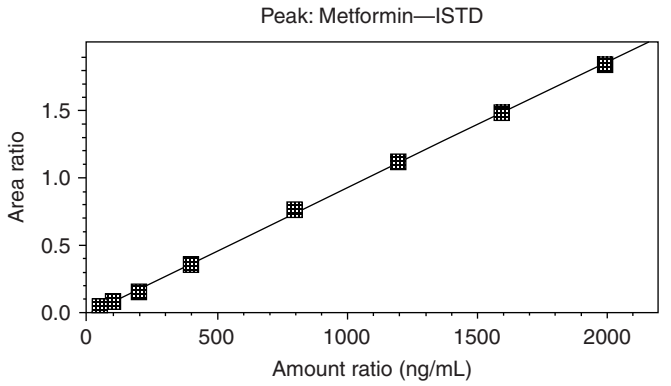


FIGURE A.1 A representative standard calibration curve.

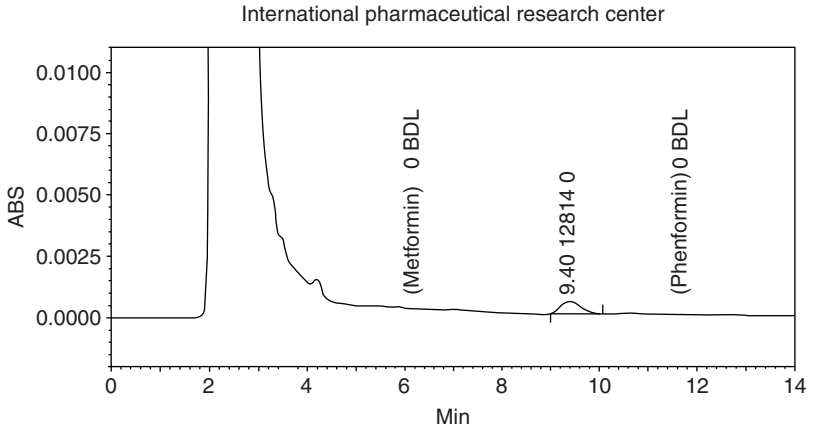


FIGURE A.2 HPLC chromatogram showing a blank human plasma sample. *Abbreviation*: HPLC, high-performance liquid chromatography.

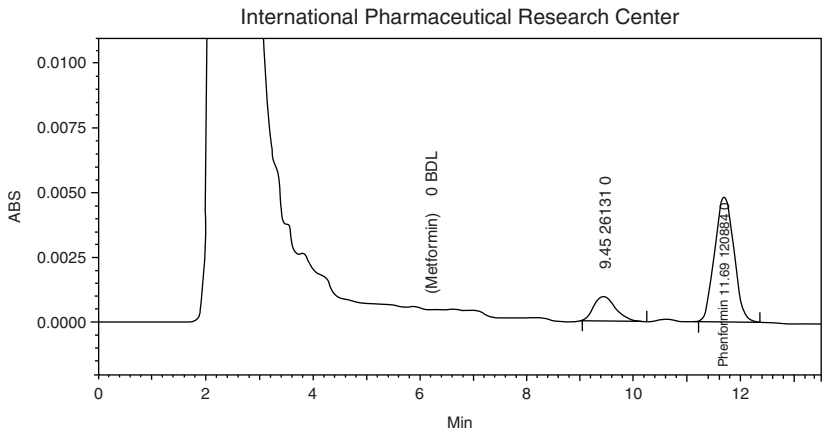


FIGURE A.3 HPLC chromatogram showing a standard zero sample containing 1500 ng/mL internal standard (phenformin). *Abbreviation*: HPLC, high-performance liquid chromatography.

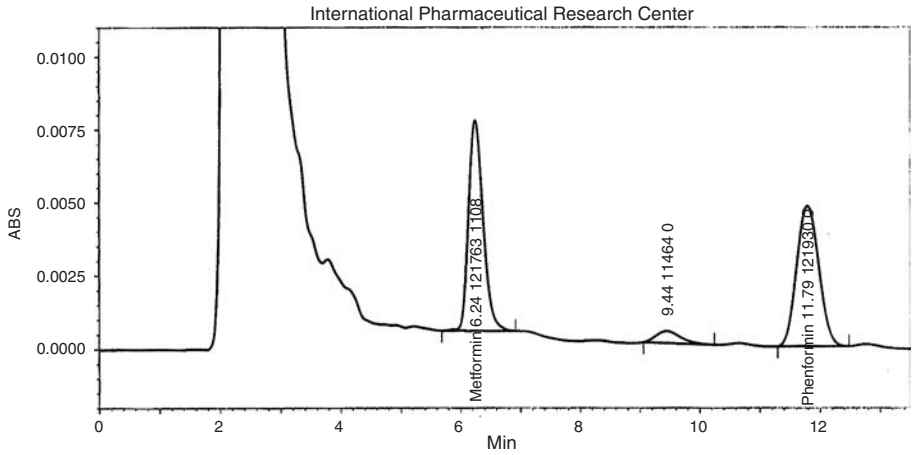


FIGURE A.4 HPLC chromatogram showing human plasma sample (LLOQ sample) containing metformin 50 ng/mL and internal standard (phenformin) 1500 ng/mL. *Abbreviation*: HPLC, high-performance liquid chromatography.

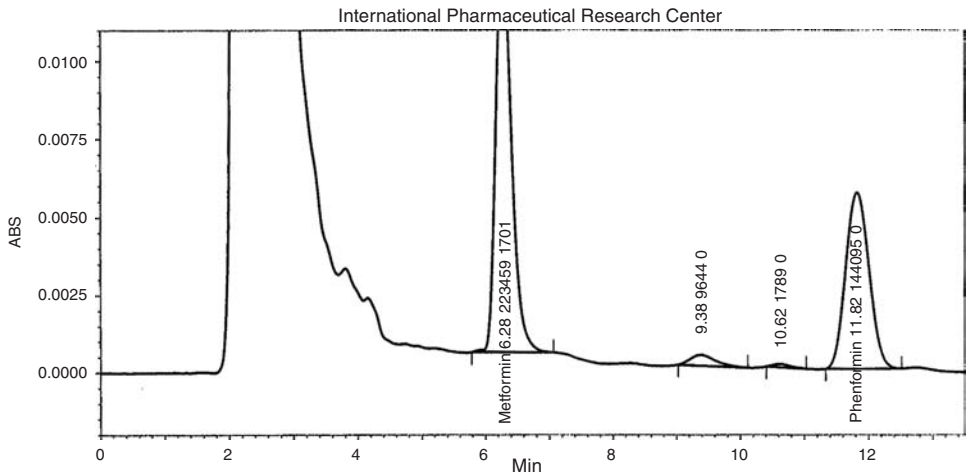


FIGURE A.5 HPLC chromatogram showing human plasma sample (QC low) containing metformin 150 ng/mL and internal standard (phenformin) 1500 ng/mL. *Abbreviation*: HPLC, high-performance liquid chromatography.

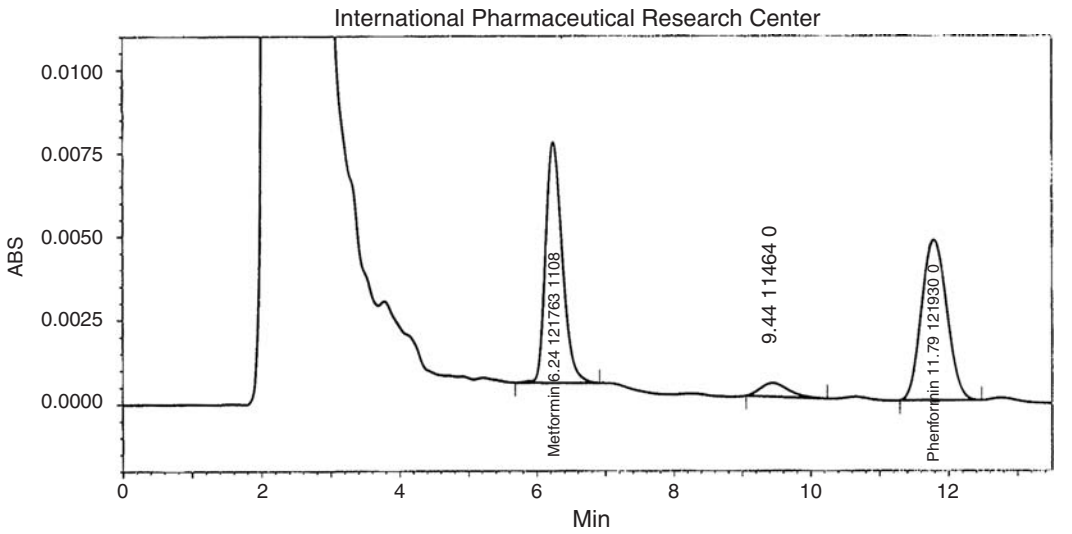


FIGURE A.6 HPLC chromatogram showing human plasma sample (QC medium) containing metformin 1000 ng/mL and internal standard (phenformin) 1500 ng/mL. *Abbreviation*: HPLC, high-performance liquid chromatography.

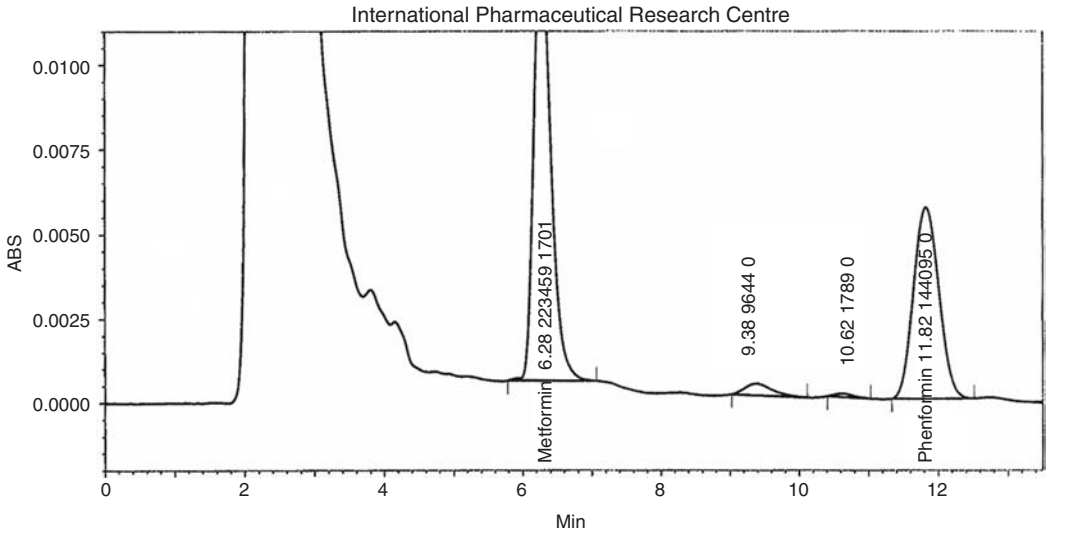


FIGURE A.7 HPLC chromatogram showing human plasma sample (QC high) containing metformin 1700 ng/mL and internal standard (phenformin) 1500 ng/mL. *Abbreviation*: HPLC, high-performance liquid chromatography.

A.9. BIOEQUIVALENCE REPORT

Comparative, Randomised, Single-Dose, Two-Way Open-Label Crossover Study To Determine The Bioequivalence of 100 mg Cyclosporine Per 1 mL Solution, From Sigmasporin Microoral Solution [Gulf Pharmaceutical Industries (Julphar)] and Sandimmun Neoral® Solution (Novartis Pharma) Given To Healthy Adult Males Under Fasting Conditions

Study synopsis	
Study title	Comparative, randomized, single-dose, two-way open-label crossover study to determine the bioequivalence of 100 mg cyclosporine per 1 mL solution, from Sigmasporin Microoral Solution [Gulf Pharmaceutical Industries (Julphar)] and Sandimmun Neoral Solution (Novartis Pharma) given to healthy adult males under fasting conditions
IPRC study code	CYC-GUL-L1001/155
Objective	To investigate the single-dose bioequivalence of Gulf Pharmaceutical Industries (Julphar) (test product, Sigmasporin Microoral Solution) and Novartis Pharma (reference product, Sandimmun Neoral Solution) 100 mg cyclosporine per 1 mL solution in healthy adult males under fasting conditions
Protocol/design	The study was an open-label, randomized, single-dose, two-way crossover design. The study was conducted on healthy adult male volunteers, and a total of 30 volunteers completed the crossover. In each period, volunteers were housed from the evening before dosing until after 24-hr blood sampling
Phases of study	Screening date: 30/12/01 Date of Phase I:05/01/02 Date of Phase II: 12/01/02
Demographic data ($N=30$)	Age: 22.70 $\pm$ 3.92 years Height: 172.43 $\pm$ 6.59 cm Weight: 68.33 $\pm$ 10.36 kg
Study medications	Treatment A (test product) Sigmasporin Microoral, 100 mg cyclosporine solution Batch no.: 015389 Treatment B (reference product) Sandimmun Neoral 100 mg cyclosporine solution Batch no.: 287
Dosage regimen	Treatment A (test product): single dose, 1 mL of Sigmasporin Microoral Solution (100 mg cyclosporine per 1 mL) Treatment B (reference product): single dose, 1 mL of Sandimmun Neoral Solution (100 mg cyclosporine per 1 mL)
Tolerance	Both products were well tolerated
Drug bioanalysis	The lower limit of quantitation for cyclosporine was 5 ng/mL
Pharmacokinetics	$t_{(1/2)e}$, K_e, C_{max}, t_{max}, $AUC_{0\rightarrow t}$, $AUC_{0\rightarrow\infty}$, and $(AUC_{0\rightarrow t}/AUC_{0\rightarrow\infty})\%$
Bioequivalence determinants	C_{max}, $AUC_{0\rightarrow t}$, and $AUC_{0\rightarrow\infty}$
Results	The results of bioequivalence determinants were as follows C_{max} 90.32% (83.82–97.31%) $AUC_{0\rightarrow t}$ 97.00% (90.61–104.79%) $AUC_{0\rightarrow\infty}$ 99.77% (92.25–107.89%)
Conclusions	The ratios of the point estimators and the 90% confidence intervals for the log-transformed ratios (test/reference) for C_{max}, $AUC_{0\rightarrow t}$, and $AUC_{0\rightarrow\infty}$ were within the 80% to 125% FDA acceptance range. Therefore, the bioequivalence of Gulf Pharmaceutical Industries (Julphar) (Sigmasporin Microoral Solution) and Novartis Pharma (Sandimmun Neoral Solution) 100 mg cyclosporine per 1 mL can be concluded

SUMMARY

This study was performed to investigate the bioequivalence of cyclosporine between a generic test product [Sigmasporin Microoral, 100 mg cyclosporine per 1 mL solution; Gulf Pharmaceutical Industries (Julphar), U.A.E.] and reference product (Sandimmun Neoral, 100 mg cyclosporine per 1 mL solution; Novartis Pharma, Switzerland). The clinical protocol called for 30 healthy volunteers, at least. The subjects received 1 mL of each product, Sigmasporin Microoral solution and Sandimmun Neoral solution (100 mg cyclosporine per 1 mL), in a randomized fashion with a washout period of seven days. Thirty healthy male volunteers completed the crossover.

The bioanalysis of clinical plasma samples was accomplished by the in-house LC–MS method, which was developed and validated in accordance with the international guidelines. The LLOQ for cyclosporine A was 5 ng/mL. Samples collection, handling, transfer, storage, and

analysis were all conducted according to the international GLPs under the supervision of IPRC's QAU.

Pharmacokinetic parameters, determined by standard noncompartmental methods, and ANOVA statistics were calculated using Kinetica 2000 statistical software.

Regarding the *pharmacokinetics*, the extent of absorption was determined by $AUC_{0\rightarrow t}$ and $AUC_{0\rightarrow \infty}$, while the rate of absorption was assessed from C_{max} and t_{max}. The adequacy of sampling time was judged from the ratio $(AUC_{0\rightarrow t}/AUC_{0\rightarrow \infty})\%$. The elimination half-life was calculated ($t_{(1/2)e}$), in addition to the elimination rate constant (K_e), which were invested for further characterization of the pharmacokinetics outcome of this study. ANOVA for both the untransformed pharmacokinetics $t_{(1/2)e}$, K_e, C_{max}, t_{max}, $AUC_{0\rightarrow t}$, and $AUC_{0\rightarrow \infty}$, and the log-transformed C_{max}, $AUC_{0\rightarrow t}$, and $AUC_{0\rightarrow \infty}$ was executed. The variance model included sequence, subjects nested in sequence, period, and product as factors, employing 5% level of significance. The significance of a sequence effect was tested using the subjects nested in sequence as the error term.

The ANOVA analysis showed no statistically significant differences between the two products with respect to the calculated pharmacokinetic parameters $t_{(1/2)e}$, K_e, C_{max}, t_{max}, $AUC_{0\rightarrow t}$, and $AUC_{0\rightarrow \infty}$. Results of ANOVA are summarized in Table C.10 of Appendix C. The geometric mean for the ratios expressed as a percentage (test product/reference product) are given in Tables C.7–C.9 (see Appendix C). Consistent with the two one-sided tests for bioequivalence, 90% confidence intervals for the ratios of means was calculated for the untransformed and the log-transformed C_{max}, $AUC_{0\rightarrow t}$, and $AUC_{0\rightarrow \infty}$. The values obtained indicated that the 90% confidence limits for all geometric means are within the recommended criteria to conclude bioequivalence (80–125% for C_{max}, $AUC_{0\rightarrow t}$ and $AUC_{0\rightarrow \infty}$) (Figs A8–A10).

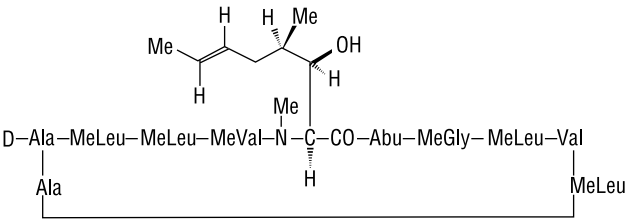
In conclusion, the study demonstrated that the test product, Sigmasporin Microoral Solution [Gulf Pharmaceutical Industries (Julphar), U.A.E.], 100 mg cyclosporine per 1 mL, is bioequivalent to the reference product, Sandimmun Neoral Solution (Novartis Pharma, Switzerland), 100 mg cyclosporine per 1 mL (Tables A.1 and A.2).

A.9.1. Introduction

A.9.1.1. Chemistry

Cyclosporine is an oral and parenteral immunosuppressive agent. Cyclosporine is a cyclic polypeptide consisting of 11 amino acids, and it is produced by the fungus *Beauveria nivea*. It has the following structural formula:

Cyclosporine A



A.9.1.2. Pharmacology

Cyclosporine induces immunosuppression by inhibiting the first phase of T-cell activation. The first phase of T-cell activation causes transcriptional activation of immediate and early gene products (e.g., interleukins—IL-2, IL-3, and IL-4, tumor necrosis factor alpha, and interferon gamma) that allow T cells to progress from the G_0 to G_1 phases. Cyclosporine binds to an immunophilin termed cyclophilin. Immunophilins (e.g., cyclophilin and FK-binding proteins) are immunosuppressant-binding proteins that are distributed in all cellular compartments and play an important role in protein regulation. The cyclosporine–cyclophilin complex then binds to and inhibits the calcium-calmodulin–activated phosphatase calcineurin. The calcineurin enzyme catalyzes critical dephosphorylation reactions necessary for early lymphokine gene transcription, and subsequent early activation of T cells. Calcineurin inhibition results in the blockade of signal transduction of the

nuclear factor of activated T cells (NF-AT). The blockade of signal transduction results in failure to activate NF-AT-regulated genes. NF-AT-activated genes include those required for B-cell activation including IL-4 and CD40 ligand and those required for T-cell activation including IL-2 and interferon gamma. Cyclosporine does not affect suppressor T cells or T-cell-independent, antibody-mediated immunity.

A.9.1.3. Pharmacokinetics

Cyclosporine is administered orally or intravenously. Cyclosporine is extremely hydrophobic. Because of the unpredictability of oral absorption, it is difficult to convert between oral and parenteral doses. Most clinicians use a 3:1 ratio when converting between oral and parenteral routes (e.g., 30 mg IV is roughly equivalent to 90 mg orally). First-pass metabolism, mode of administration, formulation, and drug interactions all affect cyclosporine absorption.

Cyclosporine is a substrate and inhibitor of P-glycoprotein, which is an energy-dependent drug-efflux pump located in intestinal epithelium and the blood–brain barrier. There appears to be overlap between inhibitors and/or substrates of cytochrome (CYP) P450 3A4 and P-glycoprotein. The P-glycoprotein efflux of cyclosporine from intestinal cells back into the gut lumen allows for CYP3A4 metabolism prior to absorption, thus limiting cyclosporine availability. When cyclosporine is administered with inhibitors of both CYP3A4 and P-glycoprotein (e.g., diltiazem, erythromycin, or ketoconazole) increased cyclosporine bioavailability leads to increased cyclosporine concentrations.

Oral absorption of cyclosporine, USP (Modified): The physical properties of the cyclosporine (modified) formulation (i.e., microemulsion) make the absorption of cyclosporine less dependent on bile, food, and other factors that assist dispersion and subsequent absorption of lipophillic substances from the gastrointestinal (GI) tract. Although, agents which influence pre-systemic metabolism (e.g., grapefruit juice) may still influence cyclosporine (modified) absorption. The absolute bioavailability of cyclosporine (modified) has not been determined in adults. Following oral administration, the t_{max} for cyclosporine (modified) ranges from 1.5 to 2 hours. Food decreases the absorption of cyclosporine (modified). The AUC of cyclosporine (modified) is linear within the therapeutic dosage range. Intersubject variability of cyclosporine exposure (AUC) ranges from about 20% to 50% when administered as cyclosporine (modified). There is less intrasubject variation in AUC with cyclosporine (modified), despite random changes in food intake, bile secretion, or time of trough concentration measurement. Intrasubject variability of AUC in renal transplant patients is 9% to 21% for cyclosporine (modified). In these same studies, the intrasubject variation in trough concentrations was similar for the two formulations.

Cyclosporine is distributed widely throughout the body, crosses the placenta, and is found in breast milk. Preferential uptake of cyclosporine occurs in the liver, pancreas, and adipose tissue, while it penetrates the CNS poorly. In blood, the distribution of cyclosporine is concentration dependent; as the hemocrit rises, the cyclosporine concentration in plasma decreases. Approximately, 22% to 47% of cyclosporine is found in plasma, 4% to 9% in lymphocytes, 5% to 12% in granulocytes, and 41% to 58% in erythrocytes. At high drug concentrations, the binding to lymphocytes and erythrocytes becomes saturated. In plasma, cyclosporine is approximately 90% bound to lipoproteins. In addition, binding to erythrocytes and lipoproteins is temperature dependent. As the temperature increases, binding to lipoproteins increases; however, binding to erythrocytes increases as the temperature decreases. Other medications that may affect the binding of cyclosporine to lipoproteins may modify the clinical response to cyclosporine. Cyclosporine is metabolized extensively by the CYP3A enzyme system in the liver and to a lesser extent in the GI tract and kidney. Agents that affect the CYP3A system may significantly alter the metabolism of cyclosporine. At least 25 metabolites of cyclosporine have been identified, some of which are biologically active. Although most cyclosporine metabolites show only 10% to 20% of the immunosuppressive activity of the parent drug, they do contribute to toxicity. The major metabolites of cyclosporine are M1, M9, and M4N, resulting from oxidation at the 1-beta, 9-gamma, and 4-*N*-desmethylated positions. At steady state, concentrations and AUCs of cyclosporine metabolites may exceed that of cyclosporine. Mean AUCs for blood concentrations of these metabolites are 70%, 21%, and 7.5%, respectively, of blood cyclosporine concentrations. The elimination half-life of cyclosporine is highly variable. In patients with normal hepatic function, the average half-life ranges from 16 to 27 hours, and in other reports 5 to 18 hours.

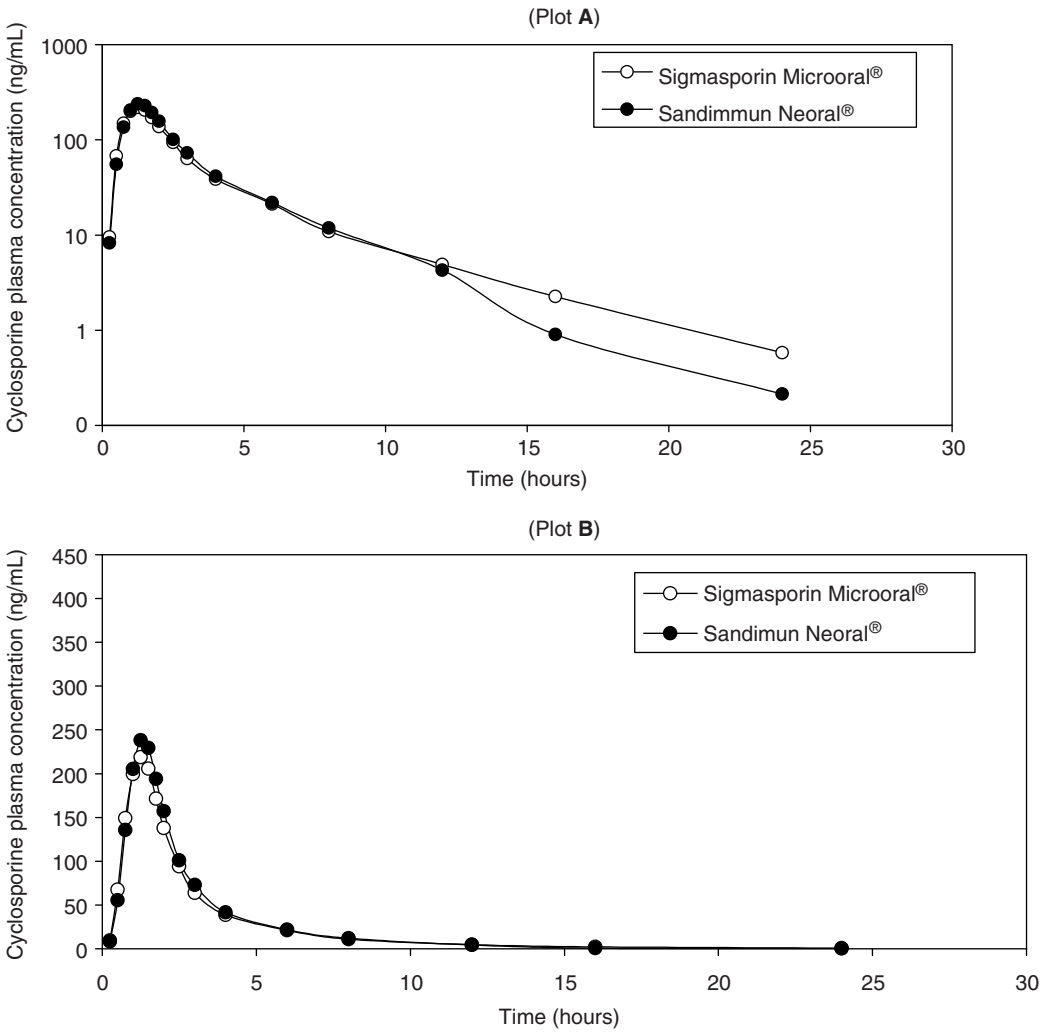


FIGURE A.8 Semi-logarithmic presentation (**A**) and linear presentation (**B**) for cyclosporine A means after single-dose administration of 100 mg cyclosporine per 1 mL for both treatments Sigmasporin Microoral® Solution (test product) and Sandimmun Neoral® Solution (reference product).

Elimination of cyclosporine and its metabolites is principally through the bile and feces. Cyclosporine undergoes enterohepatic recycling. Only 6% of the cyclosporine dose is excreted renally, of which 0.1% is excreted as unchanged cyclosporine. Although cyclosporine blood levels are widely used to assist dosing, accurate interpretation is hampered by variation in absorption, variation in protein binding, sampling error, type of assay, cross-reactivity of metabolites, enterohepatic recycling of drug, and drug interactions.

A.9.1.4. Therapeutic Uses

Cyclosporine is indicated in the following cases ([a]non-FDA-approved indication):

- Aplastic anemia[a]
- Crohn's disease[a]
- Graft-versus-host disease[a]
- Heart transplant rejection prophylaxis

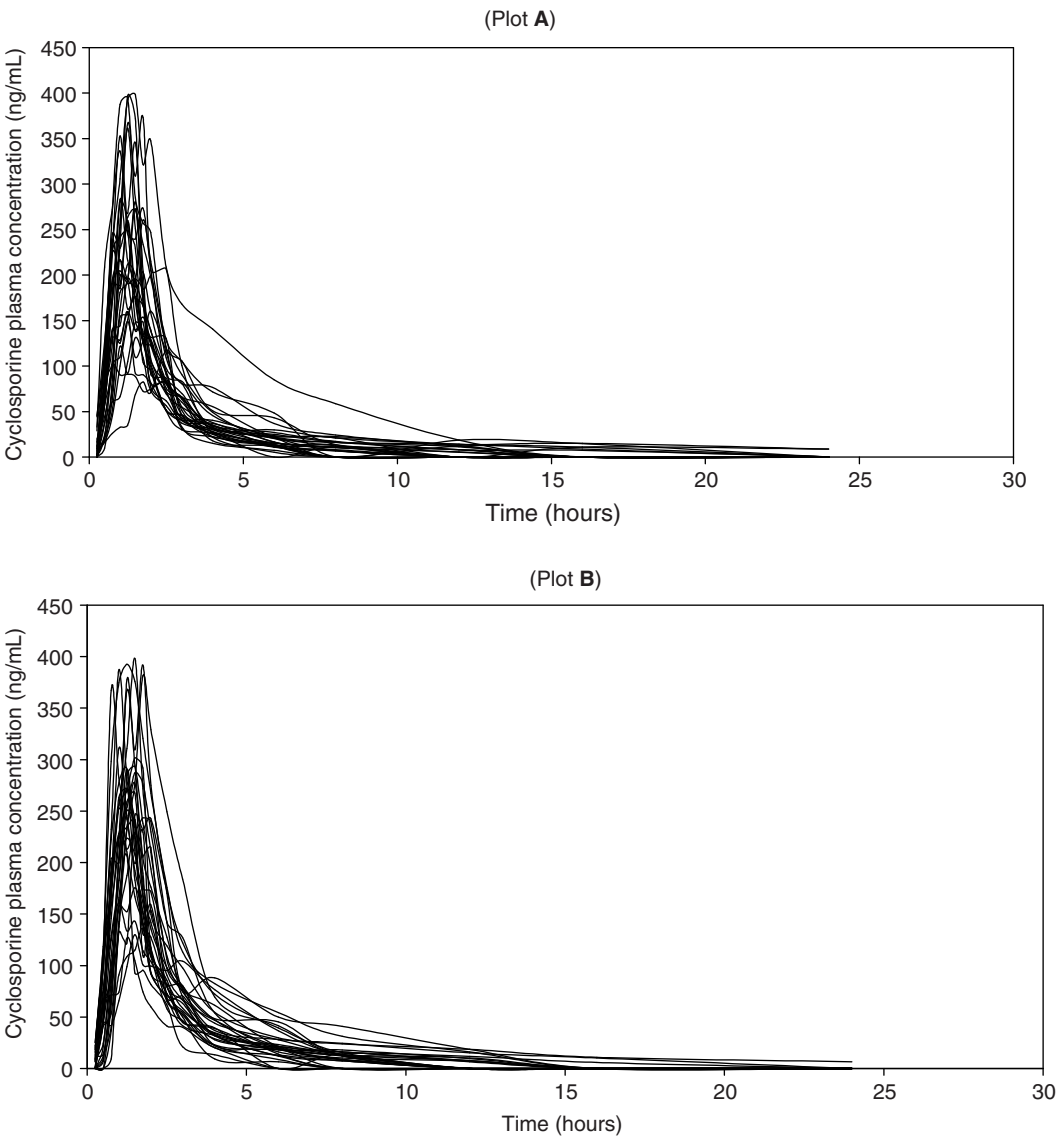


FIGURE A.9 Linear presentation for cyclosporine A individual plasma concentrations (ng/mL) versus time (hours), after single-dose administration of 100 mg cyclosporine per 1 mL for both treatments; Sigmasporin Microoral® Solution, the test product (**A**), and Sandimmun Neoral® Solution, the reference product (**B**).

- Idiopathic thrombocytopenic purpura (ITP)[a]
- Kidney transplant rejection prophylaxis
- Liver transplant rejection prophylaxis
- Lupus nephritis[a]
- Myasthenia gravis[a]
- Psoriasis
- Psoriatic arthritis[a]
- Rheumatoid arthritis
- Ulcerative colitis[a]
- Xerophthalmia[a]

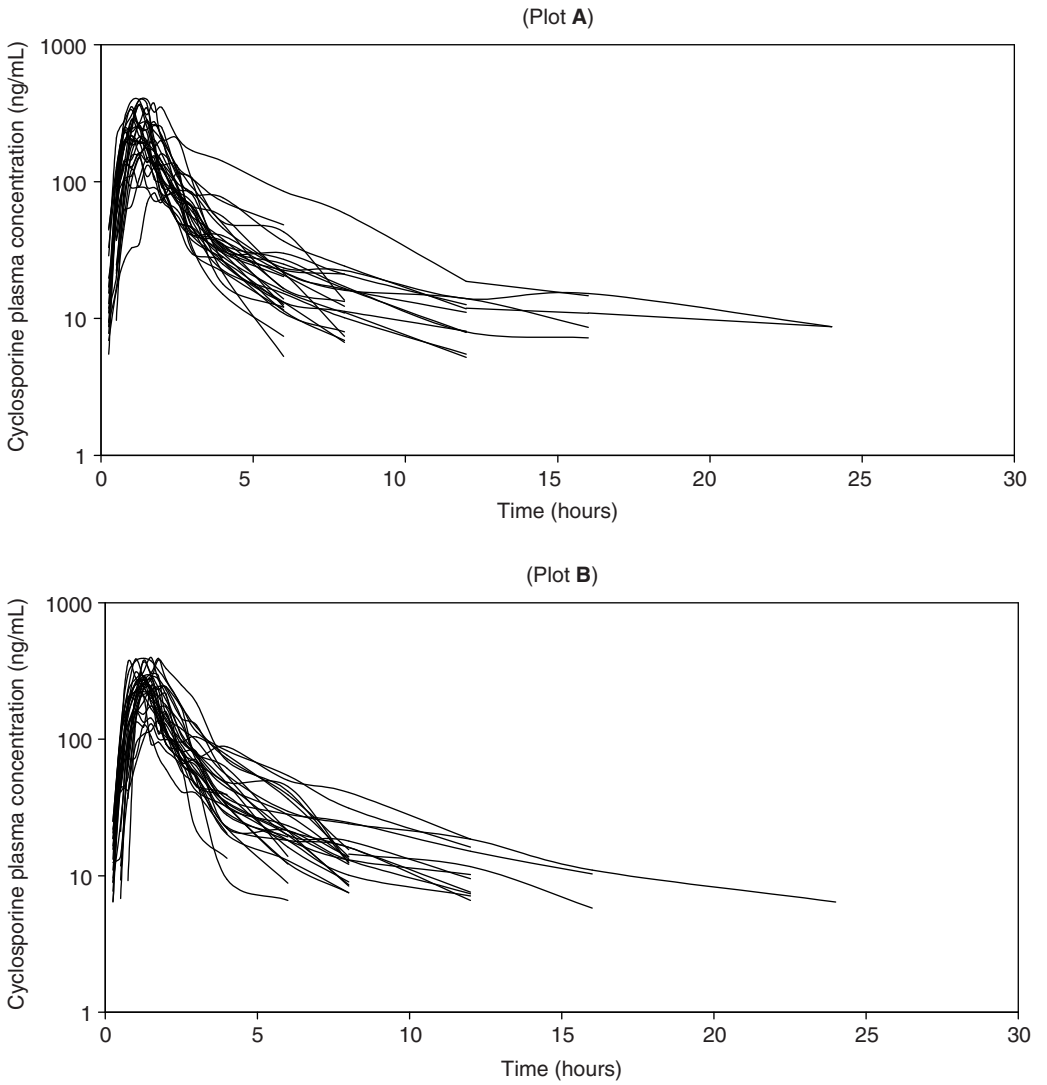


FIGURE A.10 Semi-logarithmic presentation for cyclosporine A individual plasma concentrations (ng/mL) versus time (hours), after single-dose administration of 100 mg cyclosporine per 1 mL for both treatments; Sigmasporin Microoral® Solution, the test product (**A**), and Sandimmun Neoral® Solution, the reference product (**B**).

A.9.1.5. Adverse Events

Nephrotoxicity is the most common adverse effect of cyclosporine therapy and has been documented in all types of patients receiving cyclosporine. Cyclosporine-induced nephrotoxicity is most likely due to intense renal vasoconstriction, which leads to increases in serum

TABLE A.1 Bioequivalence Parameters of Cyclosporine (Sigmasporin Microoral Solution, the Test Product, vs. Sandimmun Neoral® Solution, the Reference Product)

Pharmacokinetic parameter	Treatment (mean ± SD)		90% confidence intervals of parametric means		
	Test product	Reference product	Point estimator (%)	Lower limit (%)	Upper limit (%)
C_{max}	250.0 ± 88.8	271.7 ± 80.2	90.32	83.82	97.31
$AUC_{0\rightarrow t}$	559.775 ± 219.032	577.804 ± 213.396	97.00	90.61	104.79
$AUC_{0\rightarrow \infty}$	617.148 ± 235.742	621.125 ± 230.127	99.77	92.25	107.89

TABLE A.2 Pharmacokinetics of Cyclosporine (Sigmasporin Microoral Solution, the Test Product, vs. Sandimmun Neoral® Solution, the Reference Product)

	Treatment (mean ± SD)	
Pharmacokinetic parameter	**Test product**	**Reference product**
t_{max}	1.31 ± 0.34	1.35 ± 0.24
$t_{(1/2)e}$	4.0012 ± 3.3498	2.9325 ± 1.8906
$(AUC_{0\rightarrow t}/AUC_{0\rightarrow\infty})\%$	90.94 ± 6.01	92.96 ± 3.44
K_e	0.2858 ± 0.1825	0.3446 ± 0.2107

creatinine, arterial blood pressure, and serum potassium. The frequency and severity of serum creatinine elevations increase with dose and duration of cyclosporine therapy. Cyclosporine-induced elevations in serum creatinine and blood urea nitrogen (BUN) (azotemia) may progress to irreversible renal dysfunction if not appropriately addressed. Increased serum creatinine was reported in 16% to 20% of psoriasis patients. In patients with cyclosporine nephrotoxicity, reducing the dose of cyclosporine often leads to improvement in renal function indices. Clinical parameters associated with cyclosporine-induced nephrotoxicity include onset >six weeks posttransplant, prolonged initial nonfunction of kidney (acute renal tubular necrosis), cyclosporine trough blood level >200 ng/mL, gradual rise in serum creatinine (<0.15 mg/dL per day), serum creatinine plateau <25% above baseline, and BUN/creatinine ratio ≥20 (azotemia). Serial deterioration in renal function and morphologic changes in the kidneys characterize a form of cyclosporine-associated nephropathy. From 5% to 15% of transplant patients who have received cyclosporine will fail to show a reduction in rising serum creatinine despite a decrease or discontinuation of cyclosporine therapy. Consequences of cyclosporine nephrotoxicity include renal insufficiency with accumulation of type A (BUN, serum creatinine) and type B (potassium, uric acid) solutes.

Significant hyperkalemia (sometimes associated with hyperchloremic metabolic acidosis) and hyperuricemia have been observed in patients receiving cyclosporine. Hyperkalemia may indicate nephrotoxicity.

Occasionally patients treated with cyclosporine, especially following bone marrow transplantation, have developed a syndrome similar to idiopathic thrombotic thrombocytopenic purpura or hemolytic-uremic syndrome (HUS), which may lead to graft failure. Clinical findings include thrombosis of renal microvasculature with platelet-fibrin thrombi occluding glomerular capillaries and afferent arterioles, microangiopathic hemolytic anemia, thrombocytopenia, and decreased renal function.

During cyclosporine therapy, mild-to-moderate hypertension is encountered more frequently than severe hypertension and the incidence decreases over time. Hypertension occurs in 50% of renal transplant patients and in most cardiac transplant patients. In recipients of kidney, liver, or heart allografts treated with cyclosporine, antihypertensive therapy may be required. Besides increased sympathetic tone and the subsequent vasoconstriction of the renal afferent arteriole, cyclosporine-induced hypertension may be related to the HUS. In studies of cyclosporine in rheumatoid arthritis patients, the incidence of hypertension was 8% to 26% with 5.3% of patients discontinuing cyclosporine therapy due to hypertension. In patients with psoriasis, about 26% of patients developed new hypertension consisting of SBP >160 mm Hg and/or DBP >90 mmHg.

Hepatotoxicity in the form of elevated hepatic enzymes or hyperbilirubinemia occurs in 4% to 7% of transplant patients receiving cyclosporine. Hepatic toxicity is more common during the first month of therapy when higher doses of cyclosporine are used. Elevated hepatic enzymes and serum bilirubin levels appear to be associated with trough serum levels greater than 500 ng/mL and oral doses greater than 17 mg/kg per day. Effects usually will resolve with a reduction in cyclosporine dose.

Cyclosporine use in transplant recipients as well as in other patients has been associated with hypercholesterolemia, including hyperlipidemia with elevations in LDL-C levels and hypertriglyceridemia. Although the significance of cyclosporine-induced hypercholesterolemia is not clear, administration of lipid-lowering therapy may affect the lipoprotein binding of cyclosporine and/or cyclosporine metabolism.

Hypomagnesemia has been reported in some, but not all, patients experiencing seizures while on cyclosporine therapy. Generalized tonic-clonic seizures often occur with high cyclosporine levels. Most patients suffer a single seizure with no recurrence after dose reduction. Tremor is common in patients receiving cyclosporine and occurs in up to 55% of patients. Mild encephalopathy has been reported in up to 30% of patients receiving cyclosporine. In mild cases, symptoms tend to resolve spontaneously; however, dosage reduction may be required in more severe cases. Most cases of encephalopathy involve heart or renal transplant patients with high cyclosporine levels and diffuse white matter changes. Cyclosporine-induced dyarthria and ataxia have been reported predominately in bone marrow transplant or liver transplant patients. The onset of symptoms is usually delayed, occurring after one to six months of treatment. Other neurologic side effects include confusion, delirium, depression, dizziness, hallucinations, headache, hyperesthesia, insomnia, memory deficits, migraines, paresthesias, and visual disorders. Somnolence and coma have also been reported.

Some degree of hirsutism or hypertrichosis occurs in most patients receiving cyclosporine. Pronounced darkening and thickening of eyebrows, side burns, and other secondary hair growth occurs in both men and women. Other dermatologic effects reported in more than 3% of patients include acneiform rash, alopecia, rash (unspecified), and skin ulcers. Coarsening of facial features has been reported in children receiving cyclosporine.

Gingival hyperplasia can occur in 4% to 16% of transplant patients receiving cyclosporine. One study reported an incidence of 30%. It is reversible one to two months following discontinuance of cyclosporine therapy.

Other adverse GI effects that occur in more than 3% of patients receiving cyclosporine include abdominal pain, anorexia, diarrhea, dyspepsia, flatulence, gingivitis, nausea/vomiting, and stomatitis.

Infections are common in patients receiving cyclosporine after organ transplantation or in the treatment of other autoimmune disorders due to the immunosuppressive effects of the drug. Local and systemic infections including fungal and viral infections (e.g., herpes simplex, herpes zoster), as well as sepsis, have been reported during cyclosporine therapy. Infections reported in 1% to 3% of patients receiving cyclosporine include abscess, cellulitis, folliculitis, renal abscess, moniliasis, and tonsillitis. Respiratory tract infections including bronchitis, pharyngitis, pneumonia, rhinitis, sinusitis, or other upper respiratory tract infections may occur. Other respiratory adverse reactions reported in $>3\%$ of patients receiving cyclosporine include bronchospasm, cough, and dyspnea.

Other adverse reactions reported in at least 2% of patients receiving cyclosporine include arthralgia, dysarthria (slurred speech), fatigue, fever, flu-like symptoms, flushing, gynecomastia, hyperglycemia, leg cramps, leukopenia, myalgia, and rigors. Cyclosporine may increase serum prolactin levels (i.e., hyperprolactinemia) but decreases serum testosterone levels. These changes may lead to menstrual irregularity or spermatogenesis inhibition and associated infertility.

Hypersensitivity reactions including anaphylactoid reactions have been reported during cyclosporine administration. The IV formulation contains 33% alcohol and a castor oil vehicle, which is believed to account for occasionally severe hypersensitivity reactions.

Following immunosuppressive therapy, patients may develop a secondary malignancy. Lymphomas, lymphoproliferative disorders, skin cancers, and other malignancies have been reported in patients following treatment with cyclosporine. The risk for the development of these conditions appears to be related to the intensity and duration of immunosuppression rather than the use of specific agents.

A.9.2. Objectives

In this study, the bioavailability of single dose 1 mL of 100 mg cyclosporine per 1 m solution of Gulf Pharmaceutical Industries (Sigmasporin Microoral); and Novartis Pharma (Sandimmun Neoral) were compared, under fasting conditions. Bioequivalence was investigated by determining the 90% confidence limits for the log-transformed ratio (test product/reference product) for the bioequivalence parameters (C_{max}, $AUC_{0\rightarrow t}$, and $AUC_{0\rightarrow\infty}$), while other pharmacokinetics values of K_e, $t_{(1/2)e}$, t_{max}, and $(AUC_{0\rightarrow t}/AUC_{0\rightarrow\infty})\%$ were reported. The influence of sequence, product, and period was tested by ANOVA.

A.9.3. Rationale of Study Design

This study was a single-center, open-label, randomized, single-dose study with two-way crossover design to compare the bioavailability of 100 mg cyclosporine per 1 mL between two products, in 30 healthy, adult, male volunteers. The study was conducted in preplanned scheme, as depicted in Table A.3. Eligible subjects who fit the selection criteria were dosed according to randomization plan prescribed.

A.9.4. Justification of the Study

Since drug formulation plays a key role in drug absorption, thus variations are expected from one formula to another for the same particular drug. Moreover, drug pharmacodynamics can be affected by its pharmacokinetics, which is invariably influenced by drug product formulation. All these necessitate the need for a metric tool to proof the drug pharmaceutical equivalence or bioequivalency. Accordingly, the interchangeable use of bioequivalent products is justified and should afford the same therapeutic efficacy.

A.9.5. Study Medication

Test Product—Treatment A
Brand Name: Sigmasporin Microoral
Dosage Form: Solution
Strength: 100 mg cyclosporine per 1 mL solution
Manufacturer: Gulf Pharmaceutical Industries (Julphar)
Batch No.: 015389

Reference Product—Treatment A
Brand Name: Sandimmun Neoral
Dosage Form: Solution

TABLE A.3 Study Schematic

	Study phase[a]		
Procedure	**Screening[b]**	**Day 1 of phase I**	**Day 1 of phase II**
Subject identification	☐	☐	☐
Demographic data	☐		
Medical history	☐		
Physical examination	☐		
Vital signs	☐	☐	☐
Hepatitis B	☐		
Hematology and coagulation	☐		
Clinical chemistry	☐		
Urinalysis	☐		
Selection criteria[c]	☐		
Informed consent[d]	☐		
Study drug administration		☐	☐
Check for other medication	☐	☐	☐
Blood sampling for pharmacokinetics		☐	☐
Check for adverse effect		☐	☐
Check for variations from clinical protocol	☐	☐	☐

[a] There was a washout period of seven days between two administrations of study drugs.
[b] Between 4 weeks and 48 hours, before first study drug administration in study Phase I.
[c] To be eligible for participation in the study, subjects must meet all selection criteria before the first study drug administration in study Phase I is established.
[d] Before screening examination, the subject has to sign the informed consent form.

Strength: 100 mg cyclosporine per 1 mL solution
Manufacturer: Novartis Pharma, Switzerland
Batch No.: 287

A.9.6. Sponsor

The study was sponsored by
Gulf Pharmaceutical Industries (Julphar), U.A.E.

A.9.7. Investigators and Study Facilities

The clinical part of the study was conducted at Al-Mowasah Hospital, Amman, Jordan, between 30/12/01(first screening examination) and 13/01/02 (last blood sample collection in study Phase II). The Principal Investigator was Prof. Naji Najib. Bioanalysis of cyclosporine was performed on an HPLC equipped with MS detector. The calculations of the pharmacokinetics and statistical evaluation of data were performed by R. Tayyem (B.Sc. Pharm., M.Sc.), under the supervision of Prof. Naji Najib. The study was conducted by IPRC staff according to the international GCP guidelines and supervised by S. Al-Masri (B.Sc. Pharm.), Clinical Supervisor. The Bioanalysis was performed, according to the international GLP guidelines, under the supervision of M. Bader (B.Sc. Chem.), Laboratory Supervisor. The QAU was entirely involved in auditing and checking, throughout the study conduction and completion.

A.9.8. Experimental

A.9.8.1. Study Design and Description

The study was a single-center, open-label, randomized, two-way crossover study, to evaluate the bioequivalence of cyclosporine given as a single-dose from two products to healthy male volunteers with a washout period of seven days. The clinical part of the study is proposed in the clinical protocol [see Appendix B] and data are detailed in the clinical report. The Principal Investigator was involved throughout the propagation of the study until completion, for in advance authorization.

Treatment A: A single dose, 1 mL of Sigmasporin Microoral Solution, 100 mg cyclosporine per 1 mL solution. Gulf Pharmaceutical Industries (Julphar), U.A.E.

Treatment B: A single dose, 1 mL of Sandimmun Neoral Solution, 100 mg cyclosporine per 1 mL solution. Novartis Pharma, Switzerland

Administered treatments were sequenced according to the randomization plan shown in Table 1 (Appendix 4 of section II). Between the two administrations of the study drugs, a washout period of seven days was allowed. Per each phase, 17 blood samples were collected for analytical purposes. Safety was monitored by complete physical and medical examination with clinical laboratory testing and scheduled vital signs surveillance. The bioequivalence was studied by comparing C_{max}, $AUC_{0\rightarrow t}$, and $AUC_{0\rightarrow\infty}$ of the test product with the reference product. Other pharmacokinetic parameters (K_e, $t_{(1/2)e}$, and t_{max}) were further investigated for proper characterization of the study. The ($AUC_{0\rightarrow t}$/AUC $_{0\rightarrow\infty}$) values were devised to assess the adequacy of sampling time.

A.9.8.2. Institutional Review Board

The clinical protocol was reviewed by the IRB of Al-Mowasah Hospital, Amman, Jordan. The approval of the IRB for the clinical protocol was given on December 29, 2001, as demonstrated in Appendix A.

A.9.8.3. Subject Selection and Informed Consent

For participation in the study, subjects had to meet the selection criteria outlined in the clinical protocol [see Appendix B of this section]. Screening procedures were performed before dosing. Volunteers were informed, by IPRC representative, about the aim of the study and any potential

risk associated with the study. Before their entry to the study, eligible volunteers signed a written informed consent statement and they were free to withdraw at any time during the course of the study.

A.9.8.4. Administration of Study Drugs

Actual study drug administration times were in the morning but between 8.20 and 8.32 hours in Phase I; and 7.55 and 8.07 hours in Phase II.

The study drug was given as follows:

Treatment A: Single dose of 1 mL solution of the test product was given with 240 mL water. Water was at room temperature and was measured with a 100 mL cylinder. Visual inspection of the mouth of the subject immediately after the study drug administration was done to ensure that the solution was swallowed by the subject.

Treatment B: Single dose of 1 mL solution of the reference product was given with 240 mL water. Water was at room temperature and was measured with a 100 mL cylinder. Visual inspection of the mouth of the subject immediately after the study drug administration was done to ensure that the solution was swallowed by the subject.

A.9.8.5. Dietary Restriction and Physical Activities After Drug Intake

A.9.8.5.1. Dietary Restrictions

No consumption of alcohol was permitted for the subject from 24 hours prior to the first study drug administration until the end of the 24-hour sample. No consumption of any beverages or foods containing methylxanthines, e.g., caffeine (coffee, tea, cola, cocoa, Chocolate, etc.) was permitted for the subjects 24 hours prior to study drug administration of either study phase until the collection of last blood sample of the respective study phase. In addition, the consumption of any beverages or foods containing grapefruit was prohibited one week before study drug administration of either study phase until the collection of last blood sample of the respective study phase.

Subjects were confined from time 7.00 hours of day -1 until the 24-hour blood sample of study day 1 had been collected. The subjects were allowed to leave the clinical site after the 24-hour blood sample. In the evening before study day -1 of each study phase, all subjects received an identical meal, which had been finished at least 10 hours before study drug administration in the morning of study day 1 of each study phase.

Food and fluids given in both study phases from study day -1 until 10 hours after study drug administration were identical in composition and amount. The subjects received food as follows in each study phase:

Light standardized dinner: Finished at least 10 hours before study drug administration.
Light standardized lunch: Five hours after study drug administration.
Light standardized dinner: Twelve hours after study drug administration.

From 10 hours to 1 hour before dosing, no excessive fluid intake (>120 mL/h) was allowed. No fluid was allowed one hour prior to study drug administration. Following study drug administration with 240 mL water (room temperature), the volunteers were allowed to drink water (but not exceeding 120 mL/h) starting two hours after drug administration until study day 2. The amount and time of intake were carefully monitored for five hours following drug administration.

After study drug administration, the subjects sat or walked around and went to toilet until the five hours blood collection, except for the time of blood sampling. The room for blood collection and the toilets were in the same floor. After study drug administration, the subjects were ambulatory reading or watching television. Great care was taken by the study personnel to ensure that the physical activity of the subjects was similar in each study day of each study phase.

A.9.8.6. Collection of Blood Samples for Analysis

The following blood samples for analysis of cyclosporine in plasma were taken: immediately before (2×1 mL) and at 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.50, 3.00, 4.00, 6.00, 8.00, 12.00, 16.00, and

24.00 hours after administration of study drugs (1×10 mL). The number of blood collections for drug analysis was 17 samples in each study phase.

The volume of blood taken for determination of cyclosporine in plasma was 10 mL. In the morning of study day 1 of each study an indwelling catheter was placed into the subjects' 'forearm veins after study drug administration, but before predose sampling, in the morning of study day 1 of each study phase and remained there until the collection of 24-hour blood sample was collected.

Blood samples were collected into tubes containing lithium heparin as an anticoagulant (Dispo®, AFMA, Jordan), slightly shaken and centrifuged at approximately 3500 r.p.m. for 10 minutes. After centrifugation, plasma samples were transferred directly into a 5 mL plastic tubes (Dispo, AFMA, Jordan). These samples were immediately stored at the clinical site in a freezer at a nominal temperature of −20°C.

Transportation of samples from clinical site to IPRC was carried out after preserving the tubes in dry ice containers to guarantee a temperature at a nominal of −20°C. To maintain constant temperature during shipment time, enough dry ice was placed to preserve samples from their departure from clinical site until their delivery to the analytical site of IPRC. At IPRC, clinical plasma samples were stored immediately in a freezer at a nominal temperature −20°C.

A.9.8.7. Analysis of Cyclosporine

A specific HPLC with MS detection assay was developed in the IPRC for the determination of cyclosporine in human plasma. Details of the assay procedure are given in section III "Bioanalytical Method Validation Report." (For brevity, this report is not included in this chapter.)

A.9.8.8. Pharmacokinetic Calculations

Under the direction of the Principal Investigator Prof. Naji Najib, the pharmacokinetic parameters of cyclosporine were estimated using standard noncompartmental methods, by R. Tayyem (B.Sc. Pharm., M.Sc.). All parameters were determined from true (actual) sample collection times and assayed plasma concentrations at these times.

The maximal plasma concentration (C_{max}) and the time to peak plasma concentration (t_{max}) of cyclosporine A were taken directly from the measured data.

The area under the plasma concentration–time curve ($AUC_{0\rightarrow t}$) was calculated from measured data points from the time of administration to the time of last quantifiable concentration by the linear trapezoidal rule.

The area under the plasma concentration–time curve extrapolated to infinity ($AUC_{0\rightarrow\infty}$) was calculated according to the following formula:

$$AUC_{0\rightarrow\infty} = AUC_{0\rightarrow t} + C_{last}/[\text{In}(2)/t_{(1/2)e}],$$

where C_{last} is the last quantifiable concentration.

The ratio as a $(AUC_{0\rightarrow\infty}/AUC_{0\rightarrow t})\%$ was determined as an indicator for the adequacy of sampling times.

The elimination half-life ($t_{(1/2)e}$) was calculated as $t_{1/2e} = \ln(2)/(-b)$, where b was obtained as the slope of the linear regression of the ln-transformed plasma concentrations versus time in the terminal phase of the plasma curve.

The pharmacokinetic calculations were performed on a Pentium MMX MHz Computer using the computer program Kinetica 2000. All pharmacokinetic calculations for individual subjects, means, and standard deviations were performed without rounding. For this report, results were rounded to two decimal places.

A.9.8.9. Randomization

The study was randomized as a two-way crossover design. Administration was done according to a plan of randomization (see Table 1 found in Appendix 4 of section II).

A.9.8.10. Statistical Analysis

Statistical analysis was performed by R. Tayyem (B.Sc. Pharm., M.Sc.) using the Microsoft Excel (version 7) and the Kinetica 2000 program.

A.9.8.10.1. Assessment of Bioequivalence

The extent of absorption is determined by $AUC_{0\to t}$ and $AUC_{0\to\infty}$. The rate of absorption is determined by C_{max}. For the parametric analysis of bioequivalence for log-transformed data, the acceptance boundaries were set at 80% to 125% for $AUC_{0\to t}$, $AUC_{0\to\infty}$, and C_{max}.

A multiplicative model with respect to the untransformed bioequivalence parameters was selected. A logarithmic transformation of the original data was used. Under the assumption of a logarithmic normal distribution, a parametric approach recommended by Steinijans and Diletti based on the inclusion of the shortest 90% confidence interval in the bioequivalence range was adopted.

A.9.8.10.2. ANOVA

ANOVA tested for sequence, period, subject (sequence), and treatment effects was used. ANOVA was performed on $AUC_{0\to t}$, $AUC_{0\to\infty}$, C_{max}, $t_{(1/2)e}$, K_e, log $AUC_{0\to t}$, log $AUC_{0\to\infty}$, and log C_{max}. p-Values of more than 0.05 in the log data indicates bioequivalence.

A multiplicative linear model was used for the two-way crossover design:

$$Y_{ijk} = \log(X_{ijk}) = \mu + G_k S_{ik} + P_j + F(j,k) + e_{ijk},$$

where Y_{ijk}, a pharmacokinetic parameter of the ith subject ($i=1,2,\ldots n_k$) in the sequence ($k=1,2,\ldots k$) for the jth period ($j=1,2,\ldots j$); μ, the overall mean; G_k, the fixed effect of the kth sequence; S_{ik}, the random effect of the ith subject in the kth sequence; P_j, the fixed effect of the jth period; E (j,k), the fixed effect of the formulation in the kth sequence, which is administered at the jth period and e_{ijk}, the (within-subject) random error in observing Y_{ijk}.

It was assumed that $\{S_{ik}\}$ and $\{e_{ijk}\}$ are mutually independent and normally distributed with mean zero and variances σ_s^2 and σ_e^2.

A.9.9. Results

A.9.9.1. Study Population

The study design required 30 healthy male volunteers, aged between 18 and 45 years. The individual demographic data of the 30 subjects entering and completing the study are shown in Table C.1 of Appendix C. The actual age of the subjects ranged from 18 and 35 years with a mean value ($\pm$SD) of 22.70$\pm$3.92 years. The mean weight ($\pm$SD) at screening examination was 68.33$\pm$10.36 kg (range: 52–103 kg) and the mean height ($\pm$SD) was 172.43$\pm$6.59 cm (range: 162–190 cm).

A.9.9.2. Clinical Observations

The study was performed according to GCP/ICH guidelines by the Clinical Investigators. There were no significant deviations from the clinical protocol that could have affected the outcome of this study. All 30 subjects met the inclusion criteria described in the clinical protocol.

Adverse events were assessed using spontaneous reporting of the subjects and asking subjects after any AE. During this study, few AEs occurred and were handled by the Clinical Investigator. These findings demonstrate the excellent tolerance of the two products in these healthy volunteers (Table C.2).

A.9.9.3. Data Tabulation and Descriptive Statistics

All data and results are summarized in tables given in Appendix C, as follows:

The individual plasma concentrations of cyclosporine after administration of Treatment A (test product) and Treatment B (reference product) are listed in Tables C.3 and C.4. The individual results of cyclosporine for C_{max}, t_{max}, $AUC_{0\to t}$, $AUC_{0\to\infty}$, and $t_{(1/2)e}$ of Treatments A and B are shown in Tables C.5 and C.6, respectively. In Tables C.7, C.8, and C.9, the 90% confidence intervals based on parametric procedure for $AUC_{0\to t}$, $AUC_{0\to\infty}$, and C_{max} are depicted. The results from ANOVA of the log-transformed and untransformed data for C_{max}, $AUC_{0\to t}$, and $AUC_{0\to\infty}$; and for the untransformed data $t_{(1/2)e}$, K_e, and t_{max} are shown in Table C.10.

A.9.9.4. Figures and Diagrammatic Data Presentation

All diagrammatic data presentation for cyclosporine are given as figures depicted in Appendix C.

TABLE C.1 Demographic Data and Sequence of CYC-GUL-L1001/155 Participating Volunteers

Subject number	Sequence	Age (years)	Body weight (kg)	Body height (cm)
1	BA	24	173	70
2	AB	29	174	77
3	AB	21	164	66
4	AB	24	165	56
5	BA	19	169	78
6	AB	22	176	60
7	AB	22	167	52
8	BA	22	162	69
9	BA	25	176	63
10	BA	19	181	86
11	BA	24	169	74
12	BA	22	172	55
13	BA	24	179	71
14	BA	19	170	65
15	AB	19	178	69
16	BA	21	179	74
17	BA	23	190	103
18	BA	24	168	76
19	AB	26	55	166
20	AB	22	67	179
21	BA	35	62	179
22	AB	25	70	169
23	AB	18	62	169
24	AB	20	79	178
25	AB	19	61	169
26	AB	28	61	179
27	AB	29	59	166
28	BA	20	71	163
29	BA	18	71	167
30	AB	18	68	177
N		30	30	30
Mean		22.70	68.33	172.43
SD		3.92	10.36	6.59
SEM		0.72	1.89	1.20
CV%		17.28	15.16	3.82
Minimum		18	52	162
Median		22	68.5	171
Maximum		35	103	190

A.9.9.5. Pharmacokinetic Results

A.9.9.5.1. Treatment A: Sigmasporin Microoral Solution

- The measured peak plasma concentrations C_{max} ranged from 100.8 to 398.5 ng/mL with a mean C_{max} value of 250.0 ± 88.8 ng/mL.
- The $AUC_{0\rightarrow t}$ values ranged from 256.137 to 1382.850 ng·hr/mL with a mean $AUC_{0\rightarrow t}$ value of 559.775 ± 219.032 ng·hr/mL.
- The $AUC_{0\rightarrow \infty}$ values ranged from 291.559 to 1455.680 ng·hr/mL with a mean $AUC_{0\rightarrow t}$ value of 617.148 ± 235.742 ng·hr/mL.
- The time t_{max} to reach peak plasma concentration ranged from 0.75 to 2.50 hours with a mean t_{max} value of 1.31 ± 0.34 hours.
- The ratio $(AUC_{0\rightarrow t}/AUC_{0\rightarrow \infty})\%$ ranged from 73.75% to 98.07% with a mean value of $(AUC_{0\rightarrow t}/AUC_{0\rightarrow \infty})\%$ of 90.94 ± 6.01.

TABLE C.2 Administration Times of Study Drugs in Clinical Study of CYC-GUL-L1001/155

	Drug administration in phase I		Drug administration in Phase II	
Subject number	**Date (DD/MM/YY)**	**Time (HH.MM)**	**Date (DD/MM/YY)**	**Time (HH.MM)**
1	05/01/02	8.20	12/1/2002	7:55
2	05/01/02	8.22	12/1/2002	7:57
3	05/01/02	8.24	12/1/2002	7:59
4	05/01/02	8.26	12/1/2002	8:01
5	05/01/02	8.28	12/1/2002	8:03
6	05/01/02	8.05	12/1/2002	8:05
7	05/01/02	8.32	12/1/2002	8:07
8	05/01/02	8.20	12/1/2002	7:55
9	05/01/02	8.22	12/1/2002	7:57
10	05/01/02	8.24	12/1/2002	7:59
11	05/01/02	8.26	12/1/2002	8:01
12	05/01/02	8.28	12/1/2002	8:03
13	05/01/02	8.30	12/1/2002	8:05
14	05/01/02	8.32	12/1/2002	8:07
15	05/01/02	8.20	12/1/2002	7:55
16	05/01/02	8.22	12/1/2002	7:57
17	05/01/02	8.24	12/1/2002	7:59
18	05/01/02	8.26	12/1/2002	8:01
19	05/01/02	8.28	12/1/2002	8:03
20	05/01/02	8.30	12/1/2002	8:05
21	05/01/02	8.20	12/1/2002	7:55
22	05/01/02	8.22	12/1/2002	7:57
23	05/01/02	8.24	12/1/2002	7:59
24	05/01/02	8.26	12/1/2002	8:01
25	05/01/02	8.28	12/1/2002	8:03
26	05/01/02	8.30	12/1/2002	8:05
27	05/01/02	8.32	12/1/2002	8:07
28	05/01/02	8.20	12/1/2002	7:55
29	05/01/02	8.22	12/1/2002	7:57
30	05/01/02	8.24	12/1/2002	7:59

A.9.9.5.2. Treatment B: Sandimmun Neoral Solution

- The measured peak plasma concentrations C_{max} ranged from 126.4 to 398.1 ng/mL with a mean C_{max} value of 271.7 ± 80.2 ng/mL.
- The $AUC_{0\rightarrow t}$ values ranged from 213.650 to 1106.600 ng hr/mL with a mean $AUC_{0\rightarrow t}$ value of 577.804 ± 213.396 ng hr/mL.
- The $AUC_{0\rightarrow \infty}$ values ranged from 254.107 to 1189.230 ng hr/mL with a mean $AUC_{0\rightarrow t}$ value of 621.125 ± 230.127 ng hr/mL.
- The time t_{max} to reach peak plasma concentration ranged from 0.75 to 1.75 hours with a mean t_{max} value of 1.35 ± 0.24 hours.
- The ratio $(AUC_{0\rightarrow t}/AUC_{0\rightarrow \infty})\%$ ranged from 83.86% to 98.05% with a mean value of $(AUC_{0\rightarrow t}/AUC_{0\rightarrow \infty})\%$ of $92.96 \pm 3.44\%$.

A.9.9.5.3. Results of ANOVA

ANOVA of log-transformed data for C_{max}, $AUC_{0\rightarrow t}$ and $AUC_{0\rightarrow \infty}$ and for the untransformed data for C_{max}, $AUC_{0\rightarrow t}$, $AUC_{0\rightarrow \infty}$, $t_{(1/2)e}$, K_e, and t_{max} demonstrated that sequence effect, product effect, and period effect for all bioequivalence metrics did not significantly influence the outcome of the study. The cyclosporine results from the ANOVA table for each bioequivalence metric are located in Table C.10. Further details may be found in Appendix 4 of this section "Statistical Outputs."

(Text continues on page 402)

TABLE C.3 Individual Plasma Concentrations of Cyclosporine Versus Time After Single-Dose Administration of Treatment A: Test Product Sigmasporin Microoral® 100 mg Cyclosporine Solution

	Cyclosporine plasma concentrations (ng/mL)															
	Time (hr)															
Subject number	**0.25**	**0.50**	**0.75**	**1.00**	**1.25**	**1.50**	**1.75**	**2.00**	**2.50**	**3.00**	**4.00**	**6.00**	**8.00**	**12.00**	**16.00**	**24.00**
1	19.7	44.6	98.3	199.5	195.8	272.9	174.6	139.8	82.9	60.2	31.7	22.6	21.1	—	11.0	8.7
2	11.9	20.1	63.7	153.6	156.4	175.9	127.3	94.7	50.9	46.2	30.2	20.2	—	11.9	10.9	—
3	7.8	61.5	170.9	217.0	162.7	174.6	104.1	94.9	51.6	45.4	25.0	13.1	11.3	8.1	<	<
4	<	60.5	179.7	254.7	361.7	251.1	172.9	138.5	94.0	46.0	22.5	15.2	10.1	5.5	<	<
5	11.0	117.8	227.5	241.8	247.7	150.3	132.6	127.8	72.6	65.2	33.9	20.8	12.3	<	<	<
6	8.7	72.9	125.0	280.1	216.1	191.5	166.4	122.8	83.5	65.1	34.6	27.1	17.1	8.0	7.2	<
7	<	93.4	224.4	352.6	252.9	229.2	127.0	105.5	69.5	54.9	38.0	16.5	<	<	<	<
8	<	75.6	194.9	216.4	194.5	184.0	130.0	99.2	50.9	29.7	26.4	12.7	<	<	<	<
9	<	105.9	190.7	188.8	260.1	187.8	203.3	158.5	97.7	80.7	33.0	25.0	16.6	7.9	<	<
10	<	56.8	101.9	252.2	397.1	371.8	185.4	199.0	120.8	51.3	28.2	15.9	11.0	<	<	<
11	33.1	105.9	193.5	204.5	190.5	195.8	181.8	91.2	86.8	41.2	35.0	12.2	<	<	<	<
12	<	65.5	227.8	305.8	393.0	398.5	321.4	347.6	209.0	168.2	140.7	84.8	58.6	18.7	<	<
13	15.3	76.5	244.9	229.5	263.7	271.3	233.6	197.6	109.2	80.7	76.4	36.7	24.2	11.8	<	<
14	44.4	127.2	224.4	200.1	194.0	138.7	148.3	76.9	61.8	40.1	26.4	10.9	8.0	<	<	<
15	<	21.3	101.4	109.8	149.2	109.6	126.3	129.4	75.7	66.1	21.6	5.3	<	<	<	<
16	5.5	60.0	140.4	144.4	195.9	175.9	166.1	127.1	80.4	63.7	17.9	12.1	<	<	<	<
17	9.4	60.8	130.9	128.6	159.0	139.8	153.9	131.7	130.1	46.0	30.1	11.4	6.9	<	<	<
18	28.7	208.8	272.0	336.4	245.2	239.9	273.4	207.0	90.7	83.1	45.8	21.5	7.4	<	<	<
19	9.4	16.8	44.8	100.2	151.0	192.8	260.4	215.2	128.8	104.9	71.5	48.3	—	18.7	14.7	—
20	<	37.1	100.8	90.1	90.9	88.6	72.3	74.3	80.8	31.1	22.0	11.6	<	<	<	<
21	17.0	83.8	171.3	282.8	268.1	345.5	204.2	107.8	82.6	65.5	55.5	22.0	16.3	11.1	<	<
22	6.9	16.1	25.9	32.7	35.0	68.9	82.6	70.9	116.8	90.7	49.0	16.6	13.3	<	<	<
23	<	22.4	51.0	121.5	92.1	131.3	109.1	72.8	56.4	37.2	27.8	—	11.1	5.2	<	<
24	<	37.8	60.8	66.3	104.9	147.8	137.6	81.3	83.1	70.0	31.0	30.0	21.0	12.6	<	<
25	<	80.7	104.6	180.6	193.4	280.3	171.7	103.0	71.5	42.8	37.0	21.8	16.1	14.0	8.6	<
26	12.8	58.1	138.5	125.1	159.4	91.8	90.7	80.7	51.6	40.4	40.3	23.4	22.4	14.0	15.2	8.7
27	<	9.7	84.2	232.5	366.6	308.8	373.0	205.2	206.6	100.8	26.7	13.2	6.7	<	<	<
28	<	91.7	270.4	387.6	394.9	284.5	257.0	246.9	119.8	105.7	50.8	43.0	13.6	<	<	<
29	<	24.6	73.2	161.4	212.1	198.7	128.4	160.3	115.0	54.6	32.4	13.9	<	<	<	<
30	45.6	113.9	238.1	184.9	258.0	170.8	125.6	122.6	92.3	33.1	15.1	7.4	<	<	<	<
N	16	30	30	30	30	30	30	30	30	30	30	29	20	13	6	2
Mean	9.6	67.6	149.2	199.4	218.7	205.6	171.4	137.7	94.1	63.7	38.6	21.2	10.8	4.9	2.3	0.6
SD	13.1	42.6	72.3	85.5	93.3	83.0	70.2	62.6	38.8	29.4	23.9	15.9	12.0	6.4	4.8	2.2
SEM	136.5	63.0	48.5	42.9	42.6	40.4	41.0	45.5	41.2	46.1	62.0	75.2	110.7	129.6	211.8	380.6
CV%	2.4	7.8	13.2	15.6	17.0	15.2	12.8	11.4	7.1	5.4	4.4	2.9	2.2	1.2	0.9	0.4
Minimum	0.0	9.7	25.9	32.7	35.0	68.9	72.3	70.9	50.9	29.7	15.1	0.0	0.0	0.0	0.0	0.0
Maximum	45.6	208.8	272.0	387.6	397.1	398.5	373.0	347.6	209.0	168.2	140.7	84.8	58.6	18.7	15.2	8.7

<, below limit of quantitation; —, missing sample.

TABLE C.4 Individual Plasma Concentrations of Cyclosporine Versus Time After Single-Dose Administration of Treatment B: Reference Product Sandimmun Neoral® 100 mg Cyclosporine Solution

	Cyclosporine plasma concentrations (ng/mL)															
	Time (hr)															
Subject number	**0.25**	**0.50**	**0.75**	**1.00**	**1.25**	**1.50**	**1.75**	**2.00**	**2.50**	**3.00**	**4.00**	**6.00**	**8.00**	**12.00**	**16.00**	**24.00**
1	15.6	61.0	185.1	256.1	269.7	301.8	291.9	149.7	119.2	97.7	54.6	29.7	24.0	18.5	11.0	6.4
2	8.9	24.4	93.4	157.4	152.3	175.5	152.4	117.3	65.1	52.0	20.1	12.9	7.5	—	—	<
3	18.6	113.3	176.7	252.3	210.2	166.8	139.5	93.1	67.5	41.6	32.1	18.2	10.1	7.1	<	<
4	13.2	106.1	367.2	282.1	242.3	199.2	229.6	116.5	81.4	80.2	22.3	21.5	13.0	7.4	<	<
5	<	9.3	74.3	202.9	223.7	207.4	166.9	113.0	80.4	44.8	39.5	—	14.4	11.7	5.8	<
6	7.7	48.7	164.0	263.2	272.0	234.0	243.3	239.6	141.5	69.6	31.7	23.3	13.7	—	<	<
7	25.1	104.7	232.4	387.0	282.2	241.7	182.1	135.5	97.9	77.9	48.5	45.2	12.5	<	<	<
8	<	<	62.3	165.9	206.7	92.6	95.4	81.7	61.8	21.2	13.4	<	<	<	<	<
9	<	62.6	145.9	228.0	253.3	287.3	276.4	229.9	142.4	127.1	53.9	19.9	18.0	9.5	<	<
10	<	47.3	173.3	255.4	310.6	398.1	271.7	209.9	105.9	74.5	44.1	27.9	13.3	<	<	<
11	12.5	13.9	68.6	203.2	259.0	267.4	170.2	141.7	90.7	67.3	46.8	21.5	12.1	<	<	<
12	20.5	118.6	298.4	374.5	392.6	375.9	320.7	267.4	175.8	113.1	82.4	54.2	31.3	16.2	<	<
13	25.0	107.4	204.1	200.0	377.5	309.4	391.8	275.1	170.1	76.4	88.1	50.0	40.8	18.5	<	<
14	<	21.1	109.4	182.7	271.7	182.9	140.7	108.4	85.5	46.0	38.8	16.2	9.0	<	<	<
15	9.6	62.6	88.7	204.9	290.7	223.2	140.7	127.4	79.3	49.9	9.4	6.6	<	<	<	<
16	6.5	95.3	222.5	277.3	290.6	211.8	150.0	109.5	58.0	46.4	22.3	8.8	<	<	<	<
17	<	<	36.7	142.6	187.2	225.1	130.9	158.8	79.4	66.4	32.8	12.3	7.5	<	<	<
18	<	114.7	158.3	224.0	366.9	294.5	204.9	176.1	127.6	77.9	61.5	21.3	8.4	<	<	<
19	21.9	47.8	194.8	223.6	234.5	241.3	174.8	171.4	97.1	75.4	36.8	28.2	24.6	15.1	10.3	<
20	6.4	64.9	71.2	74.1	126.4	104.5	74.4	60.0	41.1	39.9	20.4	—	—	<	<	<
21	16.6	97.1	184.5	311.1	245.6	192.0	161.1	114.0	72.6	56.2	34.6	21.3	16.1	7.6	<	<
22	9.0	44.1	42.0	65.9	99.0	129.9	100.7	99.5	95.3	104.2	76.7	36.5	13.8	<	<	<
23	<	41.0	52.9	138.0	231.6	276.8	189.7	92.3	82.3	58.5	34.0	19.8	8.6	<	<	<
24	<	11.8	40.1	90.8	108.7	115.2	135.7	91.7	71.1	63.4	27.8	19.3	<	<	<	<
25	11.0	77.9	128.9	182.8	250.5	237.8	109.8	153.4	66.6	54.6	22.8	18.2	16.1	6.6	<	<
26	18.7	46.5	124.3	161.2	133.6	142.9	114.4	103.9	72.6	52.7	28.7	18.2	13.1	10.2	<	<
27	<	43.2	99.8	156.9	232.9	278.1	381.6	328.9	249.3	183.8	69.5	41.6	12.4	<	<	<
28	<	<	9.2	132.1	122.0	245.8	231.8	242.4	172.0	122.2	72.2	39.3	15.5	<	<	<
29	<	73.8	172.7	227.8	213.2	229.0	206.1	214.4	68.7	70.3	32.4	12.2	<	<	<	<
30	<	6.8	83.4	137.4	284.3	293.3	238.2	193.8	109.2	80.1	47.3	13.9	<	<	<	<
N	17	27	30	30	30	30	30	30	30	30	30	27	23	11	3	1
Mean	8.2	55.5	135.5	205.4	238.1	229.4	193.9	157.2	100.9	73.0	41.5	21.9	11.9	4.3	0.9	0.2
SD	8.7	38.6	81.6	77.4	75.3	73.5	80.7	67.0	44.9	32.4	20.6	14.0	9.6	6.3	2.9	1.2
SEM	105.5	69.5	60.2	37.7	31.6	32.1	41.6	42.6	44.5	44.3	49.6	63.8	81.2	148.0	316.0	547.7
CV%	1.6	7.0	14.9	14.1	13.8	13.4	14.7	12.2	8.2	5.9	3.8	2.6	1.8	1.2	0.5	0.2
Minimum	0.0	0.0	9.2	65.9	99.0	92.6	74.4	60.0	41.1	21.2	9.4	0.0	0.0	0.0	0.0	0.0
Maximum	25.1	118.6	367.2	387.0	392.6	398.1	391.8	328.9	249.3	183.8	88.1	54.2	40.8	18.5	11.0	6.4

<, below limit of quantitation; —, missing sample.

TABLE C.5 Individual Pharmacokinetics of Cyclosporine After Single-Dose Administration of Treatment A: Test Product Sigmasporin Microoral® 100 mg Cyclosporine Solution

		Pharmacokinetics[a]								
Subject number	Sequence	C_{max} (ng/mL)	t_{max} (hr)	$AUC_{0\rightarrow t}$ (ng hr/mL)	$AUC_{0\rightarrow\infty}$ (ng hr/mL)	K_e (1/hr)	K_{start}	K_{end}	$t_{(1/2)e}$ (hr)	$(AUC_{0\rightarrow t}/AUC_{0\rightarrow\infty})\%$
1	BA	272.9	1.50	711.425	868.544	0.0554	8.00	24.00	12.5180	81.91
2	AB	175.9	1.50	480.238	651.127	0.0638	6.00	16.00	10.8671	73.75
3	AB	217.0	1.00	433.888	534.422	0.0806	6.00	12.00	8.6032	81.19
4	AB	361.7	1.25	559.038	590.330	0.1758	4.00	12.00	3.9437	94.70
5	BA	247.7	1.25	520.050	568.580	0.2535	4.00	8.00	2.7348	91.46
6	AB	280.1	1.00	605.600	656.421	0.1417	4.00	16.00	4.8926	92.26
7	AB	352.6	1.00	508.863	549.799	0.4031	3.00	6.00	1.7197	92.55
8	BA	216.4	1.00	386.075	429.126	0.2950	3.00	6.00	2.3497	89.97
9	BA	260.1	1.25	618.063	659.404	0.1911	6.00	12.00	3.6273	93.73
10	BA	397.1	1.25	599.900	646.638	0.2354	4.00	8.00	2.9451	92.77
11	BA	204.5	1.00	449.475	473.580	0.5061	2.00	6.00	1.3696	94.91
12	BA	398.5	1.50	1382.850	1455.680	0.2568	6.00	12.00	2.6996	95.00
13	BA	271.3	1.50	807.125	869.975	0.1877	6.00	12.00	3.6919	92.78
14	BA	224.4	0.75	428.488	455.290	0.2985	4.00	8.00	2.3223	94.11
15	AB	149.2	1.25	328.050	334.503	0.8213	3.00	6.00	0.8439	98.07
16	BA	195.9	1.25	396.637	415.530	0.6405	1.75	6.00	1.0823	95.45
17	BA	159.0	1.25	419.387	438.125	0.3683	4.00	8.00	1.8823	95.72
18	BA	336.4	1.00	705.500	721.739	0.4557	4.00	8.00	1.5211	97.75
19	AB	260.4	1.75	840.975	939.793	0.1488	3.00	16.00	4.6595	89.49
20	AB	100.8	0.75	256.137	291.559	0.3275	3.00	6.00	2.1166	87.85
21	BA	345.5	1.50	672.375	771.971	0.1115	6.00	12.00	6.2193	87.10
22	AB	116.8	2.50	340.037	373.958	0.3921	3.00	8.00	1.7678	90.93
23	AB	131.3	1.50	339.550	361.560	0.2363	2.50	12.00	2.9339	93.91
24	AB	147.8	1.50	458.037	546.662	0.1422	6.00	12.00	4.8754	83.79
25	AB	280.3	1.50	579.900	680.928	0.0851	6.00	16.00	8.1427	85.16
26	AB	159.4	1.25	611.913	773.155	0.0540	6.00	24.00	12.8465	79.14
27	AB	373.0	1.75	672.700	692.084	0.3456	4.00	8.00	2.0054	97.20
28	BA	394.9	1.25	829.087	865.832	0.3701	2.50	8.00	1.8727	95.76
29	BA	212.1	1.25	420.663	451.459	0.4513	3.00	6.00	1.5357	93.18
30	AB	258.0	1.25	431.225	446.675	0.4790	3.00	6.00	1.4472	96.54
N		30	30	30	30	30	30	30	30	30
Mean		250.0	1.31	559.775	617.148	0.2858	4.23	10.67	4.0012	90.94
SD		88.8	0.34	219.032	235.742	0.1825	1.58	4.96	3.3498	6.01
CV%		35.5	25.92	39.129	38.199	63.8622	37.31	46.54	83.7191	6.61
SEM		16.2	0.06	39.990	43.040	0.0333	0.29	0.91	0.6116	1.10
Median		252.9	1.25	514.457	579.455	0.2551	4.00	8.00	2.7172	92.77
Minimum		100.8	0.75	256.137	291.559	0.0540	1.75	6.00	0.8439	73.75
Maximum		398.5	2.50	1382.850	1455.680	0.8213	8.00	24.00	12.8465	98.07

[a] As calculated by Kinetica TM 2000.

TABLE C.6 Individual Pharmacokinetics of Cyclosporine After Single-Dose Administration of Treatment B: Reference Product Sandimmun Neoral® 100 mg Cyclosporine Solution

		Pharmacokinetics[a]								
Subject number	Sequence	C_{max} (ng/mL)	t_{max} (hr)	$AUC_{0\rightarrow t}$ (ng hr/mL)	$AUC_{0\rightarrow \infty}$ (ng hr/mL)	K_e (1/hr)	K_{start}	K_{end}	$t_{(1/2)e}$ (hr)	$(AUC_{0\rightarrow t}/AUC_{0\rightarrow \infty})\%$
1	BA	301.8	1.50	913.212	988.074	0.0855	12.00	24.00	8.1079	92.42
2	AB	175.5	1.50	370.062	400.494	0.2465	4.00	8.00	2.8125	92.40
3	AB	252.3	1.00	498.262	536.397	0.1862	4.00	12.00	3.7229	92.89
4	AB	367.2	0.75	634.712	677.613	0.1725	6.00	12.00	4.0184	93.67
5	BA	223.7	1.25	552.050	603.074	0.1137	8.00	16.00	6.0978	91.54
6	AB	272.0	1.25	628.875	694.197	0.2097	4.00	8.00	3.3050	90.59
7	AB	387.0	1.00	697.638	733.602	0.3476	2.00	8.00	1.9943	95.10
8	BA	206.7	1.25	239.863	254.107	0.9407	1.75	4.00	0.7368	94.39
9	BA	287.3	1.50	759.762	833.717	0.1285	6.00	12.00	5.3960	91.13
10	BA	398.1	1.50	686.888	731.269	0.2997	4.00	8.00	2.3130	93.93
11	BA	267.4	1.50	522.962	558.743	0.3382	4.00	8.00	2.0497	93.60
12	BA	392.6	1.25	1106.600	1189.230	0.1960	6.00	12.00	3.5356	93.05
13	BA	391.8	1.75	1040.860	1149.510	0.1703	6.00	12.00	4.0706	90.55
14	BA	271.7	1.25	444.625	469.262	0.3653	4.00	8.00	1.8975	94.75
15	AB	290.7	1.25	400.650	408.636	0.8265	1.50	6.00	0.8387	98.05
16	BA	290.6	1.25	460.613	476.701	0.5470	2.50	6.00	1.2673	96.63
17	BA	225.1	1.50	410.975	431.307	0.3689	4.00	8.00	1.8791	95.29
18	BA	366.9	1.25	672.338	689.215	0.4977	4.00	8.00	1.3927	97.55
19	AB	241.3	1.50	720.450	815.097	0.1088	8.00	16.00	6.3694	88.39
20	AB	126.4	1.25	213.650	254.770	0.4961	2.50	4.00	1.3972	83.86
21	BA	311.1	1.00	581.200	624.870	0.1740	6.00	12.00	3.9829	93.01
22	AB	129.9	1.50	487.612	519.795	0.4288	4.00	8.00	1.6165	93.81
23	AB	276.8	1.50	451.337	473.404	0.3897	2.50	8.00	1.7785	95.34
24	AB	135.7	1.75	304.063	347.246	0.4469	1.75	6.00	1.5509	87.56
25	AB	250.5	1.25	513.550	556.213	0.1547	4.00	12.00	4.4805	92.33
26	AB	161.2	1.00	439.337	521.139	0.1247	4.00	12.00	5.5589	84.30
27	AB	381.6	1.75	883.812	912.589	0.4309	4.00	8.00	1.6086	96.85
28	BA	245.8	1.50	656.175	696.471	0.3847	4.00	8.00	1.8020	94.21
29	BA	229.0	1.50	508.925	530.323	0.5701	3.00	6.00	1.2157	95.97
30	AB	293.3	1.50	533.050	556.695	0.5879	3.00	6.00	1.1791	95.75
N		30	30	30	30	30	30	30	30	30
Mean		271.7	1.35	577.804	621.125	0.3446	4.35	9.53	2.9325	92.96
SD		80.2	0.24	213.396	230.127	0.2107	2.21	4.13	1.8906	3.44
CV%		29.5	17.93	36.932	37.050	61.1583	50.77	43.27	64.4690	3.70
SEM		14.6	0.04	38.961	42.015	0.0385	0.40	0.75	0.3452	0.63
Median		271.9	1.38	528.006	557.719	0.3429	4.00	8.00	2.0220	93.63
Minimum		126.4	0.75	213.650	254.107	0.0855	1.50	4.00	0.7368	83.86
Maximum		398.1	1.75	1106.600	1189.230	0.9407	12.00	24.00	8.1079	98.05

[a] As calculated by Kinetica TM 2000.

TABLE C.7 Ratio Analysis of Untransformed and Log-Transformed C_{max} Data of Cyclosporine After Single-Dose Administration of Treatment A: Test Product Sigmasporin Microoral® Solution and Treatment B: Reference Product Sandimmun Neoral® Solution

	C_{max}					
	Reference		Test		Test/reference	
Subject number	Untransformed data[a]	Transformed data[b]	Untransformed data[a]	Transformed data[b]	Untransformed data[a]	Transformed data[b]
1	301.8	5.7098	272.9	5.6091	90.42	−0.1007
2	175.5	5.1676	175.9	5.1699	100.23	0.0023
3	252.3	5.5306	217.0	5.3799	86.01	−0.1507
4	367.2	5.9059	361.7	5.8908	98.50	−0.0151
5	223.7	5.4103	247.7	5.5122	110.73	0.1019
6	272.0	5.6058	280.1	5.6351	102.98	0.0293
7	387.0	5.9584	352.6	5.8653	91.11	−0.0931
8	206.7	5.3313	216.4	5.3771	104.69	0.0459
9	287.3	5.6605	260.1	5.5611	90.53	−0.0995
10	398.1	5.9867	397.1	5.9842	99.75	−0.0025
11	267.4	5.5887	204.5	5.3206	76.48	−0.2682
12	392.6	5.9728	398.5	5.9877	101.50	0.0149
13	391.8	5.9708	271.3	5.6032	69.24	−0.3675
14	271.7	5.6047	224.4	5.4134	82.59	−0.1913
15	290.7	5.6723	149.2	5.0053	51.32	−0.6670
16	290.6	5.6719	195.9	5.2776	67.41	−0.3943
17	225.1	5.4165	159.0	5.0689	70.64	−0.3476
18	366.9	5.9051	336.4	5.8183	91.69	−0.0868
19	241.3	5.4860	260.4	5.5622	107.92	0.0762
20	126.4	4.8395	100.8	4.6131	79.75	−0.2263
21	311.1	5.7401	345.5	5.8450	111.06	0.1049
22	129.9	4.8668	116.8	4.7605	89.92	−0.1063
23	276.8	5.6233	131.3	4.8775	47.43	−0.7458
24	135.7	4.9104	147.8	4.9959	108.92	0.0854
25	250.5	5.5235	280.3	5.6359	111.90	0.1124
26	161.2	5.0826	159.4	5.0714	98.88	−0.0112
27	381.6	5.9444	373.0	5.9216	97.75	−0.0228
28	245.8	5.5045	394.9	5.9786	160.66	0.4741
29	229.0	5.4337	212.1	5.3571	92.62	−0.0767
30	293.3	5.6812	258.0	5.5530	87.96	−0.1282
N	30	30	30	30	30	30
Mean	271.7	5.5569	250.0	5.4551	92.69	−0.1018
SD	80.2	0.3272	88.8	0.3820	20.95	0.2371
CV%	29.5	5.8888	35.5	7.0031	22.61	−232.8515
SEM	14.6	0.0597	16.2	0.0697	3.83	0.0433
90% CI of parametric means	Point estimator %				90.32	
	Lower limit (%)[a]				83.82	
	Upper limit (%)[a]				97.31	

[a] As calculated by Kinetica TM 2000.
[b] Transformed data are results transformed to their natural logarithm values (ln).

A.9.9.5.4. Results of Bioequivalence Testing

The details of cyclosporine results of this bioequivalence study are shown in Tables C.7, C.8, and C.9. Bioequivalence could be demonstrated for cyclosporine within the prescribed 90% confidence interval of 80% to 125% for $AUC_{0\rightarrow t}$, $AUC_{0\rightarrow \infty}$, and C_{max} with respect to the parametric method on log-transformed data.

A.9.10. Data Storage

All documents related to the statistical data analysis of this study are stored electronically under CYC-GUL-L1001/155 and as a hard copy as well.

TABLE C.8 Ratio Analysis of Untransformed and Log-Transformed $AUC_{0\rightarrow t}$ Data of Cyclosporine After Single-Dose Administration of Treatment A, Test Product Sigmasporin Microoral®, and Treatment B, Reference Product Sandimmun Neoral®

	$AUC_{0\rightarrow t}$					
	Reference		Test		Test/reference	
Subject number	Untransformed data[a]	Transformed data[b]	Untransformed data[a]	Transformed data[b]	Untransformed data[a]	Transformed data[b]
1	913.212	6.8170	711.425	6.5673	77.90	−0.2497
2	370.062	5.9137	480.238	6.1743	129.77	0.2606
3	498.262	6.2111	433.888	6.0728	87.08	−0.1383
4	634.712	6.4532	559.038	6.3262	88.08	−0.1270
5	552.050	6.3136	520.050	6.2539	94.20	−0.0597
6	628.875	6.4439	605.600	6.4062	96.30	−0.0377
7	697.638	6.5477	508.863	6.2322	72.94	−0.3155
8	239.863	5.4801	386.075	5.9560	160.96	0.4760
9	759.762	6.6330	618.063	6.4266	81.35	−0.2064
10	686.888	6.5322	599.900	6.3968	87.34	−0.1354
11	522.962	6.2595	449.475	6.1081	85.95	−0.1514
12	1106.600	7.0090	1382.850	7.2319	124.96	0.2229
13	1040.860	6.9478	807.125	6.6935	77.54	−0.2543
14	444.625	6.0972	428.488	6.0603	96.37	−0.0370
15	400.650	5.9931	328.050	5.7932	81.88	−0.1999
16	460.613	6.1326	396.637	5.9830	86.11	−0.1495
17	410.975	6.0185	419.387	6.0388	102.05	0.0203
18	672.338	6.5108	705.500	6.5589	104.93	0.0481
19	720.450	6.5799	840.975	6.7346	116.73	0.1547
20	213.650	5.3643	256.137	5.5457	119.89	0.1814
21	581.200	6.3651	672.375	6.5108	115.69	0.1457
22	487.612	6.1895	340.037	5.8291	69.74	−0.3605
23	451.337	6.1122	339.550	5.8276	75.23	−0.2846
24	304.063	5.7172	458.037	6.1269	150.64	0.4097
25	513.550	6.2413	579.900	6.3629	112.92	0.1215
26	439.337	6.0853	611.913	6.4166	139.28	0.3313
27	883.812	6.7842	672.700	6.5113	76.11	−0.2729
28	656.175	6.4864	829.087	6.7203	126.35	0.2339
29	508.925	6.2323	420.663	6.0418	82.66	−0.1905
30	533.050	6.2786	431.225	6.0666	80.90	−0.2120
N	30	30	30	30	30	30
Mean	577.804	6.2917	559.775	6.2658	100.06	−0.0259
SD	213.396	0.3826	219.032	0.3481	24.38	0.2299
CV%	36.932	6.0811	39.129	5.5551	24.36	−888.4614
SEM	38.961	0.0699	39.990	0.0635	4.45	0.0420
90% CI of parametric means	Point estimator %				97.00	
	Lower limit (%)[a]				90.61	
	Upper limit (%)[a]				104.79	

[a] As calculated by Kinetica TM 2000.
[b] Transformed data are results transformed to their natural logarithm values (ln).

A.9.11. Discussion

The results of this bioequivalence study showed the equivalence of the two studied products in terms of the rate of absorption as indicated by C_{max} and the extent of absorption as indicated by $AUC_{0\rightarrow t}$ and $AUC_{0\rightarrow\infty}$. The parametric 90% confidence intervals of the mean values for the test/reference ratio were in each case well within the bioequivalence acceptable boundaries of 80% to 125% for the pharmacokinetic parameters $AUC_{0\rightarrow t}$, $AUC_{0\rightarrow\infty}$, and C_{max}. The ANOVA analysis on the log-transformed data, C_{max}, $AUC_{0\rightarrow t}$, and $AUC_{0\rightarrow\infty}$, and untransformed data for C_{max}, $AUC_{0\rightarrow t}$, $AUC_{0\rightarrow\infty}$, K_e, $t_{(1/2)e}$, and t_{max} showed that sequence effect, product, or period effect for all these parameters did not significantly influence the outcome of the study. The mean plasma curves of both products are almost superimposable suggesting that not only C_{max} and AUC but also the time course of plasma levels over the whole sampling period are identical. Since plasma levels are a meaningful surrogate for pharmacodynamic action and AEs, this demonstrates that an equivalent

TABLE C.9 Ratio Analysis of Untransformed and Log-Transformed $AUC_{0\rightarrow\infty}$ Data of Cyclosporine After Single-Dose Administration of Treatment A, Test Product Sigmasporin Microoral®, and Treatment B, Reference Product Sandimmun Neoral®

	$AUC_{0\rightarrow\infty}$					
	Reference		Test		Test/reference	
Subject number	Untransformed data[a]	Transformed data[b]	Untransformed data[a]	Transformed data[b]	Untransformed data[a]	Transformed data[b]
1	988.074	6.8958	868.544	6.7668	87.90	−0.1289
2	400.494	5.9927	651.127	6.4787	162.58	0.4860
3	536.397	6.2849	534.422	6.2812	99.63	−0.0037
4	677.613	6.5186	590.330	6.3807	87.12	−0.1379
5	603.074	6.4020	568.580	6.3431	94.28	−0.0589
6	694.197	6.5428	656.421	6.4868	94.56	−0.0560
7	733.602	6.5980	549.799	6.3096	74.95	−0.2884
8	254.107	5.5378	429.126	6.0618	168.88	0.5240
9	833.717	6.7259	659.404	6.4913	79.09	−0.2346
10	731.269	6.5948	646.638	6.4718	88.43	−0.1230
11	558.743	6.3257	473.580	6.1603	84.76	−0.1654
12	1189.230	7.0811	1455.680	7.2832	122.41	0.2022
13	1149.510	7.0471	869.975	6.7685	75.68	−0.2786
14	469.262	6.1512	455.290	6.1209	97.02	−0.0302
15	408.636	6.0128	334.503	5.8126	81.86	−0.2002
16	476.701	6.1669	415.530	6.0296	87.17	−0.1373
17	431.307	6.0668	438.125	6.0825	101.58	0.0157
18	689.215	6.5356	721.739	6.5817	104.72	0.0461
19	815.097	6.7033	939.793	6.8457	115.30	0.1424
20	254.770	5.5404	291.559	5.6752	114.44	0.1349
21	624.870	6.4375	771.971	6.6489	123.54	0.2114
22	519.795	6.2534	373.958	5.9241	71.94	−0.3293
23	473.404	6.1599	361.560	5.8904	76.37	−0.2695
24	347.246	5.8500	546.662	6.3038	157.43	0.4538
25	556.213	6.3212	680.928	6.5235	122.42	0.2023
26	521.139	6.2560	773.155	6.6505	148.36	0.3945
27	912.589	6.8163	692.084	6.5397	75.84	−0.2766
28	696.471	6.5460	865.832	6.7637	124.32	0.2177
29	530.323	6.2735	451.459	6.1125	85.13	−0.1610
30	556.695	6.3220	446.675	6.1018	80.24	−0.2202
N	30	30	30	30	30	30
Mean	621.125	6.3653	617.148	6.3630	102.93	−0.0023
SD	230.127	0.3756	235.742	0.3529	27.60	0.2477
CV%	37.050	5.9008	38.199	5.5455	26.82	−10796.7713
SEM	42.015	0.0686	43.040	0.0644	5.04	0.0452
LL (90% CI)	254.770	5.5404	291.559	5.6752	114.44	0.1349
UL (90% CI)	624.870	6.4375	771.971	6.6489	123.54	0.2114
90% CI of parametric means	Point estimator %				99.77	
	Lower limit (%)[a]				92.25	
	Upper limit (%)[a]				107.89	

[a] As calculated by Kinetica TM 2000.
[b] Transformed data are results transformed to their natural logarithm values (ln).

therapeutic activity and tolerance to be expected from Sigmasporin Microoral [Gulf Pharmaceutical Industries (Julphar), U.A.E.] generic product when compared with the reference product.

A.9.12. Clinical Consequences

The test product, Sigmasporin Microoral [Gulf Pharmaceutical Industries (Julphar), U.A.E.; 100 mg cyclosporine per 1 mL], investigated in this study was shown to be bioequivalent with the reference product; Sandimmun Neoral Solution (Novartis Pharma, Switzerland; 100 mg cyclosporine per 1 mL). Plasma levels may be used as surrogate parameters for clinical activity. Therefore, the data obtained in this study proving, by appropriate statistical methods, the similarity of plasma levels of cyclosporine A from the test product Sigmasporin Microoral [Gulf Pharmaceutical Industries (Julphar), U.A.E.] and from the reference product Sandimmun Neoral solution (Novartis Pharma,

TABLE C.10 Summary of ANOVA Results Obtained from Cyclosporine After Single-Dose Administration of Treatment A, Test Product Sigmasporin Microoral®, and Treatment B, Reference Product Sandimmun Neoral®

	DF	SS	*F*-value	*p*
C_{max}				
Phase	1	180.96×10	0.11	0.7448
Subject (sequence)	28	3.3344×10^{5}	7.11	7.7260×10^{-7}
Drug	1	7.0417×10^{3}	4.20	0.0498
Sequence	1	3.4589×10^{4}	2.90	0.0994
Error	28	4.6892×10^{4}		
Log C_{max}				
Phase	1	0.0074	0.26	0.6158
Subject (sequence)	28	5.7325	7.10	7.8640×10^{-7}
Drug	1	0.1555	5.39	0.0277
Sequence	1	0.7903	3.86	0.0595
Error	28	0.8075		
$AUC_{0\rightarrow t}$				
Phase	1	2.4387×10^{2}	0.03	0.8693
Subject (sequence)	28	2.2382×10^{6}	9.04	5.3710×10^{-8}
Drug	1	4.8755×10^{3}	0.55	0.4640
Sequence	1	2.2582×10^{5}	2.83	0.1039
Error	28	2.4765×10^{5}		
Log $AUC_{0\rightarrow t}$				
Phase	1	6.8081×10^{-4}	2.49×10^{-2}	0.8758
Subject (sequence)	28	6.3837	8.34	1.3410×10^{-7}
Drug	1	1.0045×10^{-2}	3.67×10^{-1}	0.5494
Sequence	1	6.0836×10^{-1}	2.67	0.1136
Error	28	0.7658		
$AUC_{0\rightarrow\infty}$				
Phase	1	3.7246×10^{2}	3.25×10^{-2}	0.8582
Subject (sequence)	28	2.6236×10^{6}	8.18	1.6660×10^{-7}
Drug	1	2.3725×10^{2}	2.07×10^{-2}	0.8866
Sequence	1	2.0259×10^{5}	2.16	0.1526
Error	28	3.2089×10^{5}		
Log $AUC_{0\rightarrow\infty}$				
Phase	1	2.9492×10^{-4}	9.29×10^{-3}	0.9239
Subject (sequence)	28	6.3795	7.17	7.0250×10^{-7}
Drug	1	7.8947×10^{-5}	2.49×10^{-3}	9.6060×10^{-1}
Sequence	1	4.3309×10^{-1}	1.90	0.1789
Error	28	8.8932×10^{-1}		
K_e				
Phase	1	9.5590×10^{-3}	8.68×10^{-1}	0.3596
Subject (sequence)	28	1.9251	6.24	3.0950×10^{-6}
Drug	1	5.1880×10^{-2}	4.71	3.8660×10^{-2}
Sequence	1	1.0742×10^{-2}	1.56×10^{-1}	0.6956
Error	28	3.0853×10^{-1}		
$t_{(1/2)e}$				
Phase	1	1.1977×10^{1}	4.21	4.9580×10^{-2}
Subject (sequence)	28	3.3096×10^{2}	4.16	1.6190×10^{-4}
Drug	1	1.7132×10^{1}	6.03	2.0570×10^{-2}
Sequence	1	6.5211	5.52×10^{-1}	0.4638
Error	28	7.9607×10^{1}		
t_{max}				
Phase	1	1.7604×10^{-1}	3.54	0.0703
Subject (sequence)	28	3.4417	2.47	0.0097
Drug	1	2.6042×10^{-2}	0.52	0.4752
Sequence	1	2.6042×10^{-2}	0.21	0.6489
Error	28	1.3917		

Values were calculated by Kinetica TM 2000.
Abbreviations: DF, degrees of freedom; SS, sum of squares.

Switzerland) suggests equal clinical efficacy of these products. The product, Sigmasporin Microoral developed by Gulf Pharmaceutical Industries (Julphar), U.A.E., may be used interchangeably with the reference product Sandimmun Neoral solution (Novartis Pharma, Switzerland) which was shown to have an acceptable therapeutic efficacy.

APPENDIX C

Study Forms

Screening record

This page must be filed separately

Subject's Identification Data

First name: _____

Middle name: _____

Third name: _____

Street Address: _____

City: _____

Country: _____

Occupation: _____

Telephone Number: _____ _____

Sex: ❒ **Male** ❒ **Female**

Date of Birth: |_____|_____|_____| (DD | MM | YY)

Age: |_____| Years

Interviewer: _____ Date: _____/_____/_____/

Confirmation by the Principal Investigator

I confirm that the data included here is in complete accordance with the source documents of this subject

Name of the Principal Investigator: _____

Signature:_____

Date: |_____|_____|_____| **(DD| MM | YY)**

Subject's Identification Data (*continued*)

Height |_____| cm

Weight |_____| Kg

Body frame ❒ Small ❒ Medium ❒ Large ❒ N/A

Refer to the study master file for more details about the body frame

Date of Birth: |_____|_____|_____| (DD| MM | YY)

Age: |_____| years

Recorded by: _____ Date: _____

(See height-weight ranges in the master file of the study)

Vital signs

Study Day		**Screening**	
Date (DD | MM | YY)		|_____|_____|_____|	
Actual Time (hh|mm)		|_____|_____|	
Blood Pressure	**After at least 3 minutes sitting**	|_____| / |_____|	
Pulse	**After at least 3 minutes sitting**	|_____|	
Respiration rate (/min)		|_____|	
Temperature (°C)		|_____|	❒ oral ❒ rectal ❒ axillary

Performed by: _____ Date: _____ / _____ / _____

Clinical Investigator Signature: _____ Date: _____ / _____ / _____

Medical History

			Notes
1. Family history	❒ Normal	❒ Abnormal	
2. Allergy (*including drug allergy)*	❒ Normal	❒ Abnormal	
3. Cardiovascular	❒ Normal	❒ Abnormal	
4. Respiratory	❒ Normal	❒ Abnormal	
5. Renal	❒ Normal	❒ Abnormal	
6. Hepatic	❒ Normal	❒ Abnormal	
7. Gastrointestinal	❒ Normal	❒ Abnormal	
8. Special diet in the last 30 days (***Excessive vitamin intake, popular diets, significant weight gain or loss, vegetarian or psychological eating disorders***)	❒ Normal	❒ Abnormal	
9. Surgery	❒ No	❒ Yes	(MM | YY)
10. Psychiatric disease	❒ No	❒ Yes	
11. Epilepsy or other seizures	❒ No	❒ Yes	
12. G-6-PD deficiency	❒ No	❒ Yes	
13. Bleeding/coagulation disorders or anemia	❒ No	❒ Yes	
14. Acute infection within the last week	❒ No	❒ Yes	
15. Thyroid gland abnormalities	❒ No	❒ Yes	
16. Skin abnormalities	❒ No	❒ Yes	
17. Eye/ear/ nose/ throat abnormalities	❒ No	❒ Yes	
18. Diabetes	❒ No	❒ Yes	
19. Neurological abnormalities	❒ No	❒ Yes	
20. Musculoskeletal disorders	❒ No	❒ Yes	

Interviewer: _____ Date: _____ / _____ / _____

Clinical Investigator Signature: _____ Date: _____ / _____ / _____

Medical History (*continued*)

			Notes
21. Latest blood donation	❒ No	❒ Yes	QTY:_____ Date:_____
22. Latest participation in another clinical trial	❒ No	❒ Yes	
23. Planned hospitalization (*within the next 3 months*)	❒ No	❒ Yes	
24. Tobacco use	❒ No	❒ Yes	QTY:_____
	❒ ex-consumer		
25. Caffeine use	❒ No	❒ Yes	
Consumer of			
cola	❒ No	❒ Yes	QTY_____
tea	❒ No	❒ Yes	QTY_____
coffee	❒ No	❒ Yes	QTY_____
	❒ ex-consumer		
26. Alcohol consumption	❒ No	❒ Yes	QTY_____
	❒ ex-consumer		
27. Drug abuse	❒ No	❒ Yes	
28. Others	❒ No	❒ Yes	

Interviewer: _____ Date: _____ / _____ / _____

Clinical Investigator Signature: _____ Date: _____ / _____ / _____

Alcohol Screen (if applicable)

Alcohol Screen:

Result ❑ Negative ❑ Positive Date |____|____|____| (DD.MM.YY)

Comments:

_____

_____

Screening for Drugs of Abuse (if applicable)

Drug name	***Drugs of abuse***	
	❑ Negative	❑ Positive
	❑ Negative	❑ Positive
	❑ Negative	❑ Positive
	❑ Negative	❑ Positive
	❑ Negative	❑ Positive
	❑ Negative	❑ Positive

This form can be expanded depending on the number of drugs that will be screened.

Recorded by: _____ Date: _____ / _____ / _____

Clinical Investigator Signature: _____ Date: _____ / _____ / _____

Physical Examination

			Notes
1. Appearance	❒ Normal	❒ Abnormal	_____
2. Skin	❒ Normal	❒ Abnormal	_____
3. Eye	❒ Normal	❒ Abnormal	_____
4. Nose	❒ Normal	❒ Abnormal	_____
5. Throat	❒ Normal	❒ Abnormal	_____
6. Mouth	❒ Normal	❒ Abnormal	_____
7. Neck	❒ Normal	❒ Abnormal	_____
8. Breast	❒ Normal	❒ Abnormal	_____
9. Lungs	❒ Normal	❒ Abnormal	_____
10. Heart	❒ Normal	❒ Abnormal	_____
11. Abdomen	❒ Normal	❒ Abnormal	_____
12. Kidney	❒ Normal	❒ Abnormal	_____
13. Spine	❒ Normal	❒ Abnormal	_____
14. Lymph nodes	❒ Normal	❒ Abnormal	_____
15. Extremities	❒ Normal	❒ Abnormal	_____
16. Neurological and reflex	❒ Normal	❒ Abnormal	_____
17. Genitalia	❒ Normal	❒ Abnormal	_____
18. Rectum	❒ Normal	❒ Abnormal	_____
19. Mental status	❒ Normal	❒ Abnormal	_____
20. Other	❒ No	❒ Yes	_____

If "Other," please specify: _____

_____

Result of ECG Examination ❒ Normal ❒ Abnormal ❒ NA

See attached ECG record *(if applicable)*

_____

_____

_____

Recorded by: _____ Date: _____ / _____ / _____

Clinical Investigator Signature: _____ Date: _____ / _____ / _____

Selection Criteria

A. Inclusion Criteria			**Notes**
1. Male, age 18 to 45 years, inclusive.	❒ Yes	❒ No	
2. Body weight within the limits for height (see "Height-weight-ranges" Study master file).	❒ Yes	❒ No	
3. Medical history, vital signs, physical examiniation (including neurological assessment), ECG, (it applicable without evidence of clinically significant deviation from normal medical condition, performed not longer than two weeks before the initiation of the clinical study.	❒ Yes	❒ No	
4. Clinical laboratory tests are performed no longer than one month from the initiation of the clinical study	❒ Yes	❒ No	
5. Results of blood and urine examinations are within the normal range or deviation is not considered clinically significant by the Clinical Investigator.	❒ Yes	❒ No	
6. Subject does not have allergy to the drugs under investigation.	❒ Yes	❒ No	

B. Exclusion Criteria			**Notes**
1. Meidical histry, physical examination and laboratory tests with evidence of clinically significant deviation from normal medical condition	❒ Yes	❒ No	
2 Evidence for any abnormality in carbohydrate metabolism.	❒ Yes	❒ No	
3. Results of blood and/or urine examinations, which are clinically significant.	❒ Yes	❒ No	
4. Acute infection within one week preceding first Study Drug administration.	❒ Yes	❒ No	
5. History of drug or alcohol abuse.	❒ Yes	❒ No	
6. Subject is a heavy smoker.	❒ Yes	❒ No	
7. Subject does not agree not to take any prescription or non-prescription drug within 1 week before first Study Drug administration.	❒ Yes	❒ No	
8. Subject is on a special diet (for example subject is vegetarian).	❒ Yes	❒ No	

Checked by: _____ Date: _____ / _____ / _____

Clinical Investigator Signature: _____ Date: _____ / _____ / _____

B. Exclusion Criteria (continued)			**Notes**
9. Subject consumes large quantities of alcohol or beverages containing methylxanthines, e.g., caffeine.	❒ Yes	❒ No	
10. Subject does not agree not to consume any beverages or foods containing alcohol 24 hours prior to first Study Drug administration until the end of Study.	❒ Yes	❒ No	
11. Subject does not agree not to consume any beverages or foods containing methylxanthines e. g. caffeine (coffee, tea, cola, chocolate etc.) 24 hours (or as specified in the study protocol) prior to the Study Drug administration of either Study Phase until the last blood sample of the respective Study Phase was collected.	❒ Yes	❒ No	
12. Subject has a history of severe diseases which have direct impact on the study.	❒ Yes	❒ No	
13. Participation in a bioequivalence study within the last month before first Study Drug administration.	❒ Yes	❒ No	
14. Participation in a clinical study within the last 3 months before first Study Drug administration.	❒ Yes	❒ No	
15. Subject intends to be hospitalized within 3 months after first Study Drug administration.	❒ Yes	❒ No	
16. Subjects who, through completion of this study, would have donated more than 500 mL of blood in 14 days, 750 mL in 30 days, 1000 mL in 90 days, 1250 mLin 120 days 1500 mL of blood in 180 days, 2000 mL of blood in 270 days and 2500 mL of blood in 1 year.	❒ Yes	❒ No	

Checked by: _____ Date: _____ / _____ / ____

Subject can be included ❒ Yes ❒ No _____

If no (reason for exclusion) _____

_____

_____

_____

Clinical Investigator Signature: _____ Date: _____ / _____ / ____

Phase I Clinical Record

Study Phase: Phase I

1. Admission Time |_____|_____| Date : _____/_____/_____
   (hh:mm) (DD.MM.YY)

2. Drugs taken within the last week _____

   _____

3. Alcohol consumption within the last 24 hours: _____

4. Alcohol screening result: ❒ Negative ❒ Positive ❒ N/A

5. Caffeine consumption within the last 24 hours (or as specified in the study protocol) (coffee, tea, cola, chocolate, etc):_____

6. Meals leftovers (see meal composition in the master file of the study):

Meal	*Date (DD.MM.YY)*	*Time (hrs)* From - To	*Meals leftovers*
Dinner (day 1)			_____
Lunch (day 1)			_____
Dinner (day 1)			_____

Vital Signs Measurement Form

Study Phase: Phase II

Time from drug administration	Date	Actual Time (hh/mm)	Blood Pressure (mmHg) After at least 3 minutes sitting	Pulse (/min)	Respiration rate (/min)	Temperature (°C)		Performed by/Date:	Clinical Investigator Signature/Date:
Before study drug administration.		|____|____|	|____|/|____|	|____|	|____|	|____|	❑ oral ❑ rectal ❑ axillary		
4.00 hrs		|____|____|	|____| / |____|	|____|	|____|	|____|	❑ oral ❑ rectal ❑ axillary		
10.00 hrs.		|____|____|	|____| / |____|	|____|	|____|	|____|	❑ oral ❑ rectal ❑ axillary		

Drug Administration Form

Study Phase: Phase I

Treatment Given

❒ Treatment A ❒ Treatment B ❒ Treatment C

	Time (*hh.mm*)	Date (*DD.MM.YY*)
Administration of study drug	|____|____|	____/____/____

Volume of water:________ mL Temperature of water:____________ ºC

Hand check ❒ ❒ N/A *Others* ❒ ❒ N/A

Mouth check ❒ ❒ N/A Specify

____

Comments:____

____

____

Administered by: ______________________________________ Date: _____/_____/_____

Checked by: ____ Date: _____/_____/_____

Blood Glucose Strip (applicable for anti-diabetic drugs only)

	Actual test time	Value (normal <100 mg/dL)	Performed by/date
Immediately before study drug administration	|____|____| (hh:mm)	|__________| (mg/dL)	____________
( ) hour after study drug administration	|____|____| (hh:mm)	|__________| (mg/dL)	____________
( ) hour after study drug administration	|____|____| (hh:mm)	|__________| (mg/dL)	____________
( ) hour after study drug administration	|____|____| (hh:mm)	|__________| (mg/dL)	____________
( ) hour after study drug administration	|____|____| (hh:mm)	|__________| (mg/dL)	____________
( ) hour after study drug administration	|____|____| (hh:mm)	|__________| (mg/dL)	____________
( ) hour after study drug administration	|____|____| (hh:mm)	|__________| (mg/dL)	____________

Blood Sampling Form

Study Phase: Phase I

Stopwatch code no.: _____

Date (DD.MM.YY)	Sample ID	Sample collection time			Signature	Comments if necessary
		Time (hrs)	Theoretical	Actual		
	PRE-DOSE SAMPLE		NA			
	STUDY DRUG					
CGL	CGL (I)	0.25				
CGL	(I)	0.50				
CGL	(I)	0.75				
CGL	(I)	1.00				
CGL	(I)	1.25				
CGL	(I)	1.50				
CGL	(I)	1.75				
CGL	(I)	2.00				
CGL	(I)	2.50				
CGL	(I)	3.00				
CGL	(I)	4.00				
CGL	(I)	6.00				
CGL	(I)	8.00				
CGL	(I)	12.00				
CGL	(I)	16.00				
CGL	(I)	24.00				

Check for Adverse Events

Study Phase: Phase I

Clinical assessment

1. No adverse event occurred during Phase I (treatment was well tolerated by the subject):

❒ Yes ❒ No If no, specify: _____

_____

_____

_____

Interviewer: _____ Date: |_____|_____|_____|

Clinical Investigator Signature: _____ Date: |_____|_____|_____|

2. The subject left the study without any changes of the baseline condition:

❒ Yes ❒ No If no, specify:

_____

_____

_____

Clinical Investigator Signature: _____ Date: |_____|_____|_____|

For Clinical Investigator: Please refer to the Adverse Events Registration Record and use an Adverse Events form to record any adverse events as per CLP-010.

Comments

Any clinically important observation, not reported in other parts of the Case Report Form, including observations toward a possible activity outside the studied indication?

❒ Yes ❒ No

Comments

Interviewer: _____ Date: |_____|_____|_____|

Clinical Investigator Signature: _____ Date : |_____|_____|_____|

Phase II Clinical Record

Study Phase: Phase II

1. Admission Time |_____|_____| Date: _____/_____/_____
(hh:mm) (DD.MM.YY)

2. *Drugs taken within the wash out period* _____

_____

3. *Alcohol consumption within the last 24 hours:* _____

4. *Alcohol screening result:* ❒ *Negative* ❒ *Positive* ❒ *N/A*

5. *Caffeine consumption within the last 24 hours (or as specified in the study protocol) (coffee, tea, cola, chocolate, etc):* _____

6. *Meals leftovers (see meal composition in the master file of the study):*

Meal	*Date (DD.MM.YY)*	*Time (hrs)* From - To	*Meals leftovers*
Dinner (day 1)			_____ _____
Lunch (day 1)			_____ _____
Dinner (day 1)			_____ _____

Vital Signs Measurement Form

Study Phase: Phase II

Time from drug administration	Date	Actual Time (hh/mm)	Blood Pressure (mmHg)	Pulse (/min)	Respiration rate (/min)	Temperature (°C)		*Performed by/ Date:*	*Clinical Investigator Signature / Date:*
			After at least 3 minutes sitting						
Before study drug administration.		|_____|_____|	|_____| / |_____|	|_____|	|_____|	|_____|	❑ oral ❑ rectal ❑ axillary		
V4.00 hrs		|_____|_____|	|_____| / |_____|	|_____|	|_____|	|_____|	❑ oral ❑ rectal ❑ axillary		
10.00 hrs.		|_____|_____|	|_____| / |_____|	|_____|	|_____|	|_____|	❑ oral ❑ rectal ❑ axillary		

Drug Administration Form

Study Phase: Phase II

Treatment Given

❒ Treatment A ❒ Treatment B ❒ Treatment C

Administration of study drug

Time (*hh.mm*) |____|____|

Date (*DD.MM.YY*) ___/___/___

Volume of water:_____ mL Temperature of water:_____ ºC

Hand check ❒ ❒ N/A *Others* ❒ ❒ N/A

Mouth check ❒ ❒ N/A Specify _____

Comments:_____

_____

_____

Administered by: _____ Date: ____/____/____

Checked by: _____ Date: ____/____/____

Blood Glucose Strip (applicable for anti-diabetic drugs only)

	Actual test time	Value (normal <100 mg/dL)	Performed by/date
Immediately before study drug administration	|____|____| (hh:mm)	|_______| (mg/dL)	________
( ) hour after study drug administration	|____|____| (hh:mm)	|_______| (mg/dL)	________
( ) hour after study drug administration	|____|____| (hh:mm)	|_______| (mg/dL)	________
( ) hour after study drug administration	|____|____| (hh:mm)	|_______| (mg/dL)	________
( ) hour after study drug administration	|____|____| (hh:mm)	|_______| (mg/dL)	________
( ) hour after study drug administration	|____|____| (hh:mm)	|_______| (mg/dL)	________
( ) hour after study drug administration	|____|____| (hh:mm)	|_______| (mg/dL)	________

Blood Sampling Form

Study Phase: Phase I

Stopwatch code no.: __________

Date (DD.MM.YY)	Sample ID	Sample collection time			Signature	Comments, if necessary
		Time (hrs)	Theoretical	Actual		
	PRE-DOSE SAMPLE		NA			
	STUDY DRUG					
	CGL (II) 1	0.25				
	CGL (II) 2	0.50				
	CGL (II) 3	0.75				
	CGL (II) 4	1.00				
	CGL (II) 5	1.25				
	CGL (II) 6	1.50				
	CGL (II) 7	1.75				
	CGL (II) 8	2.00				
	CGL (II) 9	2.50				
	CGL (II) 10	3.00				
	CGL (II) 11	4.00				
	CGL (II) 12	6.00				
	CGL (II) 13	8.00				
	CGL (II) 14	12.00				
	CGL (II) 15	16.00				
	CGL (II) 16	24.00				

Check for Adverse Events

Study Phase: Phase I

Clinical assessment

No adverse event occurred during Phase I (treatment was well tolerated by the subject):

❒ Yes ❒ No If no, specify: _____

_____

_____

_____

Interviewer: _____ Date: |_____|_____|_____|

Clinical Investigator Signature: _____ Date: |_____|_____|_____|

The subject left the study without any changes of the baseline condition:

❒ Yes ❒ No If no, specify:

_____

_____

_____

Clinical Investigator Signature: _____ Date: |_____|_____|_____|

For Clinical Investigator: Please refer to the Adverse Events Registration Record and use an Adverse Events form to record any adverse events as per CLP-010.

Comments

Any clinically important observation, not reported in other parts of the Case Report Form, including observations toward a possible activity outside the studied indication?

❒ Yes ❒ No

Comments

Interviewer: _____ Date: |_____|_____|_____|

Clinical Investigator Signature: _____ Date: |_____|_____|_____|

Subject Evaluation Form (Close Out)

❒ **Used after completion of the study (24 hours after donating all the samples*)**

The Case Report Form of this subject has been reviewed and the subject was asked for any adverse event (see relevant forms) that occurred during the study. The subject completed the study and he /she:

❍ **left the study without any changes of the baseline condition:**

❍ **left the study with some changes of the baseline condition:**

Specify:

_____

_____

_____

_____

❒ **Used in case of withdrawal**

Reason for withdrawal:

_____

_____

_____

_____

The following measures were taken to evaluate the above case (see attached examinations):

_____

_____

_____

_____

(In case of vital signs measurement use additional Vital Signs Form)

Clinical Investigator Signature: _____ Date : |_____|_____|_____|

* ***Unless otherwise specified in the study protocol***

Follow Up Record

1. Physical Examination

			Notes
1. Appearance	❒ Normal	❒ Abnormal	
2. Skin	❒ Normal	❒ Abnormal	
3. Eye	❒ Normal	❒ Abnormal	
4. Nose	❒ Normal	❒ Abnormal	
5. Throat	❒ Normal	❒ Abnormal	
6. Mouth	❒ Normal	❒ Abnormal	
7. Neck	❒ Normal	❒ Abnormal	
8. Breast	❒ Normal	❒ Abnormal	
9. Lungs	❒ Normal	❒ Abnormal	
10. Heart	❒ Normal	❒ Abnormal	
11. Abdomen	❒ Normal	❒ Abnormal	
12. Kidney	❒ Normal	❒ Abnormal	
13. Spine	❒ Normal	❒ Abnormal	
14. Lymph nodes	❒ Normal	❒ Abnormal	
15. Extremities	❒ Normal	❒ Abnormal	
16. Neurological and reflex	❒ Normal	❒ Abnormal	
17. Genitalia	❒ Normal	❒ Abnormal	
18. Rectum	❒ Normal	❒ Abnormal	
19. Mental status	❒ Normal	❒ Abnormal	
20. Other	❒ No	❒ Yes	
21. If "Other" please specify			

2. Result of ECG Examination ❒ Normal ❒ Abnormal

See attached ECG record (*if applicable*)

Recorded by: _____ Date: _____ / _____ / _____

Clinical Investigator Signature: _____ Date: _____ / _____ / _____

Comparative, Randomized, Open-Label, Two-Way Crossover Bioequivalence Study to Compare the Bioequivalence of Carbamazepine from Gulf Pharmaceutical Industries (Julphar) (Fitzecalm 100 mg Carbamazepine Suspension), and Novartis Pharma (Tegretol® 100 mg Carbamazepine Syrup), Each Given as a Single Dose to Healthy Adult Males Under Fasting Conditions

Study synopsis	
Title of study	Comparative, randomized, open-label, two-way crossover bioequivalence study to compare the bioequivalence of carbamazepine from Fitzecalm 100 mg carbamazepine suspension [Gulf Pharmaceutical Industries (Julphar)], and Tegretol 100 mg carbamazepine syrup (Novartis Pharma.), each given as a single dose to healthy adult males under fasting conditions
Study code	CAR-GUL-SY0202/172
Principal Investigator	Naji Najib, B. Sc. (Pharm.), Ph.D.
Sponsor	Gulf Pharmaceutical Industries (Julphar), U.A.E.
Purpose of trial	To assess the bioequivalence of a test product with a reference product by measurement of plasma concentrations of carbamazepine and calculation of the bioequivalence parameters from those measurements
Period of trial	Screening date: 26/02/02 Phase I: 02/03/02 Phase II: 23/03/02
Protocol/design	Single-center, open, randomized two-way crossover bioequivalence study with a washout period of 21 days
Number of subjects	Twenty-four subjects
Demographic data	Age: 24.04 $\pm$ 5.49 years, height: 170.13 $\pm$ 4.52 cm, weight at screening examination: 67.42 $\pm$ 6.43 kg (from n = 24)
Study medication	Treatment A (test formulation) Fitzecalm batch no.: 0012, manufacturing date: 01/02, expiry date: (1/4) Manufacturer: Gulf Pharmaceutical Industries (Julphar), U.A.E. Treatment B (reference formulation) Tegretol batch no.: H1057, manufacturing date: 08/01, expiry date: 08/06 Manufacturer: Novartis Pharma, Switzerland
Dosage regimen	Each healthy volunteer received each of the following treatments as a single dose in accordance with a randomization scheme Treatment A: 10 mL of Fitzecalm, 100 mg carbamazepine per 5 mL suspension Treatment B: 10 mL of Tegretol, 100 mg carbamazepine per 5 mL syrup
Drug analysis	By HPLC analysis on-line with a UV–Visible detector; the limit of quantitation during sample analysis for carbamazepine was 0.05 μg/mL
Pharmacokinetic parameters	$t_{1/2}$, K_e, C_{max}, t_{max}, $Auc_{0\rightarrow t}$, $Auc_{0\rightarrow\infty}$, and $(Auc_{0\rightarrow t}/Auc_{0\rightarrow\infty})\%$
Bioequivalence parameters	C_{max}, $Auc_{0\rightarrow t}$, and $Auc_{0\rightarrow\infty}$
Tolerance	Both products were well tolerated by the volunteers
Conclusion	Bioequivalence of the test and reference products was shown

Summary

This study was performed to investigate the bioequivalence of carbamazepine between a test formulation (Fitzecalm) suspensions; Manufacturer: Gulf Pharmaceutical Industries (Julphar) (U.A.E.) and a reference formulation (Tegretol); Manufacturer: Novartis Pharma (Switzerland).

The following blood samples for analysis of carbamazepine were taken: immediately before (2× 10 mL) and at 0.33, 0.66, 1.00, 1.50, 2.00, 2.50, 3.00, 4.00, 6.00, 8.00, 12.00, 24.00, 48.00, 72.00, 96.00, 120.00, 144.00, 168.00, and 192.00 hours. The clinical protocol called for 24 healthy subjects. Samples from the first 24 subjects were analyzed for plasma carbamazepine. The subjects received an oral dose of 100 mg carbamazepine during each study phase in a randomized fashion and with a washout period of 21 days.

Drug analysis of carbamazepine in plasma was performed by HPLC coupled on-line with a UV–Visible detector. The limit of quantitation during sample analyses being 0.05 μg/mL for carbamazepine. The plasma assay procedures were validated at IPRC according to the international guidelines. The pharmacokinetics was determined by standard noncompartmental methods and ANOVA statistics was used for bioequivalence calculations.

The extent of absorption was determined by $AUC_{0\to t}$ and $AUC_{0\to\infty}$ of carbamazepine. The rate of absorption was determined by C_{max} and t_{max}. The adequacy of the sampling time was determined by the ratio $(AUC_{0\to t}/AUC_{0\to\infty})\%$. The half-life of elimination ($t_{(1/2)e}$) and the rate of elimination (K_e) of carbamazepine were used to further characterize the pharmacokinetic outcome of this study. $AUC_{0\to t}$, $AUC_{0\to\infty}$, and C_{max} values of carbamazepine were used for bioequivalence estimations.

ANOVA of the untransformed pharmacokinetic parameters t_{max}, C_{max}, $t_{(1/2)e}$, K_e, $AUC_{0\to t}$, and $AUC_{0\to\infty}$, and of the log transformed data for C_{max}, $AUC_{0\to t}$, and $AUC_{0\to\infty}$ was performed using the Kinetica 2000 statistical program. The variance model included sequence, subjects nested in sequence, period, and product as factors. The significance of the sequence effect was tested using the subjects nested in sequence as the error term. A 5% level of significance was used for all comparisons (period, product, and sequence). This analysis showed that no statistically significant differences were obtained between the two products with respect to the calculated pharmacokinetic parameters: t_{max}, C_{max}, $t_{(1/2)e}$, K_e, $AUC_{0\to t}$, and $AUC_{0\to\infty}$ (Tables C.11 and C.12).

TABLE C.11 Pharmacokinetics—Bioequivalence Parameters of Carbamazepine

	Treatments				
	Means ± SD				
	A	B	90% confidence intervals (based on parametric testing)		
Parameter	Test formulation	Reference formulation	Point estimator (%)	Lower limit (%)	Upper limit (%)
$AUC_{0\to t}$ (μg h/mL)	168.52 ± 37.45	174.89 ± 42.51	96.73	93.42	100.16
$AUC_{0\to\infty}$ (μg h/mL)	176.91 ± 43.53	183.06 ± 48.79	97.05	93.64	100.59
C_{max} (μg/mL)	3.08 ± 0.42	3.17 ± 0.37	96.82	92.26	101.61

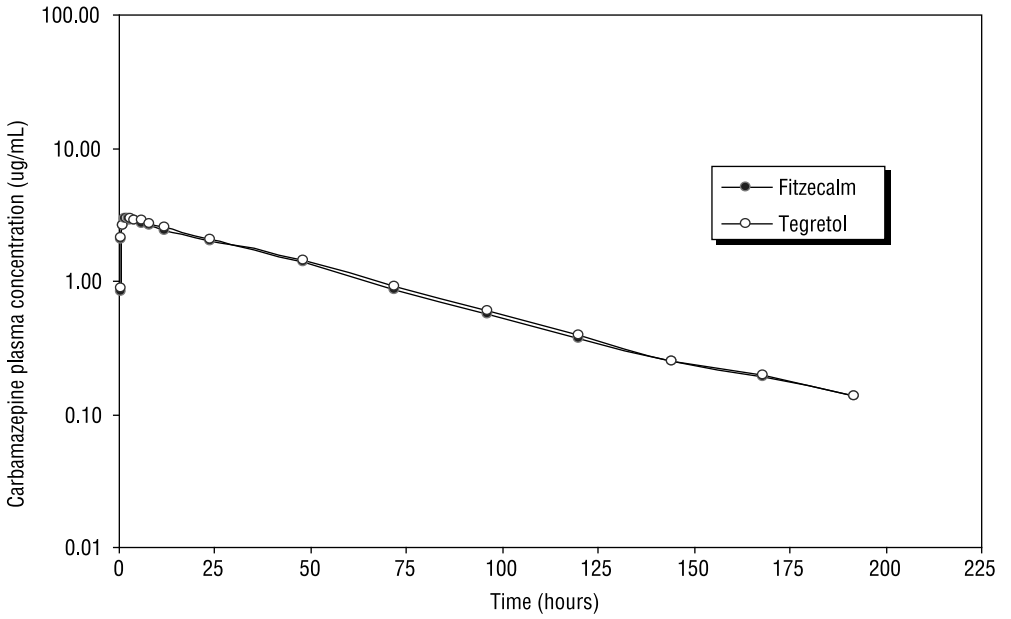
TABLE C.12 Pharmacokinetics—Further Parameters of Carbamazepine

	Treatments	
	Means ± SD	
Parameter	A Test formulation	B Reference formulation
t_{max} (hr)	2.27 ± 1.22	2.16 ± 1.24
$AUC_{0\to t}/AUC_{0\to\infty}$ (%)	95.70 ± 2.22	96.02 ± 2.39
$t_{1/2e}$ (hr)	41.22 ± 10.47	38.76 ± 10.46
K_e	0.0180 ± 0.0053	0.0194 ± 0.0062

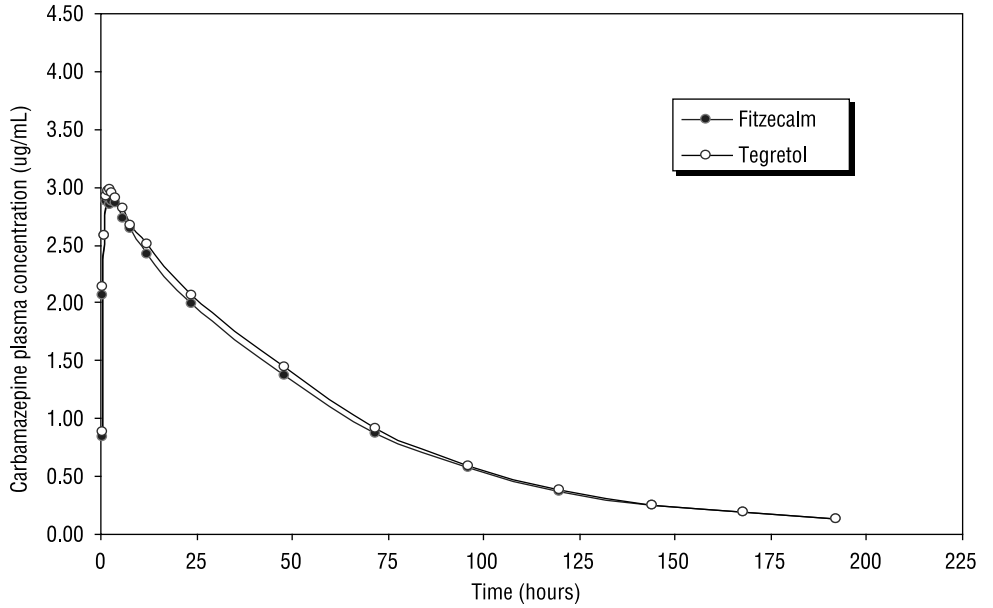
Consistent with the two one-sided tests for bioequivalence, 90% confidence intervals for the ratios of means was calculated for both untransformed and log-transformed $AUC_{0\rightarrow t}$, $AUC_{0\rightarrow\infty}$, and C_{max}. The geometric mean for the ratios expressed as a percentage (test product/reference product) is shown in Table C.11. The values obtained indicated that 90% confidence limits for all geometric means are within the recommended range of bioequivalence of 80% to 125% for $AUC_{0\rightarrow t}$, $AUC_{0\rightarrow\infty}$, and C_{max}.

Mean ($N=24$) of plasma concentrations carbamazepine for Treatment A (test formulation, Fitzecalm) and Treatment B (reference formulation, Tegretol) after a single oral dose of 2× 100 mg carbamazepine.

Semi-logarithmic Scale

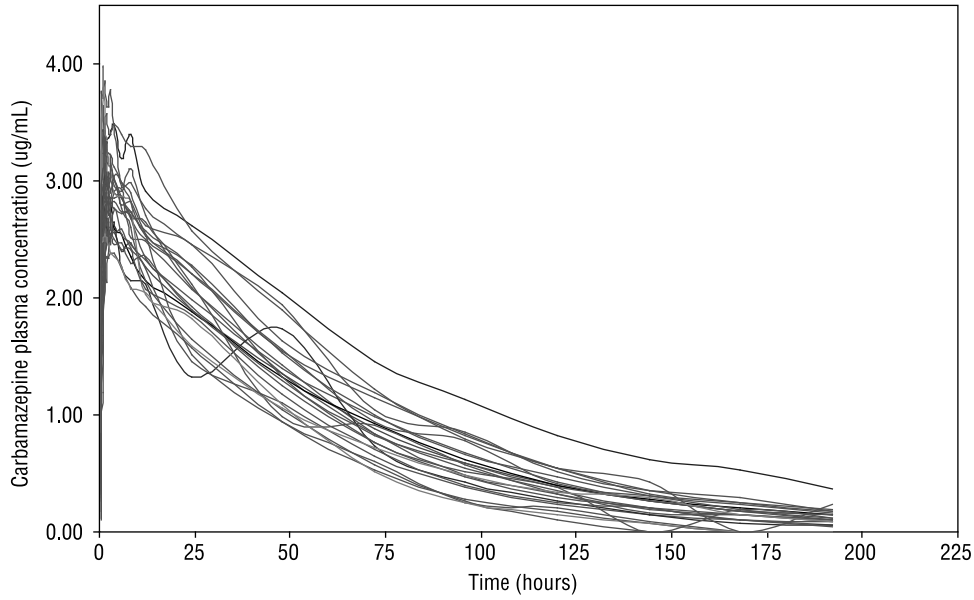


Linear Scale

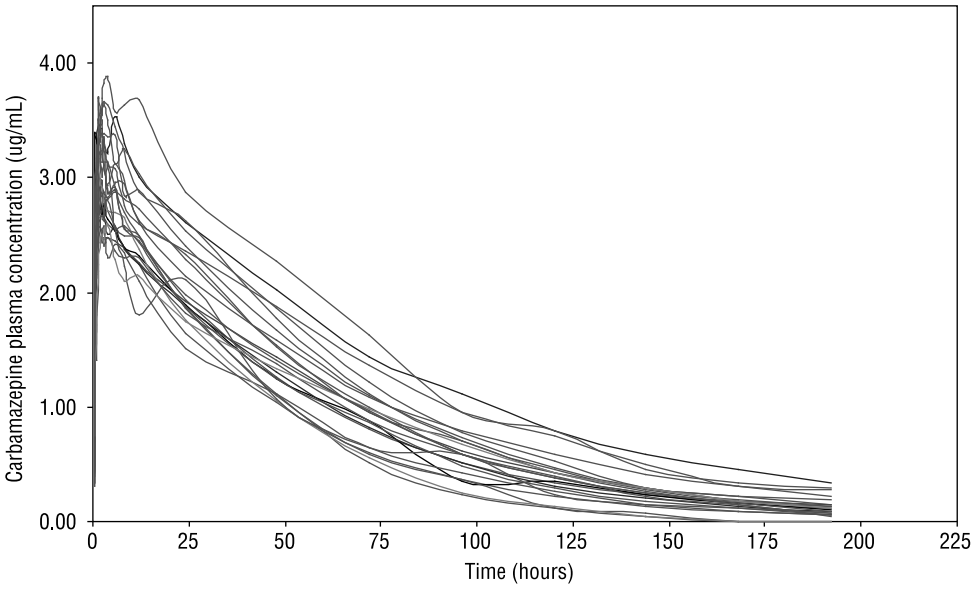


Individual plasma concentrations ($N=24$) of carbamazepine for Treatment A (test formulation, Fitzecalm) and Treatment B (reference formulation, Tegretol) after a single oral dose of 2× 100 mg carbamazepine.

Test formulation
Linear Scale



Reference formulation
Linear Scale



Conclusion:
This study demonstrated that the test product, Fitzecalm® (Gulf Pharmaceutical Industries (Julphar), United Arab Emirates), 100 mg carbamazepine per 5 ml suspension, is bioequivalent to the reference product, Tegretol® (Novartis Pharma, Switzerland) 100mg carbamazepine

Comparative, Randomized, Open-Label, Two-Way Crossover Bioequivalence Study to Compare the Bioequivalence of Aceclofenac from Gulf Pharmaceutical Industries (Julphar) (Aceclofar 100 mg Aceclofenac Tablets), and Bristol-Myers Squibb (Bristaflam® 100 mg Aceclofenac Tablets), Each Given as a Single Dose to Healthy Adult Males Under Fasting Conditions

Study synopsis	
Title of study	Comparative, randomized, open-label, two-way crossover bioequivalence study to compare the bioequivalence of aceclofenac from Gulf Pharmaceutical Industries (Julphar) (Aceclofar 100 mg aceclofenac tablets), and Bristol-Myers Squibb (Bristaflam 100 mg aceclofenac tablets), each given as a single dose to healthy adult males under fasting conditions
Study code	ACE-GUL-T0701/138
Principal Investigator	Naji Najib, B.Sc. (Pharm.), Ph.D.
Sponsor	Gulf Pharmaceutical Industries (Julphar), U.A.E.
Purpose of trial	To assess the bioequivalence of a generic test product with a reference product by measurement of plasma concentrations of aceclofenac and calculation of the bioequivalence parameters from those measurements
Period of trial	Screening date: 28/08/01 Phase I: 01/09/01 Phase II: 08/09/01
Protocol/design	Single-center, open, randomized two-way crossover bioequivalence study with a washout period of seven days
Number of subjects	Twenty-four subjects
Demographic data	Age: 21.75 $\pm$ 2.97 years, height: 174.46 $\pm$ 6.21 cm, weight at screening examination: 72.63 $\pm$ 9.56 kg (from $n=24$)
Study medication	Treatment A (test formulation) Aceclofar batch no.: 0002, manufacturing date: 09/00, expiry date: 09/0 Manufacturer: Gulf Pharmaceutical Industries (Julphar), U.A.E. Treatment B (reference formulation) Bristaflam batch no.: D00794, manufacturing date: 04/00, expiry date: 04/03 Manufacturer: Bristol-Myers Squibb
Dosage regimen	Each healthy volunteer received each of the following treatments as a single dose in accordance with a randomization scheme Treatment A: One tablet of Aceclofar, 100 mg aceclofenac Treatment B: One tablet of Bristaflam, 100 mg aceclofenac
Drug analysis	By HPLC analysis on-line with a UV–Visible detector; the limit of quantitation during sample analysis for aceclofenac was 0.20 μg/mL
Pharmacokinetic parameters	$t_{1/2}$, K_e, C_{max}, t_{max}, $Auc_{0\rightarrow t}$, $Auc_{0\rightarrow\infty}$, and $(Auc_{0\rightarrow t}/Auc_{0\rightarrow\infty})\%$
Bioequivalence parameters	C_{max}, $Auc_{0\rightarrow t}$, and $Auc_{0\rightarrow\infty}$
Tolerance	Both products were well tolerated by the volunteers
Conclusion	Bioequivalence of the test and reference products was shown

Summary

This study was performed to investigate the bioequivalence of aceclofenac between a generic test formulation (Aceclofar) tablets; manufacturer: Gulf Pharmaceutical Industries (Julphar), U.A.E., and a reference formulation (Bristaflam) tablets; manufacturer: Bristol-Myers Squibb. The clinical protocol called for 24 healthy subjects. The following blood samples for analysis of aceclofenac concentrations in blood were taken: immediately before (2 $\times$ 10 mL) and at 0.33, 0.66, 1.00, 1.33, 1.66, 2.00, 2.33, 2.66, 3.00, 3.50, 4.00, 4.50, 5.00, 6.00, 8.00, 10.00, 12.00, and 24.00 hours after administration of study drugs (1 $\times$ 10 mL). Samples from the first 24 subjects were analyzed for plasma aceclofenac. The subjects received an oral dose of 100 mg aceclofenac during each study phase in a randomized fashion and with a washout period of seven days.

Drug analysis of aceclofenac in plasma was performed by HPLC coupled on-line with a UV–Visible detector. The limit of quantitation during sample analyses being 0.20 μg/mL for aceclofenac. The plasma assay procedures were validated at IPRC according to international guidelines. The pharmacokinetics was determined by standard noncompartmental methods and ANOVA statistics was used for bioequivalence calculations.

The extent of absorption was determined by $AUC_{0\rightarrow t}$ and $AUC_{0\rightarrow\infty}$ of aceclofenac. The rate of absorption was determined by C_{max} and t_{max}. The adequacy of the sampling time was determined by the ratio $(AUC_{0\rightarrow t}/AUC_{0\rightarrow\infty})\%$. The half-life of elimination ($t_{(1/2)e}$) and the rate of elimination (K_e) of aceclofenac were used to further characterize the pharmacokinetic outcome of this study. $AUC_{0\rightarrow t}$, $AUC_{0\rightarrow\infty}$, and C_{max} values of aceclofenac were used for bioequivalence estimations.

ANOVA of the untransformed pharmakokinetic parameters t_{max}, C_{max}, $t_{(1/2)e}$, K_e, $AUC_{0\rightarrow t}$, and $AUC_{0\rightarrow\infty}$, and of the log-transformed data for C_{max}, $AUC_{0\rightarrow t}$, and $AUC_{0\rightarrow\infty}$ was performed using the Kinetica 2000 statistical program. The variance model included sequence, subjects nested in sequence, period, and product as factors. The significance of the sequence effect was tested using the subjects nested in sequence as the error term. A 5% level of significance was used for all comparisons (period, product, and sequence). This analysis showed that no statistically significant differences were obtained between the two products with respect to the calculated pharmacokinetic parameters: t_{max}, C_{max}, $t_{(1/2)e}$, K_e, $AUC_{0\rightarrow t}$, and $AUC_{0\rightarrow\infty}$ (Tables C.13 and C.14).

Consistent with the two one-sided tests for bioequivalence, 90% confidence intervals for the ratios of means was calculated for both untransformed and log-transformed $AUC_{0\rightarrow t}$, $AUC_{0\rightarrow\infty}$, and C_{max}. The geometric mean for the ratios expressed as a percentage (test product/reference product) is shown in Table C.13. The values obtained indicated that the 90% confidence limits for all geometric means are within the recommended range of bioequivalence of 80% to 125% for $AUC_{0\rightarrow t}$, $AUC_{0\rightarrow\infty}$, and C_{max}.

Mean ($N=24$) of plasma concentrations aceclofenac for Treatment A (test formulation, Aceclofar) and Treatment B (reference formulation, Bristaflam) after a single oral dose of 100 mg aceclofenac tablets.

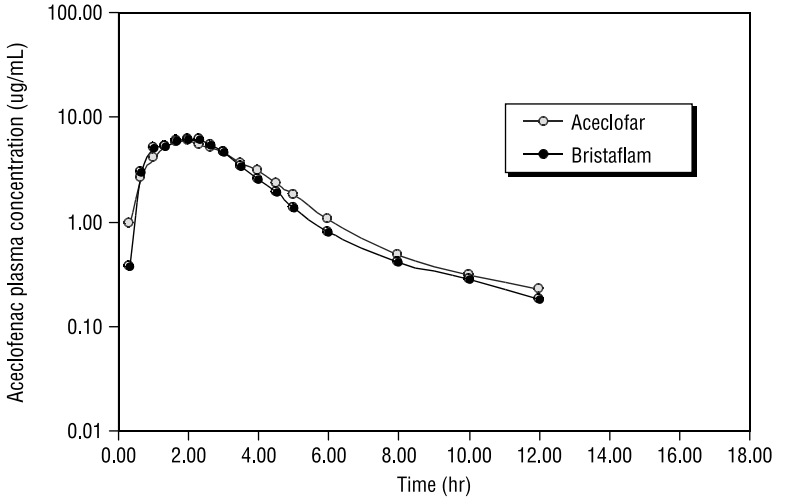
TABLE C.13 Pharmacokinetics—Bioequivalence Parameters of Aceclofenac

	Treatments				
	Means ± SD		90% confidence intervals (based on parametric testing)		
Parameter	A Test formulation	B Reference formulation	Point estimator (%)	Lower limit (%)	Upper limit (%)
$AUC_{0\rightarrow t}$ (μg hr/mL)	22.65 ± 4.48	21.88 ± 3.91	103.16	100.04	106.37
$AUC_{0\rightarrow\infty}$ (μg hr/mL)	24.02 ± 4.74	23.17 ± 4.28	103.42	100.18	106.76
C_{max} (μg/mL)	8.64 ± 1.86	9.36 ± 2.20	92.52	83.31	102.75

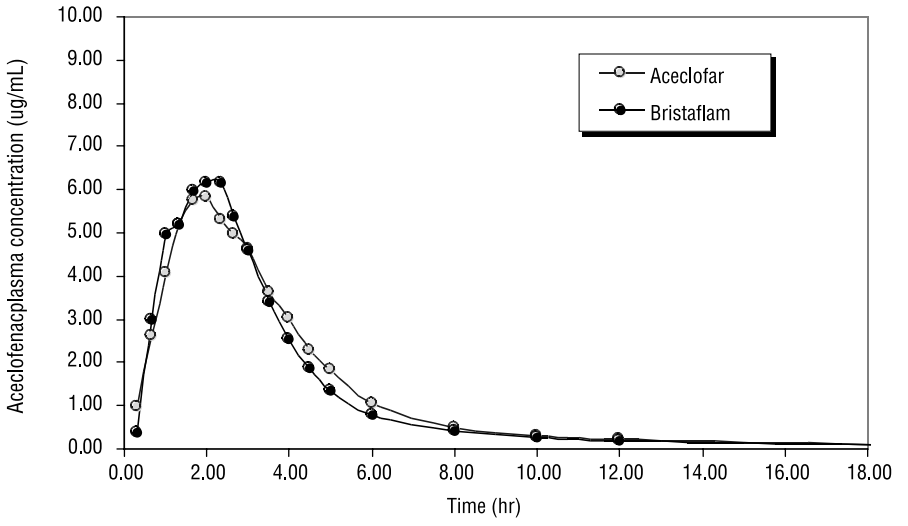
TABLE C.14 Pharmacokinetics—Further Parameters of Aceclofenac

	Treatments	
	Means ± SD	
Parameter	A Test formulation	B Reference formulation
t_{max} (hr)	1.99 ± 0.80	1.91 ± 0.75
$AUC_{0\rightarrow t}/AUC_{0\rightarrow\infty}$ (%)	94.30 ± 1.59	94.53 ± 1.55
$t_{1/2e}$ (hr)	3.30 ± 0.68	3.36 ± 0.90
K_e	0.2207 ± 0.0560	0.2254 ± 0.0811

Semi-logarithmic scale

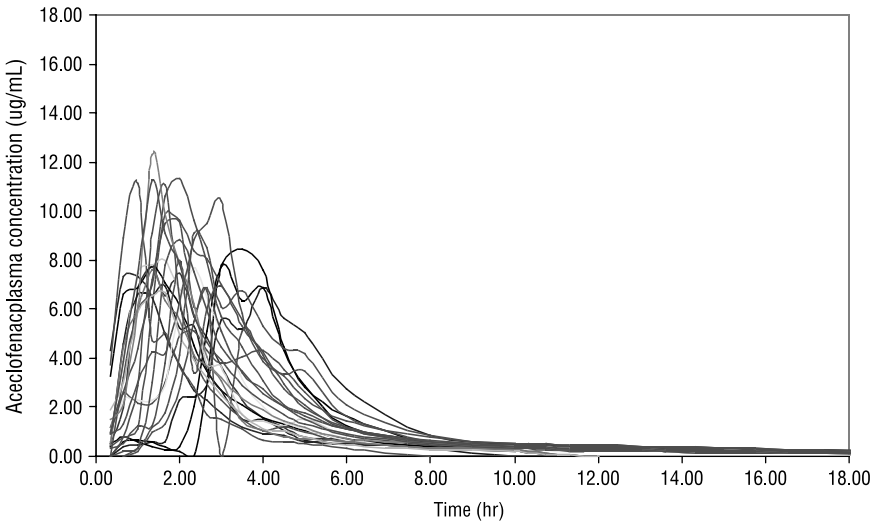


Linear scale

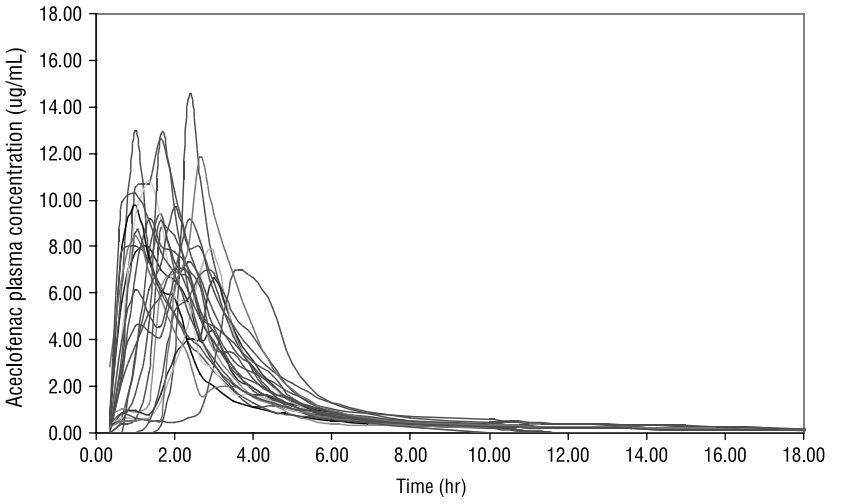


Individual plasma concentrations ($N=24$) of aceclofenac for Treatment A (test formulation, Aceclofar) and Treatment B (reference formulation, Bristaflam) after a single oral dose of 100 mg aceclofenac tablets.

Linear scale



Linear scale



Conclusion:
This study demonstrated that the TEST product, Aceclofar (Gulf Pharmaceutical Industries (Julphar), United Arab Emirates), 100 mg aceclofenac per tablet, is bioequivalent to the REFERENCE product, Bristaflam® (Bristol-Myers Squibb) 100 mg aceclofenac per tablet.

Comparative, Randomized, Open-Label, Two-Way Crossover Bioequivalence Study to Compare the Bioequivalence of Enalapril from Narapril 20 mg Enalapril Maleate Tablets [Gulf Pharmaceutical Industries (Julphar)], and Renitec® 20 mg Enalapril Maleate Tablets (Merck Sharp and Dohme B.V.), Each Given as a Single Dose to Healthy Adults Under Fasting Conditions.

Study synopsis	
Title of study	Comparative, randomized, open-label, two-way crossover bioequivalence study to compare the bioequivalence of enalapril from Narapril 20 mg enalapril maleate tablets [Gulf Pharmaceutical Industries (Julphar)], and Renitec 20 mg enalapril maleate tablets (Merck Sharp and Dohme B.V.), each given as a single dose to healthy adults under fasting conditions
Study code	ENA-GUL-T0601/133
Principal Investigator	Naji Najib, B.Sc. (Pharm.), Ph.D.
Sponsor	Gulf Pharmaceutical Industries (Julphar), U.A.E.
Purpose of trial	To assess the bioequivalence of a generic test product with a reference product by measurement of plasma concentrations of enalapril and its metabolite enalaprilat and calculation of the bioequivalence parameters from those measurements
Period of trial	Screening date: 21/08/2001 Phase I: 25/08/2001 Phase II: 01/09/2001
Protocol/design	Single-center, open, randomized two-way crossover bioequivalence study with a washout period of seven days
Number of subjects	Twenty-four subjects
Demographic data	Age: 23.25 ± 4.55 years, height: 175.96 ± 7.66 cm, weight at screening examination: 73.38 ± 9.39 kg (from $n = 24$)
Study medication	Treatment A (test formulation) Narapril batch no.: 0004, expiry date: 09/02 Manufacturer: Gulf Pharmaceutical Industries (Julphar), U.A.E. Treatment B (reference formulation) Renitec batch no.: HN01020, expiry date: 03/03 Manufacturer: Merck Sharp and Dohme B.V., Harlem-Netherlands
Dosage regimen	Each healthy volunteer received each of the following treatments as a single dose in accordance with a randomization scheme Treatment A: One tablet of Narapril, 20 mg enalapril maleate Treatment B: One tablet of Renitec, 20 mg enalapril maleate
Drug analysis	By HPLC analysis on-line with MS–MS detector. The limit of quantitation during sample analysis for both enalapril and its metabolite enalaprilat was 0.500 ng/mL
Pharmacokinetic parameters	$t_{1/2}$, K_e, C_{max}, t_{max}, $Auc_{0\rightarrow t}$, $Auc_{0\rightarrow\infty}$, and $(Auc_{0\rightarrow t}/Auc_{0\rightarrow\infty})\%$
Bioequivalence parameters	C_{max}, $Auc_{0\rightarrow t}$, and $Auc_{0\rightarrow\infty}$
Tolerance	Both products were well tolerated by the volunteers
Conclusion	Bioequivalence of the test and reference products was shown

Summary

This study was performed to investigate the bioequivalence of enalapril between a generic test formulation (Narapril) tablets; manufacturer: Gulf Pharmaceutical Industries (Julphar), U.A.E., and a reference formulation (Renitec) tablets; manufacturer: Merck Sharp and Dohme B.V. (MSD) (Harlem-Netherlands). The clinical protocol called for 24 healthy subjects. The following blood samples for analysis of enalapril and its metabolite enalaprilat concentrations in blood were taken: immediately before drug administration (1×20 mL) and at 0.25, 0.50, 0.75, 1.00, 1.33, 1.66, 2.00, 2.50, 3.00, 3.50, 4.00, 6.00, 8.00, 10.00, 12.00, 16.00, 24.00, and 48.00 hours after administration of study drugs (1×10 mL). Samples from the first 24 subjects were analyzed for enalapril and its active metabolite enalaprilat in plasma. The subjects received an oral dose of 20 mg enalapril maleate during each study phase in a randomized fashion and with a washout period of seven days.

Drug analysis of enalapril and its active metabolite enalaprilat in plasma was performed by HPLC coupled on-line with MS–MS detector. The limit of quantitation during sample analyses being 0.500 ng/mL for both enalapril and enalaprilat. The plasma assay procedures were validated at Cartesius Analytical Unit—Institute of Biomedical Sciences-USP Brazil according to the international guidelines. The pharmacokinetics was determined by standard noncompartmental methods and ANOVA -statistics was used for bioequivalence calculations.

The extent of absorption was determined by $AUC_{0\to t}$ and $AUC_{0\to\infty}$ of enalapril. The rate of absorption was determined by C_{max} and t_{max}. The adequacy of the sampling time was determined by the ratio $(AUC_{0\to t}/AUC_{0\to\infty})\%$. The half-life of elimination ($t_{(1/2)e}$) and the rate of elimination (K_e) were used to further characterize the pharmacokinetic outcome of this study. $AUC_{0\to t}$, $AUC_{0\to\infty}$, and C_{max} values of enalapril and enalaprilat were used for bioequivalence estimations.

ANOVA of the untransformed pharmacokinetic parameters t_{max}, C_{max}, $t_{(1/2)e}$, K_e, $AUC_{0\to t}$, and $AUC_{0\to\infty}$ and of the log-transformed data for C_{max}, $AUC_{0\to t}$, and $AUC_{0\to\infty}$ was performed using the Kinetica 2000 statistical program. The variance model included sequence, subjects nested in sequence, period, and product as factors. The significance of the sequence effect was tested using the subjects nested in sequence as the error term. A 5% level of significance was used for all comparisons (period, product, and sequence). This analysis showed that no statistically significant differences were obtained between the two products with respect to the calculated pharmacokinetic parameters: t_{max}, C_{max}, $t_{(1/2)e}$, K_e, $AUC_{0\to t}$, and $AUC_{0\to\infty}$ (Tables C.15 and C.16).

Consistent with the two one-sided tests for bioequivalence, 90% confidence intervals for the ratios of means was calculated for both untransformed and log-transformed $AUC_{0\to t}$, $AUC_{0\to\infty}$, and C_{max}. The geometric mean for the ratios expressed as a percentage (test product/reference product) is shown in Table C.15. The values obtained indicated that the 90% confidence limits for all geometric means are within the recommended range of bioequivalence of 80% to 125% for $AUC_{0\to t}$, $AUC_{0\to\infty}$, and C_{max}.

Mean ($N=24$) of plasma concentrations enalapril for Treatment A (test formulation, Narapril) and Treatment B (reference formulation, Renitec) after a single oral dose of 20 mg enalapril maleate tablets.

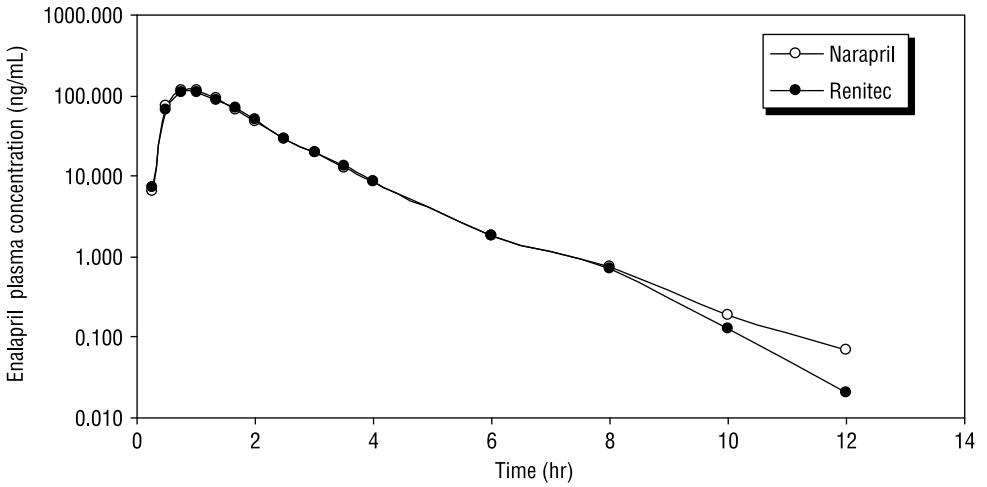
TABLE C.15 Pharmacokinetics—Bioequivalence Parameters of Enalapril

	Treatments				
	Means ± SD		90% confidence intervals (based on parametric testing)		
Parameter	A Test formulation	B Reference formulation	Point estimator (%)	Lower limit (%)	Upper limit (%)
$AUC_{0\to t}$ (ng hr/mL)	199.96 ± 67.77	196.78 ± 63.57	100.79	94.43	107.57
$AUC_{0\to\infty}$ (ng hr/mL)	201.72 ± 67.68	198.51 ± 63.32	100.79	94.51	107.49
C_{max} (ng/mL)	123.99 ± 43.77	121.28 ± 37.54	100.72	91.81	110.48

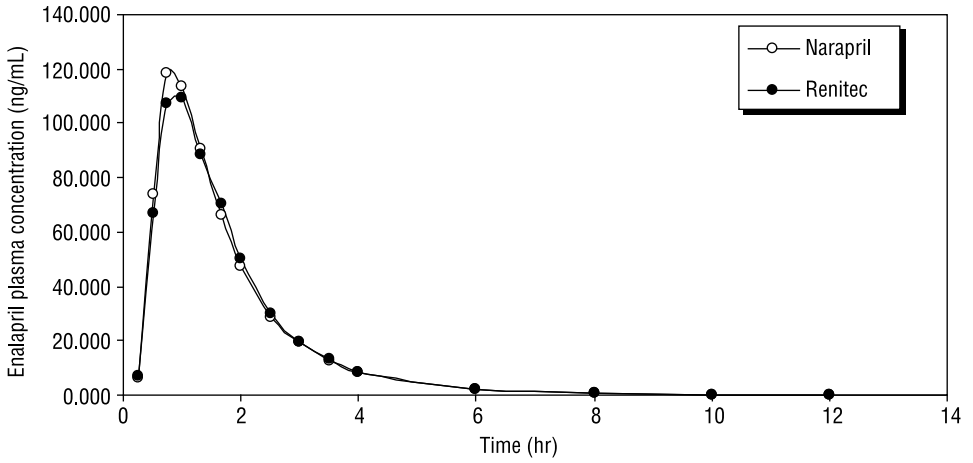
TABLE C.16 Pharmacokinetics—Further Parameters of Enalapril

	Treatments	
	Means ± SD	
Parameter	**A Test formulation**	**B Reference formulation**
t_{max} (hr)	0.86 ± 0.16	0.96 ± 0.30
$AUC_{0\rightarrow t}/AUC_{0\rightarrow\infty}$ (%)	99.00 ± 0.80	99.01 ± 0.86
$t_{1/2e}$ (hr)	1.28 ± 0.72	1.24 ± 0.50
K_e	0.65 ± 0.26	0.65 ± 0.24

Semi-logarithmic scale

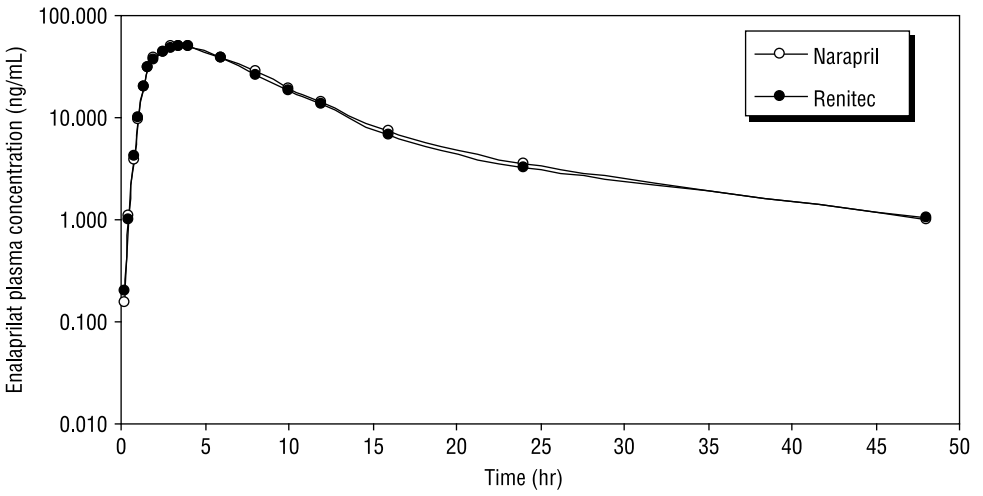


Linear scale

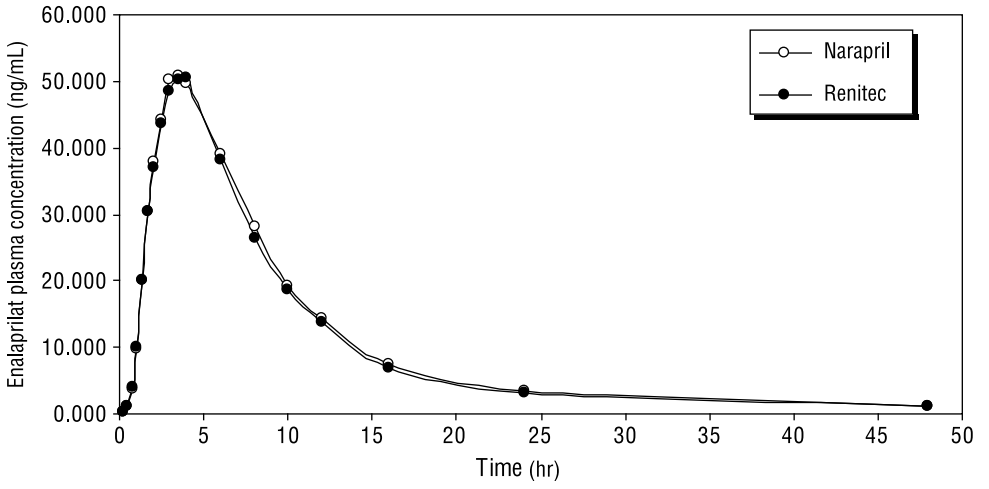


Mean ($N=24$) of plasma concentrations enalaprilat for Treatment A (test formulation, Narapril) and Treatment B (reference formulation, Renitec) after a single oral dose of 20 mg enalapril maleate tablets.

Semi-logarithmic scale

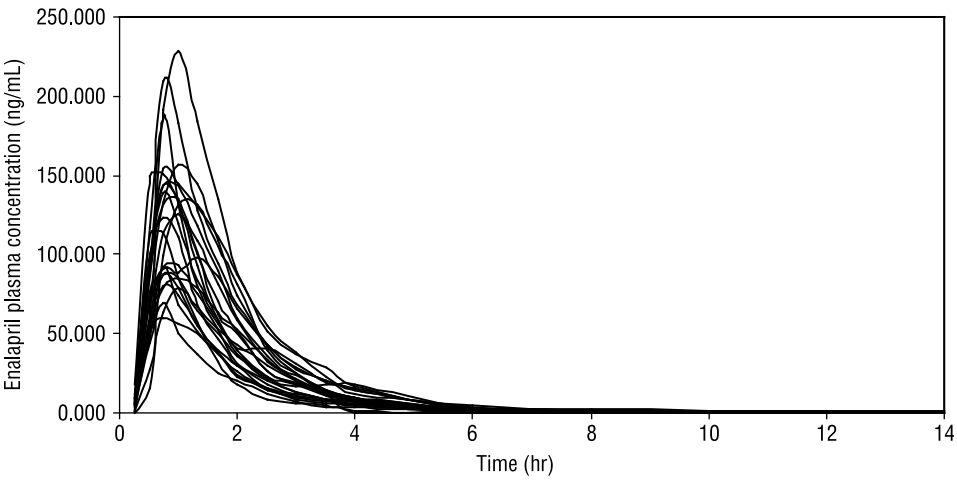


Linear scale

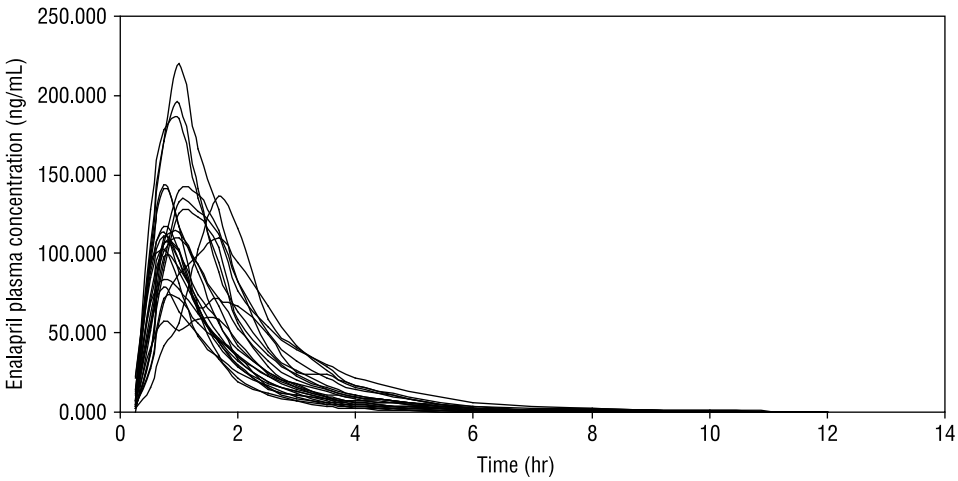


Individual plasma concentrations ($N=24$) of enalapril for Treatment A (test formulation, Narapril) and Treatment B (reference formulation, Renitec) after a single oral dose of 20 mg enalapril maleate tablets.

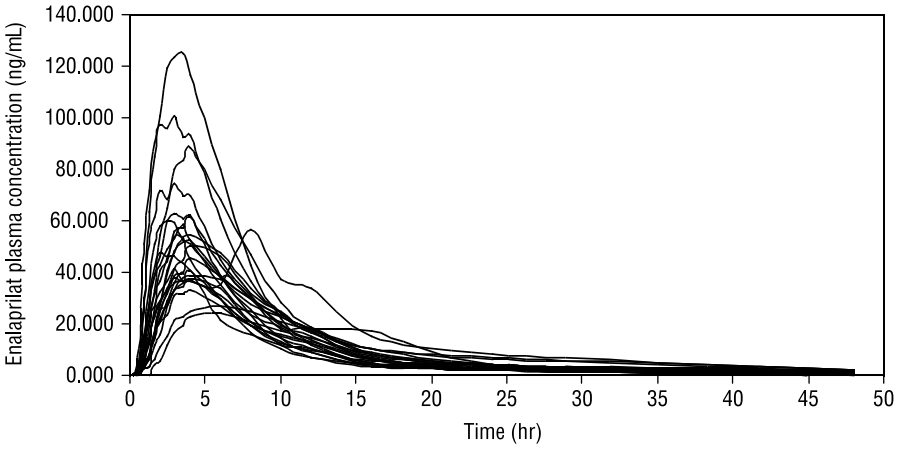
Test formulation
Linear scale

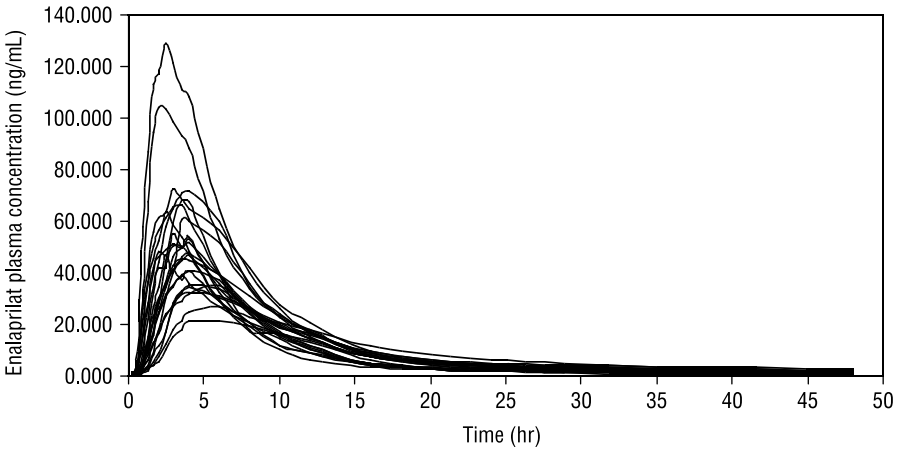


Reference formulation
Linear scale



Individual plasma concentrations ($N=24$) of enalaprilat for Treatment A (test formulation, Narapril) and Treatment B (reference formulation, Renitec) after a single oral dose of 20 mg enalapril maleate tablets.





C.1. BIOEQUIVALENCE EVALUATION OF LANSOPRAZOLE 30 MG CAPSULES (LANFAST® AND LANZOR®) IN HEALTHY VOLUNTEERS

Abstract

The bioequivalence of two lansoprazole 30 mg capsules was determined in healthy human, adult volunteers after a single dose in a randomized crossover study. The study was conducted at Pharmaconsult, Flemington Pharmaceutical Corporation, New Jersey, U.S.A. Reference (Lanzor®, Laboratoires Houde, Paris, France) and test (Lanfast®, Julphar, U.A.E.) products were administered to volunteers with 240 mL water after overnight fasting. Blood samples were collected at specified time intervals, plasma was separated, and analyzed for lansoprazole using a validated HPLC method. The pharmacokinetic parameters $AUC_{0\rightarrow t}$, $AUC_{0\rightarrow\infty}$, C_{max}, t_{max},$t_{1/2}$, and elimination rate constant were determined from plasma concentration–time profile of both formulations and found to be in good agreement with previously reported values. The calculated pharmacokinetic parameters were compared statistically to evaluate bioequivalence between the two brands, using the statistical modules recommended by FDA. The ANOVA did not show any significant difference between the two formulations and 90% confidence intervals fell within the acceptable range (80–120%) for bioequivalence. Based on these statistical inferences, it was concluded that the two formulations exhibited comparable pharmacokinetic profiles and that Julphar's Lanfast is bioequivalent to Lanzor of Laboratoires Houde.

C.1.1. Introduction

Bioequivalence of two formulations of the same drug comprises equivalence with respect to the rate and extent of their absorption. While the area under concentration–time curve (AUC) generally serves as the characteristic of the extent of absorption, the peak concentration (C_{max}) and the time of its occurrence (t_{max}) reflect the rate of absorption, especially in fast-releasing drug formulations (1,2). The present study was conducted to evaluate the bioequivalence of two brands of lansoprazole 30 mg capsules in fasting, healthy human volunteers. Although several studies have been published regarding lansoprazole pharmacokinetics, very few of them have focussed on the proof of bioequivalence between two formulations.

Chemically lansoprazole is 2-({3-Methyl-4-(2,2,2-trifluoroethoxy)-2-pyridyl)methyl} sulphinylbenzimidazole as shown in Figure C.1 (3).

It is a benzimidazole derivative with antisecretory and antiulcer activities. It inhibits the acid pump activity in the final stage of the enzyme process and therefore reduces the acid secretion of parietal cells. Lansoprazole is converted to active sulphenamide metabolites in the acidic environment of parietal cells; these metabolites inactivate H^+,K^+-ATPase (4). In vitro inhibition of H^+,K^+-ATPase activity and acid secretion by lansoprazole were found to be concentration dependent (5). Although lansoprazole alone has relatively low eradication effects on *Helicobacter pylori*, it may enhance the ability of other agents to eradicate the organism (6,7). Orally 30 mg/day of lansoprazole provided effective symptoms relief and healing of duodenal ulcer in 75% to 100% of patients after four weeks of therapy in non-comparative and comparative trials (8). In healthy volunteers, single and multiple oral doses of lansoprazole inhibited both basal and stimulated gastric acid secretions (9).

Since lansoprazole is acid labile, it is usually administered as capsules containing enteric-coated granules to prevent gastric decomposition and to increase the bioavailability. Its absolute bioavailability is 80% to 91%, which may be decreased if administered within 30 minutes of food intake. It has high protein binding (97%) which is decreased in renal function impairment (7,10). Lansoprazole has a half-life of about 1.5 hours; renal impairment decreases the half-life. After oral administration, peak concentration is achieved within one to two hours; a single 30 mg oral dose gives a peak concentration of 750 to 1150 ng/mL (7). Peak maxima (C_{max}) and bioavailability were not significantly altered by administration of multiple doses of the drug for seven days (11) when compared with the first day of treatment, although bioavailability showed marked interindividual variability (11,12).

No significant difference was reported in the pharmacokinetics of lansoprazole in typical healthy individuals when compared with patient groups (13). The peak blood levels over the dosage range of 15, 30, and 60 mg appear relatively dose proportional. Time-to-peak concentration ranged from 1.0 to 2.0 hours and half-life ranged from 1.3 to 2.1 hours (12). Significant difference was observed in half-life when compared in young (1.4 hours) and elderly (1.9–2.9 hours) after multiple-day dosing. No effect of food on half-life was observed, although food delayed the time to peak concentration (3.3–3.7 hours) (13). Lansoprazole is extensively metabolized in the liver to two main excretory metabolites that are inactive (13,14). In the acid environment, lansoprazole is converted to two active metabolites that inhibit the acid secretion by H^+,K^+-ATPase within the parietal cells canaliculus, but that are not present in the systemic circulation (14). Therefore, in this study only the parent drug was estimated in plasma samples.

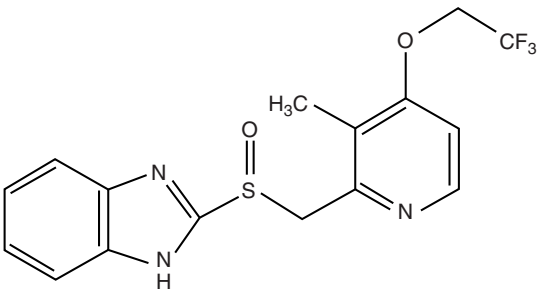


FIGURE C.1 Molecular structure of lansoprazole. *Source*: From Ref. 3.

C.1.1.1. Objectives of the Study

The purpose of this study was to determine the pharmacokinetic parameters of two brands of lansoprazole 30 mg capsules and then to compare these parameters statistically to evaluate the bioequivalence between the two brands. Lanfast (Julphar, U.A.E.) was used as test product while Lanzor (Laboratoires Houde, Paris, France) was used as reference product.

C.1.2. Material and Methods

C.1.2.1. Study Products

Test Product: Lanfast—Lansoprazole 30 mg capsules
Batch No.: 0001; Expiration date: 10/97
Manufacturer: Gulf Pharmaceutical Industries (Julphar), U.A.E.
Reference Product: Lanzor Lansoprazole 30 mg capsules
Batch No.: 147; Expiration date: 06/97
Manufacturer: Laboratoires Houde, Paris, France

C.1.2.2. Study Design

Considering the reported pharmacokinetic data (8) of lansoprazole, considering $\alpha = 0.05$, and the bioequivalence range (0.8–1.2), a total number of 26 volunteers is expected to be sufficient to obtain a statistical power greater than 80%. Based on this estimation, 26 healthy male volunteers completed this pharmacokinetic study at Flemington Pharmaceutical Corporation, New Jersey, U.S.A. Their mean age was 25.1 ± 7.2 years with a range of 18 to 45 years and mean body weight was 76.7 ± 10.9 kg with a range of 56.6 to 97.4 kg. Every subject completed an acceptable medical history, medication history, physical examination, an electrocardiogram, screens for HIV 1 and 2 antibodies and hepatitis B surface antigen, and a urine drug screen prior to study initiation. Selected routine clinical laboratory measurements were performed during screening. Upon completion of study, the physical examination and clinical laboratory measurements were repeated. The subjects were instructed to abstain from taking any medication for one week prior to and during the study period. Informed consents were obtained from the subjects after explaining the nature and purpose of the study. The study protocols were approved by the IRB of PRACS Institute, Fargo, North Dakota, U.S.A.

C.1.2.3. Drug Administration and Sample Collection

This study was based on a single-dose, randomized, two-treatment, two-period crossover design. In the morning of Phase I, after an overnight fasting (10 hours), volunteers were given single dose of either formulation (reference or test) of lansoprazole 30 mg with 240 mL of water. No food was allowed until four hours after dose administration. Water intake was allowed after two hours of dose; water, lunch, and dinner were given to all volunteers according to a time schedule. The volunteers were continuously monitored by PRACS Institute Ltd. Staff throughout the confinement period of study. They were not be permitted to lie down or sleep for the first four hours after the dose. Approximately, 10 mL of blood samples for lansoprazole assay were drawn into heparinized tubes through indwelling canula before (0 hours) and at 0.33, 0.67, 1.0, 1.67, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0,8.0, 10, 12, and 16 hours after dosing. The blood samples were centrifuged at 2400 r.p.m. for 15 minutes at 4°C, plasma was separated and kept frozen at -20°C until assayed. After a washout period of seven days, the study was repeated in the same manner to complete the crossover design.

C.1.2.4. Chromatographic Conditions

An HPLC method was developed and validated at PRACS Institute Analytical Laboratory Ltd. for lansoprazole assay in plasma samples. Lansoprazole reference standard was obtained from Chemo Iberica S.A.; internal standard megestrol acetate from Sigma; acetonitrile (HPLC grade), methyl-*t*-butyl ether (HPLC grade), and methanol (HPLC grade) from Burdick & Jackson; and KH_2PO_4 (ACS grade) from Fischer Scientific.

HPLC system was an isocratic system consisting of a solvent delivery pump, diode-array detector (Hewlett-Packard 1090), and a chromatograph (Hewlett-Packard 1090 Chemstation). The separation was performed using a stainless steel Dupont SB-CN column. The mobile phase consisted of 46% acetonitrile and 54% KH_2PO_4 buffer (pH 4.5) and was pumped at a flow rate of 1.5 mL/min. Effluent was monitored at a wave length of 285 nm and corresponding peak areas were recorded.

The method was validated by following the international guidelines (15). The limit of quantitation for lansoprazole was 20 ng/mL plasma; at this concentration, the accuracy was 96.9% while precision was 12.7%. During validation within-batch accuracy ranged from 92.5% to 107.0%, while within-batch precision remained below 16.6%. The between-batch accuracy was between 94.6% and 105.1%, while precision remained below 12.7%. Short-term stability showed that lansoprazole is stable in plasma at least 16 hours at room temperature, while long-term stability studies showed that lansoprazole is stable in plasma for at least 64 days when stored at −20°C.

C.1.2.5. Extraction of Lansoprazole from Plasma

A 1-mL aliquot of plasma was extracted with 6 mL of extraction solution containing internal standard (egestrol acetate, 0.13 μg/mL in methyl-*t*-butyl ether). After mixing and centrifugation, the organic phase was removed and evaporated to dryness under nitrogen stream and residue was reconstituted in 100 μL of methanol; 10 μL was injected onto the HPLC system equipped with a diode-array UV detector and peak areas were recorded.

C.1.2.6. Pharmacokinetic Analysis

Pharmacokinetic analysis was performed by means of a model-independent method. The maximum lansoprazole concentration (C_{max}) and the corresponding peak times (t_{max}) were determined by the inspection of the individual drug plasma concentration–time profiles. The elimination rate constant (λ_Z) was obtained from the least square fitted terminal log-linear portion of the plasma concentration–time profile. The elimination half-life ($t_{1/2}$) was calculated as $0.693/\lambda_Z$. The area under the curve to the last measurable concentration ($AUC_{0\to t}$) was calculated by the linear trapezoidal rule. The area under the curve extrapolated to infinity ($AUC_{0\to\infty}$) was calculated as $AUC_{0\to t}+C_t/\lambda_Z$, where C_t is the last measurable concentration.

C.1.2.7. Statistical Analysis

For the purpose of bioequivalence analysis, $AUC_{0\to t}$, $AUC_{0\to\infty}$, and C_{max} were considered as primary variables. Bioequivalence was assessed by means of an ANOVA GLM model (16) for crossover design and calculating standard 90% confidence intervals (17–19) of the ratio test/reference (T/R). The products were considered bioequivalent if the difference between two compared parameters was found statistically insignificant ($p \geq 0.05$) and 90% confidence intervals for these parameters fell within 80% to 120%. The acceptance range for C_{max} may be wider than that for AUC, particularly for drugs having highly variable peak concentrations; the recommended range for C_{max} is 70% to 143% (20–22). Anderson–Hauck test (23–25) was also applied, which computes the probability in the two one-sided *t*-tests based on the null hypothesis.

C.1.3. Results and Discussion

The mean concentration–time profiles for the two brands of lansoprazole 30 mg capsules are shown in Figure C.2. All calculated pharmacokinetic parameter values were in good agreement with the previously reported values (4–13). The pharmacokinetic parameters for both formulations are shown in Table C.17. For bioequivalence, evaluation various statistical modules were applied to $AUC_{0\to t}$, $AUC_{0\to\infty}$, and C_{max} as per current FDA guidelines (24). Table C.18 shows the results of the statistical analysis for $AUC_{0\to t}$, $AUC_{0\to\infty}$, and C_{max}. Due to lack of normality of the ln-transformed data, the final conclusions of this study were based on the analysis done on the non-transformed data.

According to the mean plasma levels of 26 subjects completing the study, the relative bioavailability was found to be 101.8%, 101.9%, and 110.4% on the basis of mean $AUC_{0\to t}$, $AUC_{0\to\infty}$, and C_{max}, respectively.

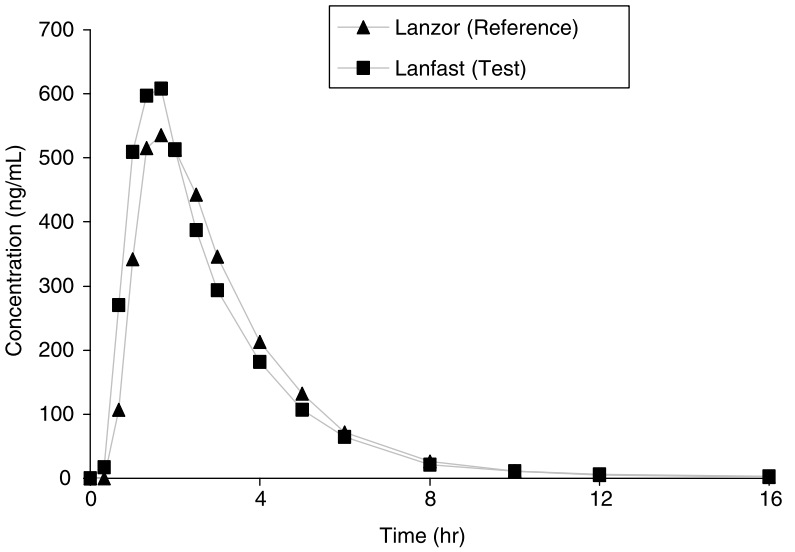


FIGURE C.2 Plasma concentration–time profile of lansoprazole 30 mg capsules.

C.1.3.1. Area Under the Curve ($AUC_{0\to t}$)

The mean $AUC_{0\to t}$ was 1741 and 1709 ng hr/mL for test and reference products, respectively; these values were in good agreement with reported ones (9,12). On the basis of these values, it was concluded that the two products did not show any unusual pharmacokinetics values for lansoprazole.

ANOVA did not show any significant differences for period effects and treatment (formulations). 90% confidence interval also fell within the bioequivalence acceptance criteria. Two one-sided *t*-tests (23–25) were also performed on the ratio (r) of mean $AUC_{0\to t}$ of test to mean $AUC_{0\to t}$ of reference. These tests showed the $p(r < 0.8) = 0.0054$ and $p(r > 1.2) = 0.0167$; so both tests were rejected and it was accepted that the probability for the ratio (T/R) to lie within 0.8 and 1.2 was 0.98.

C.1.3.2. Area Under the Curve ($AUC_{0\to\infty}$)

The mean $AUC_{0\to\infty}$ was 1813 and 1779 ng hr/mL for test and reference products, respectively; these values were in good agreement with reported ones (9,12). These values again confirmed the conclusion that the two products did not show any unusual pharmacokinetics for lansoprazole.

ANOVA did not show any significant differences for period effects and treatment (formulations). 90% confidence interval ranges also fell within the bioequivalence acceptance criteria. Two one-sided *t*-tests (23–25) were also performed on the ratio of mean $AUC_{0\to\infty}$ of test to mean $AUC_{0\to\infty}$ of reference. These tests showed the $p(r<0.8) = 0.0038$ and $p(r>1.2) = 0.012$; so both tests were rejected and it was accepted that the probability for the ratio (T/R) to lie within 0.8 and 1.2 was 0.98.

C.1.3.3. Peak Plasma Concentration (C_{max})

The mean C_{max} was 784 and 710 ng/mL for test and reference products, respectively; these values were in good agreement with the reported ones (9,12), assuring further the lack of any unusual pharmacokinetics for lansoprazole.

ANOVA did not show any significant difference; for period effects, the observed *F*-value was 0.092 while table *F*-value at the corresponding degree of freedom was 4.26 ($p>0.05$). In terms of treatment (formulations), no significant difference was observed; the observed *F*-value was 1.06 while table *F*-value at the corresponding degree of freedom was 4.26. 90% confidence interval ranges among the reference and test products also fell within the bioequivalence acceptance criteria for C_{max} (70% to 143%) (20–22). Two one-sided *t*-tests (23–25) were also performed on the ratio of mean C_{max} of test to mean C_{max} of reference. These tests showed the $p(r<0.8) = 0.003$ and $p(r> 1.2) = 0.183$; probability for this ratio lie within 0.8 to 1.2 was 0.81.

TABLE C.17 Pharmacokinetics—Parameters of the Two Brands of Lansoprazole Capsules

Volunteer number	Parameters											
	$AUC_{0\rightarrow t}$ (ng hr/mL)		$AUC_{0\rightarrow \infty}$ (ng hr/mL)		C_{max}(ng/mL)		t_{max} (hr)		λ_Z (hr)		$T_{1/2}$ (hr)	
	Test	Reference	Test	Reference	Test	Reference	Test	Reference	Test	Reference	Test	Reference
1	5724.40	5204.49	6107.77	5479.24	775.71	1093.93	2.50	1.00	0.19	0.19	3.68	3.59
2	1518.37	1172.63	1559.37	1218.05	1088.93	448.64	1.00	4.00	0.66	0.77	1.06	0.90
3	1186.72	1720.13	1247.21	1753.86	549.46	650.23	2.00	3.00	0.67	0.66	1.04	1.06
4	805.70	2390.36	869.29	2445.39	259.24	1002.91	1.00	1.67	0.56	0.58	1.24	1.19
5	1155.86	762.54	1210.83	794.06	617.60	302.34	0.67	2.50	0.63	0.80	1.10	0.87
6	2380.60	3050.71	2449.14	3110.23	723.69	923.12	1.67	2.50	0.40	0.41	1.73	1.70
7	1077.61	1044.55	1101.32	1087.05	676.98	631.89	1.33	1.33	0.92	0.81	0.75	0.86
8	1755.64	1760.97	1811.35	1798.06	1027.63	899.53	1.33	1.33	0.80	0.72	0.86	0.96
9	973.11	1196.18	997.42	1220.28	726.50	842.71	1.00	1.33	0.86	0.97	0.81	0.72
10	689.05	702.22	728.86	730.07	402.61	498.46	1.33	2.50	1.00	1.10	0.70	0.63
11	1323.86	1399.50	1446.42	1470.66	517.65	720.23	1.67	1.67	0.48	0.63	1.46	1.09
12	1367.10	232.78	1404.92	382.08	724.68	101.81	1.67	2.50	0.79	0.31	0.88	2.26
13	3092.67	3354.46	3153.61	3435.88	1000.02	1130.11	1.33	1.33	0.43	0.41	1.61	1.70
14	1730.24	1475.54	1783.64	1537.40	859.90	739.26	1.00	2.00	0.70	0.75	0.99	0.92
15	1070.01	1381.20	1135.48	1414.83	704.49	674.09	1.67	1.33	0.79	0.74	0.88	0.94
16	1803.08	1454.36	1849.72	1516.90	1101.85	597.21	0.67	1.00	0.65	0.61	1.07	1.14
17	2507.96	2160.75	2575.22	2258.86	977.05	781.99	1.33	2.00	0.52	0.50	1.34	1.38
18	2051.32	1192.23	2089.22	1332.68	1040.51	465.25	1.67	1.67	0.60	0.51	1.16	1.36
19	1181.96	1220.58	1237.80	1266.78	558.82	699.28	1.67	1.33	0.86	0.90	0.81	0.77
20	1097.87	395.90	1118.80	439.70	804.68	249.90	1.33	1.67	1.06	0.96	0.65	0.72
21	1339.46	1431.47	1392.86	1476.91	653.11	705.10	2.00	1.33	0.73	0.67	0.95	1.03
22	3610.07	2995.25	3715.98	3095.12	1872.63	1093.00	0.67	1.33	0.48	0.48	1.45	1.45
23	847.34	869.04	879.00	911.96	623.53	523.03	1.00	1.33	0.80	0.80	0.86	0.86
24	1655.91	1436.13	1739.99	1508.53	864.15	865.76	1.00	1.33	0.58	0.62	1.20	1.12
25	1285.73	3253.84	1413.65	3337.19	708.90	1009.81	1.00	1.00	0.81	0.36	0.86	1.91
26	2044.78	1185.02	2121.10	1244.11	536.57	818.97	1.67	1.33	0.30	0.76	2.32	0.91
Mean	1741.40	1709.34	1813.08	1779.46	784.50	710.33	1.35	1.74	0.66	0.65	1.21	1.23
SD	1072.43	1099.15	1127.72	1131.09	307.51	265.86	0.45	0.71	0.21	0.22	0.63	0.62
CV%	61.58	64.30	62.20	63.56	39.20	37.43	33.48	40.82	31.70	33.33	51.72	50.51

TABLE C.18 Statistical Analysis of Pharmacokinetic Data

Statistical analysis	$AUC_{0\rightarrow t}$	$AUC_{0\rightarrow\infty}$	C_{max}
ANOVA GLM (Prob. *F*)	>0.30 (>0.30)	>0.30 (>0.30)	>0.31 (>0.3)
90% CI	88.1–115.6% (89.6–117.3%)	89.0–114.8% (91.1–117.2%)	93.0–128.1% (86.1–119.9%)
Two one-sided *t*-tests probability[a]	0.98	0.98	0.81

Values in parentheses indicate analysis for periods.
[a] Probability for *T*/*R* ratio (*r*) to be within 0.8 and 1.2.

TABLE C.19 Pharmacokinetics—Bioequivalence Parameters of Enalaprilat

	Treatments				
	Means ± SD				
	A	B	90% confidence intervals (based on parametric testing)		
Parameter	Test formulation	Reference formulation	Point estimator (%)	Lower limit (%)	Upper limit (%)
$AUC_{0\rightarrow t}$ (ng hr/mL)	492.94 ± 148.77	476.89 ± 117.47	101.62	94.27	109.55
$AUC_{0\rightarrow\infty}$ (ng hr/mL)	518.36 ± 148.91	505.54 ± 115.97	100.87	93.69	108.64
C_{max} (ng/mL)	54.41 ± 23.50	53.87 ± 24.12	101.62	93.26	110.72

TABLE C.20 Pharmacokinetics—Further Parameters of Enalaprilat

	Treatments	
	Means ± SD	
Parameter	A Test formulation	B Reference formulation
t_{max} (hr)	3.92 ± 1.21	3.71 ± 0.91
$AUC_{0\rightarrow t}/AUC_{0\rightarrow\infty}$ (%)	94.79 ± 2.44	94.18 ± 4.38
$t_{1/2e}$ (hr)	11.79 ± 4.83	12.35 ± 5.66
K_e	0.07 ± 0.04	0.07 ± 0.04

For t_{max}, the parametric point estimate of difference (test—reference) was 0.39 hours, which showed an improved rate of bioavailability, though it was very close to acceptance limits (±20% of reference mean) (Tables C.19 and C.20).

C.1.4. Summary and Conclusions

The statistical comparison of $AUC_{0\rightarrow t}$, $AUC_{0\rightarrow\infty}$ and C_{max} clearly indicated no significant difference in the two brands of lansoprazole 30 mg capsules. 90% confidence intervals for the mean ratio (*T*/*R*) of $AUC_{0\rightarrow t}$, $AUC_{0\rightarrow\infty}$, and C_{max} were entirely within the FDA acceptance range. Based on the pharmacokinetic and statistical results of this study, we can conclude that Lanfast 30 mg capsules (Julphar, U.A.E.) is bioequivalent to Lanzor 30 mg capsules (Laboratoires Houde, France), and that the two products can be considered interchangeable in medical practice.

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Appendix I: Glossary of Terms

Accuracy: (IEEE) (1) A qualitative assessment of correctness or freedom from error. (2) A quantitative measure of the magnitude of error. Contrast with precision (Center for Devices and Radiological Health, CDRH). (3) The measure of an instrument's capability to approach a true or absolute value. It is a function of precision and bias.

Act: The Federal Food, Drug, and Cosmetic Act, as amended [Sections 201–902, 52 Stat. 1040 et seq., as amended (21 U.S.C. 321–392)].

Adverse drug reaction (ADR): In the pre-approval clinical experience with a new medicinal product or its new usages, particularly as the therapeutic dose(s) may not be established: all noxious and unintended responses to a medicinal product related to any dose should be considered ADRs. The phrase responses to a medicinal product mean that a causal relationship between a medicinal product and an adverse event is at least a reasonable possibility, i.e., the relationship cannot be ruled out. Regarding marketed medicinal products: a response to a drug which is noxious and unintended and which occurs at doses normally used in man for prophylaxis, diagnosis, or therapy of diseases or for modification of physiologic function (see the ICH Guideline for Clinical Safety Data Management: Definitions and Standards for Expedited Reporting).

Adverse event (AE): Any untoward medical occurrence in a patient or clinical investigation subject administered with a pharmaceutical product does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product (see the ICH Guideline for Clinical Safety Data Management: Definitions and Standards for Expedited Reporting).

Algorithm: (IEEE) (1) A finite set of well-defined rules for the solution of a problem in a finite number of steps. (2) Any sequence of operations for performing a specific task.

Algorithm analysis: (IEEE) A software V&V task to ensure that the algorithms selected are correct, appropriate, and stable, and meet all accuracy, timing, and sizing requirements.

Analysis: (1) To separate into elemental parts or basic principles so as to determine the nature of the whole. (2) A course of reasoning showing that a certain result is a consequence of assumed premises. (3) (American National Standards Institute, ANSI) The methodical investigation of a problem, and the separation of the problem into smaller related units for further detailed study.

Analyte: A specific chemical moiety being measured, which can be intact drug, biomolecule or its derivative, metabolite, and/or degradation product in a biologic matrix.

Analytical laboratory: A facility used by a pharmaceutical sponsor or contract research organization to determine the nature and proportionate quantities of the constituents of a compound for an in vivo bioequivalence study. An analytical laboratory typically completes an assay to determine the drug concentration in body fluids.

Analytical run (or batch): A complete set of analytical and study samples with appropriate number of standards and QCs for their validation. Several runs (or batches) may be completed in one day, or one run (or batch) may take several days to complete.

ANDA: Abbreviated new drug application.

Anomaly: (IEEE) Anything observed in the documentation or operation of software that deviates from expectations based on previously verified software products or reference documents.

ANSI: American National Standards Institute.

API: Active pharmaceutical ingredients.

Applicable regulatory requirement(s): Any law(s) and regulation(s) addressing the conduct of clinical trials of investigational products.

Approval (in relation to Institutional Review Boards, IRB): The affirmative decision of the IRB that the clinical trial has been reviewed and may be conducted at the institution site within the constraints set forth by the IRB, the institution, Good Clinical Practice (GCP), and the applicable regulatory requirements.

ASCII: American Standard Code for Information Interchange.

Attributable data: Data that can be traced to individuals responsible for observing and recording the data. In an automated system, attributability could be achieved by a computer system designed to identify individuals responsible for any input.

Audit: A systematic and independent examination of trial-related activities and documents to determine whether the evaluated trial-related activities were conducted, and the data were recorded, analyzed, and accurately reported according to the protocol, sponsor's standard operating procedures (SOPs), Good Clinical Practice (GCP), and the applicable regulatory requirement(s). (1) (IEEE) An independent examination of a work product or set of work products to assess compliance with specifications, standards, contractual agreements, or other criteria. (2) (ANSI) To conduct an independent review and examination of system records and activities in order to test the adequacy and effectiveness of data security and data integrity procedures, to ensure compliance with established policy and operational procedures, and to recommend any necessary changes.

Audit certificate: A declaration of confirmation by the auditor that an audit has taken place.

Audit report: A written evaluation by the sponsor's auditor of the results of the audit.

Audit trail: Documentation that allows reconstruction of the course of events. (1) (ISO) Data in the form of a logical path linking a sequence of events, used to trace the transactions that have affected the contents of a record. (2) A chronological record of system activities that is sufficient to enable the reconstruction, reviews, and examination of the sequence of environments and activities surrounding or leading to each event in the path of a transaction from its inception to output of final results. An audit trail is a secure, computer generated, time-stamped electronic record that allows reconstruction of the course of events relating to the creation, modification, and deletion of an electronic record.

Baseline: (National Institute of Standards and Technology, NIST) A specification or product that has been formally reviewed and agreed upon, that serves as the basis for further development, and that can be changed only through formal change control procedures.

Batch: A specific quantity or lot of a test or control article that has been characterized according to Section 58.105(a). (IEEE) Pertaining to a system or mode of operation in which inputs are collected and processed all at one time, rather than being processed as they arrive, and a job, once started, proceeds to completion without additional input or user interaction. Contrast with conversational, interactive, on-line, real time.

Batch processing: Execution of programs serially with no interactive processing. Contrast with real time processing.

BCS: Biopharmaceutics Classification System.

Benchmark: A standard against which measurements or comparisons can be made.

Bias: A measure of how closely the mean value in a series of replicate measurements approaches the true value.

Bioavailability (BA): The rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the action site. For drug products

not intended to be absorbed into the bloodstream, BA may be assessed by measurements intended to reflect the rate and extent to which the active ingredient or active moiety becomes available at the action site.

Bioequivalence (BE): The absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study. Where there is an intentional rate difference (e.g., in certain extended release dosage forms), certain pharmaceutical equivalents or alternatives may be considered bioequivalent if there is no significant difference in the extent to which the active ingredient or moiety from each product becomes available at the site of drug action. This applies only if the difference in the rate at which the active ingredient or moiety becomes available at the site of drug action is intentional and is reflected in the proposed labeling, is not essential to the achievement of effective body drug concentrations on chronic use, and is considered medically insignificant for the drug.

Bioequivalence requirement: A requirement imposed by the Food and Drug Administration for in vitro and/or in vivo testing of specified drug products, which must be satisfied as a condition of marketing.

Bioequivalent drug products: Pharmaceutical equivalent or pharmaceutical alternative products that display comparable bioavailability when studied under similar experimental conditions. The Regulatory Authorities describes one set of conditions under which a test and reference listed drug shall be considered bioequivalent: the rate and extent of absorption of the test drug do not show a significant difference from the rate and extent of absorption of the reference drug when administered at the same molar dose of the therapeutic ingredient under similar experimental conditions in either a single dose or multiple doses; or the extent of absorption of the test drug does not show a significant difference from the extent of absorption of the reference drug when administered at the same molar dose of the therapeutic ingredient under similar experimental conditions in either a single dose or multiple doses and the difference from the reference drug in the rate of absorption of the drug is intentional, is reflected in its proposed labeling, is not essential to the achievement of effective body drug concentrations on chronic use, and is considered medically insignificant for the drug.

Biologic matrix: A discrete material of biologic origin that can be sampled and processed in a reproducible manner. Examples are blood, serum, plasma, urine, feces, saliva, sputum, and various discrete tissues.

Bioresearch Monitoring (BIMO) Goal Date (or Inspection Summary Goal Date): The date by which the Office of Generic Drugs (OGD) Division of Bioequivalence (DBE) project manager anticipates a review of the inspection results from the Drug Safety Institute (DSI). This date is determined in consultation with DSI staff and includes time to assess the inspection results.

Blank: A sample of a biologic matrix to which no analytes have been added that is used to assess the specificity of the bioanalytical method.

Blinding/masking: A procedure in which one or more parties to the trial are kept unaware of the treatment assignment(s). Single-blinding usually refers to the subject(s) being unaware, and double-blinding usually refers to the subject(s), investigator(s), monitor, and, in some cases, data analyst(s) being unaware of the treatment assignment(s).

Bomb: A trojan horse that attacks a computer system upon the occurrence of a specific logical event (logic bomb), the occurrence of a specific time-related logical event (time bomb), or is hidden in virus, andelectronic mail or data and is triggered when read in a certain way (letter bomb).

Boolean: Pertaining to the principles of mathematical logic developed by George Boole, a nineteenth century mathematician. Boolean algebra is the study of operations carried out on variables that can have only one of two possible values, i.e., 1 (true) and 0 (false).

Bootstrap: (IEEE) A short computer program that is permanently resident or easily loaded into a computer and whose execution brings a larger program, such an operating system or its loader, into memory.

Boundary value: (1) (IEEE) A data value that corresponds to a minimum or maximum input, internal, or output value specified for a system or component. (2) A value which lies at, or just inside or just outside a specified range of valid input and output values.

Boundary value analysis: (NBS) A selection technique in which test data are chosen to lie along "boundaries" of the input domain (or output range) classes, data structures, procedure parameters, etc. Choices often include maximum, minimum, and trivial values or parameters. This technique is often called stress testing.

Bulk drug substance: Any substance represented for use in a drug and when in the manufacturing, processing, or packaging of a drug becomes an active ingredient of a finished dosage form. This does not include intermediates used in the synthesis of such substances.

Calibration: Ensuring continuous adequate performance of sensing, measurement, and actuating equipment with regard to specified accuracy and precision requirements.

Calibration standard: A biologic matrix to which a known amount of analyte has been added or spiked. Calibration standards are used to construct calibration curves from which the concentrations of analytes in QCs and in unknown study samples are determined.

Case report form (CRF): A printed, optical, or electronic document designed to record all of the protocol required information to be reported to the sponsor on each trial subject.

Certified copy: A copy of original information that has been verified, as indicated by dated signature, as an exact copy having all of the same attributes and information as the original.

CFR: Code of Federal Regulations.

Change control: The processes, authorities for, and procedures to be used for all changes that are made to the computerized system and/or the system's data. Change control is a vital subset of the Quality Assurance (QA) program within an establishment and should be clearly described in the establishment's SOPs.

Clinical facility: A site where patients or subjects are examined and observed during an in vivo bioequivalence study.

Clinical trial/study: Any investigation in human subjects intended to discover or verify the clinical, pharmacologic and/or other pharmacodynamic effects of an investigational product(s), and/or to identify any adverse reactions to an investigational product(s), and/or to study absorption, distribution, metabolism, and excretion of an investigational product(s) with the object of ascertaining its safety and/or efficacy. The terms clinical trial and clinical study are synonymous.

Clinical trial/study report: A written description of a trial/study of any therapeutic, prophylactic, or diagnostic agent conducted in human subjects, in which the clinical and statistical description, presentations, and analyses are fully integrated into a single report (see the ICH Guideline for Structure and Content of Clinical Study Reports).

CPMP: Commission for Proprietary Medicinal Products.

Comparator (**product**): An investigational or marketed product (i.e., active control), or placebo, used as a reference in a clinical trial.

Compliance (**in relation to trials**): Adherence to all the trial-related requirements, Good Clinical Practice (GCP) requirements, and the applicable regulatory requirements.

Compliance classification: The compliance status of an inspection.

Contract: A written, dated, and signed agreement between two or more involved parties that sets out any arrangements on delegation and distribution of tasks and obligations and, if appropriate, on financial matters. The protocol may serve as the basis of a contract.

Contract Research Organization (**CRO**): A person or an organization (commercial, academic, or other) contracted by the sponsor to perform one or more of a sponsor's trial-related duties and functions.

Control article: It means any food additive, color additive, drug, biologic product, electronic product, medical device for human use, or any article other than a test article, feed, or water that is administered to the test system in the course of a nonclinical laboratory study for the purpose of establishing a basis for comparison with the test article.

Coordinating committee: A committee that a sponsor may organize to coordinate the conduct of a multicentre trial.

Coordinating investigator: An investigator assigned the responsibility for the coordination of investigators at different centers participating in a multicenter trial.

Correctness: (IEEE) The degree to which software is free from faults in its specification, design, and coding. The degree to which software, documentation, and other items meet specified requirements. The degree to which software, documentation, and other items meet user needs and expectations, whether specified or not.

Critical control point: (QA) A function or an area in a manufacturing process or procedure, the failure of which, or loss of control over, may have an adverse affect on the quality of the finished product and may result in a unacceptable health risk.

Critical design review: (IEEE) A review conducted to verify that the detailed design of one or more configuration items satisfy specified requirements; to establish the compatibility among the configuration items and other items of equipment, facilities, software, and personnel; to assess risk areas for each configuration item; and, as applicable, to assess the results of producibility analyses, review preliminary hardware product specifications, evaluate preliminary test planning, and evaluate the adequacy of preliminary operation and support documents.

Critical drugs: "Critical dose drugs" are defined as those drugs where comparatively small differences in dose or concentration lead to dose- and concentration-dependent, serious therapeutic failures and/or adverse drug reactions that may be persistent, irreversible, slowly reversible, or life-threatening events.

Criticality: (IEEE) The degree of impact that a requirement, module, error, fault, failure, or other item has on the development or operation of a system. Synonym: severity.

Criticality analysis: (IEEE) Analysis which identifies all software requirements that have safety implications, and assigns a criticality level to each safety-critical requirement based upon the estimated risk.

Cross-validation: Comparison validation parameters of two bioanalytical methods.

Data: Representations of facts, concepts, or instructions in a manner suitable for communication, interpretation, or processing by humans or by automated means.

Data analysis: (IEEE) (1) Evaluation of the description and intended use of each data item in the software design to ensure that the structure and intended use will not result in a hazard. *Data structures*: They are assessed for data dependencies that circumvent isolation, partitioning, data aliasing, and fault containment issues affecting safety, and the control or mitigation of hazards. (2) Evaluation of the data structure and usage in the code to ensure each is defined and used properly by the program. Usually performed in conjunction with logic analysis.

Data validation: (1) (ISO) A process used to determine if data are inaccurate, incomplete, or unreasonable. The process may include format checks, completeness checks, check key tests, reasonableness checks, and limit checks. (2) The checking of data for correctness or compliance with applicable standards, rules, and conventions.

Direct access: Permission to examine, analyze, verify, and reproduce any records and reports that are important to evaluation of a clinical trial. Any party (e.g., domestic and foreign regulatory authorities, sponsor's monitors, and auditors) with direct access should take all reasonable precautions within the constraints of the applicable regulatory requirement(s) to maintain the confidentiality of subjects' identities and sponsor's proprietary information.

Direct entry: Recording data where an electronic record is the original capture of the data. Examples are the keying by an individual of original observations into the system, or automatic recording by the system of the output of a balance that measures subject's body weight.

Directed inspection: An inspection based on substantive information suggesting scientific misconduct, major human subject protection violations, or compromised bioequivalence data.

Distributor: The distributor of a product under a custom or own label. The product is manufactured and labeled by a registered establishment.

Documentation: All records, in any form (including, but not limited to, written, electronic, magnetic, and optical records, and scans, X-rays, and electrocardiograms) that describe or record the methods, conduct, and/or results of a trial, the factors affecting a trial, and the actions taken. (ANSI) The aids provided for the understanding of the structure and intended uses of an information system or its components, such as flowcharts, textual material, and user manuals.

Documentation, level of: (NIST) A description of required documentation indicating its scope, content, format, and quality. Selection of the level may be based on project cost, intended usage, extent of effort, or other factors, e.g., level of concern.

Documentation, software: (NIST) Technical data or information, including computer listings and printouts, in human readable form, that describe or specify the design or details, explain the capabilities, or provide operating instructions for using the software to obtain desired results from a software system.

Documentation plan: (NIST) A management document describing the approach to a documentation effort. The plan typically describes what documentation types are to be prepared, what their contents are to be, when this is to be done and by whom, how it is to be done, and what are the available resources and external factors affecting the results.

Dosage form: The form of the completed pharmaceutical product, e.g., tablet, capsule, injection, elixir, and suppository.

DRA: Drug Regulatory Authority.

Driver: A program that links a peripheral device or internal function to the operating system, and providing for activation of all device functions. Synonym: device driver. Contrast with test driver.

Drug: Any substance or pharmaceutical product for human or veterinary use that is intended to modify or explore physiologic systems or pathologic states for the benefit of the recipient.

Drug master file (DMF): A master file that provides a full set of data on an active pharmaceutical ingredients. In some countries, the term may also comprise data on an excipient or a component of a product such as a container.

Drug product: A finished dosage form, e.g., tablet, capsule, or solution that contains the active drug ingredient, generally, but not necessarily, in association with inactive ingredients.

Drug Regulatory Authority: A national body that administers the full spectrum of drug regulatory activities, including at least all of the following functions: marketing authorization of new products and variation of existing products, quality control laboratory testing, adverse drug reaction monitoring.

Electronic record: Any combination of text, graphics, data, audio, pictorial, or other information representation in digital form that is created, modified, maintained, archived, retrieved, or distributed by a computer system.

Electronic signature: A computer data compilation of any symbol or series of symbols executed, adopted, or authorized by an individual to be the legally binding equivalent of the individual's handwritten signature.

Embedded computer: A device which has its own computing power dedicated to specific functions, usually consisting of a microprocessor and firmware. The computer becomes an integral part of the device as opposed to devices which are controlled by an independent, stand-alone computer. It implies software that integrates operating system and application functions.

Embedded software: (IEEE) Software that is part of a larger system and performs some of the requirements of that system, e.g., software used in an aircraft or rapid transit system. Such software does not provide an interface with the user.

End user: (ANSI) (1) A person, device, program, or computer system that uses an information system for the purpose of data processing in information exchange. (2) A person whose occupation requires the use of an information system but does not require any knowledge of computers or computer programming.

Essential documents: Documents which individually and collectively permit evaluation of the conduct of a study and the quality of the data produced.

Essential drugs: Drugs that satisfy the health care needs of the majority of the population. As indicated by the Expert Committee on the Use of Essential Drugs, each country may generate its own list of essential drugs.

Establishment evaluation request (EER): A request made to evaluate establishments listed in an application.

Event table: A table which lists events and the corresponding specified effect(s) of or reaction(s) to each event.

Excipient: Any component of a finished dosage form other than the claimed therapeutic ingredient or ingredients.

Failure analysis: Determining the exact nature and location of a program error in order to fix the error, to identify and fix other similar errors, and to initiate corrective action to prevent future occurrences of this type of error. Contrast with debugging.

FDA: Food and Drug Administration.

FDA Compliance Program Guidance Manual (CPGM), Compliance Program 7348.001: Bioresearch Monitoring (in vivo bioequivalence): The program describing the procedures used by FDA staff in performing inspections of bioequivalence studies.

Feasibility study: Analysis of the known or anticipated need for a product, system, or component to assess the degree to which the requirements, designs, or plans can be implemented.

File: (1) (ISO) A set of related records treated as a unit, e.g., in stock control, a file could consists of a set of invoices. (2) The largest unit of storage structure that consists of a named collection of all occurrences in a database of records of a particular record type. Synonym: data set.

File maintenance: (ANSI) The activity of keeping a file up to date by adding, changing, or deleting data.

File transfer protocol: (1) Communications protocol that can transmit binary and ASCII data files without loss of data. (2) TCP/IP protocol that is used to log onto the network, list directories, and copy files. It can also translate between ASCII and EBCDIC.

Finished product: A product that has undergone all stages of production, including packaging in its final container and labeling.

Firmware: (IEEE) The combination of a hardware device, e.g., an IC; and computer instructions and data that reside as read-only software on that device. Such software cannot be modified by the computer during processing.

Flowchart or flow diagram: (1) (ISO) A graphical representation in which symbols are used to represent such things as operations, data, flow direction, and equipment, for the definition, analysis, or solution of a problem. (2) (IEEE) A control flow diagram in which suitably annotated geometrical figures are used to represent operations, data, or equipment, and arrows are used to indicate the sequential flow from one to another.

Full validation: Establishment of all validation parameters to apply to sample analysis for the bioanalytical method for each analyte.

Generic products: Generic products may be marketed either under the approved non-proprietary name or under a brand (proprietary) name. They may be marketed in dosage forms and/or strengths different from those of the innovator products. Where the term generic product is used, it means a pharmaceutical product, usually intended to be interchangeable with the innovator product, which is usually manufactured without a license from the innovator company and marketed after expiry of the patent or other exclusivity rights. The term should not be confused with generic names for active pharmaceutical ingredients (APIs). The term generic product has somewhat different meanings in different jurisdictions. Use of this term is therefore

avoided as much as possible, and the term multisource pharmaceutical product (see below) is used instead.

GMP: Good Manufacturing Practices.

Good Clinical Practice (GCP): A standard for the design, conduct, performance, monitoring, auditing, recording, analyses, and reporting of clinical trials that provides assurance that the data and reported results are credible and accurate, and that the rights, integrity, and confidentiality of trial subjects are protected.

Good Laboratory Practice and Bioequivalence Investigations Branch (**GBIB**): The unit within the Division of Scientific Investigations responsible for assigning and/or performing inspections of facilities conducting bioequivalence and nonclinical studies.

Hard copy: Printed, etc., output on paper.

Immediate release dosage form: A dosage form that is intended to release the entire active ingredient on administration with no enhanced, delayed, or extended release effect.

IND: Investigational new drug.

Independent Data-Monitoring Committee (**IDMC**) (**Data and Safety Monitoring Board**, **Monitoring Committee**, **Data Monitoring Committee**): An independent data-monitoring committee that may be established by the sponsor to assess at intervals the progress of a clinical trial, the safety data, and the critical efficacy endpoints, and to recommend to the sponsor whether to continue, modify, or stop a trial.

Independent Ethics Committee (**IEC**): An independent body (a review board or a committee, institutional, regional, national, or supranational), constituted of medical professionals and non-medical members, whose responsibility is to ensure the protection of the rights, safety, and well-being of human subjects involved in a trial and to provide public assurance of that protection, by, among other things, reviewing and approving/providing favorable opinion on, the trial protocol, the suitability of the investigator(s), facilities, and the methods and material to be used in obtaining and documenting informed consent of the trial subjects. The legal status, composition, function, operations, and regulatory requirements pertaining to Independent Ethics Committees may differ among countries, but should allow the Independent Ethics Committee to act in agreement with GCP as described in this guideline.

Informed consent: A process by which a subject voluntarily confirms his or her willingness to participate in a particular trial, after having been informed of all aspects of the trial that are relevant to the subject's decision to participate. Informed consent is documented by means of a written, signed, and dated informed consent form.

Innovator pharmaceutical product: A pharmaceutical product that was first authorized for marketing (normally as a patented drug) based on documentation of its safety, efficacy, and pharmaceutical quality (according to contemporary regulatory requirements). When drugs have been available in the marketplace for many years, it may not be possible to identify an innovator pharmaceutical product. In these cases an innovator product may be defined as a medicinal authorized and marketed on the basis of a full dossier, i.e., including chemical, biologic, pharmacologic–toxicologic, and clinical data.

Inspection: The act by a regulatory authority(ies) of conducting an official review of documents, facilities, records, and any other resources that are deemed by the authority(ies) to be related to the clinical trial and that may be located at the site of the trial, at the sponsor's and/or contract research organization's (CRO's) facilities, or at other establishments deemed appropriate by the regulatory authority(ies).

Installation: (ANSI) The phase in the system life cycle that includes assembly and testing of the hardware and software of a computerized system. Installation includes installing a new computer system, new software or hardware, or otherwise modifying the current system.

Institutional Review Board (**IRB**): An independent body constituted of medical, scientific, and non-scientific members, whose responsibility is to ensure the protection of the rights, safety, and well-being of human subjects involved in a trial by, among other things, reviewing, approving, and providing continuing review of trial protocol and

amendments and of the methods and material to be used in obtaining and documenting informed consent of the trial subjects.

Interchangeability: An interchangeable pharmaceutical product is one that is therapeutically equivalent to a comparator (reference) product.

Interim clinical trial/study report: A report of intermediate results and their evaluation based on analyses performed during the course of a trial.

Internal standard: Test compound(s) (e.g., structurally similar analog, stable labeled compound) added to both calibration standards and samples at known and constant concentration to facilitate quantification of the target analyte(s).

Investigational product: A pharmaceutical form of an active ingredient or placebo being tested or used as a reference in a clinical trial, including a product with a marketing authorization when used or assembled (formulated or packaged) in a way different from the approved form, or when used for an unapproved indication, or when used to gain further information about an approved use.

Investigator/institution: An expression meaning "the investigator and/or institution, where required by the applicable regulatory requirements." **Investigator**: A person responsible for the conduct of the clinical trial at a trial site. If a trial is conducted by a team of individuals at a trial site, the investigator is the responsible leader of the team and may be called the principal investigator.

Investigator's brochure: A compilation of the clinical and nonclinical data on the investigational product(s) that is relevant to the study of the investigational product(s) in human subjects.

IR: Immediate release.

IS: Internal standards.

ISO: International Organization for Standardization.

Key element: (QA) An individual step in an critical control point of the manufacturing process.

Legally acceptable representative: An individual, juridical, or other body authorized under applicable law to consent, on behalf of a prospective subject, to the subject's participation in the clinical trial.

Life cycle methodology: The use of any one of several structured methods to plan, design, implement, test, and operate a system from its conception to the termination of its use.

Limit of detection (**LOD**): The lowest concentration of an analyte that the bioanalytical procedure can reliably differentiate from background noise.

Lower limit of quantification (**LLOQ**): The lowest amount of an analyte in a sample that can be quantitatively determined with suitable precision and accuracy.

Method: A comprehensive description of all procedures used in sample analysis.

Methods validation: The analytical process of actual use testing of the applicant's proposed regulatory method(s) in an FDA laboratory.

Methods verification: The process of testing a compendial ANDA (abbreviated new drug application) drug substance or drug product by compendial procedures in an FDA laboratory for purposes of ensuring compliance with compendial specifications and evaluating the appropriateness of a particular formulation for analysis by the compendial methods.

Monitoring: The act of overseeing the progress of a clinical trial, and of ensuring that it is conducted, recorded, and reported in accordance with the protocol, Standard Operating Procedures (SOPs), Good Clinical Practice (GCP), and the applicable regulatory requirement(s).

Monitoring report: A written report from the monitor to the sponsor after each site visit and/or other trial-related communication according to the sponsor's standard operating procedures.

Multicenter trial: A clinical trial conducted according to a single protocol but at more than one site, and therefore, carried out by more than one investigator.

Multisource and single-source drug products: In most instances it refers to those pharmaceutical equivalents available from more than one manufacturer that may or may not be

therapeutically equivalent. Multisource pharmaceutical products that are therapeutically equivalent are interchangeable.

Multi-tasking: (IEEE) A mode of operation in which two or more tasks are executed in an interleaved manner. Synonym: parallel processing.

Narrow therapeutic index: Less than a 2-fold difference in median lethal dose (LD 50) and median effective does (ED 50) values, or less than 2-fold difference in the minimum toxic concentration and minimum effective concentration in the blood; steep dose response.

Nonclinical laboratory study: In vivo or in vitro experiments in which test articles are studied prospectively in test systems under laboratory conditions to determine their safety. The term does not include studies utilizing human subjects or clinical studies or field trials in animals. The term does not include basic exploratory studies carried out to determine whether a test article has any potential utility or to determine physical or chemical characteristics of a test article.

Nonclinical study: Biomedical studies not performed on human subjects.

Octal: The base 8 number system. Digits are 0, 1, 2, 3, 4, 5, 6, and 7.

OEM: Original equipment manufacturer.

Official action indicated (OAI): Objectionable conditions or practices were found that represented significant departures from the regulations and could require administrative or regulatory sanctions.

Operating system: (ISO) Software that controls the execution of programs, and that provides services such as resource allocation, scheduling, input/output control, and data management. Usually, operating systems are predominantly software, but partial or complete hardware implementations are possible.

Operation and maintenance phase: (IEEE) The period of time in the software life cycle during which a software product is employed in its operational environment, monitored for satisfactory performance, and modified as necessary to correct problems or to respond to changing requirements.

Opinion (in relation to Independent Ethics Committee): The judgment and/or the advice provided by an Independent Ethics Committee (IEC).

Original data: Original data are those values that represent the first recording of study data. Food and Drug Administration is allowing original documents and the original data recorded on those documents to be replaced by certified copies provided the copies are identical and have been verified as such.

Partial validation: Modification of validated bioanalytical methods that do not necessarily call for full revalidation.

Perfective maintenance: (IEEE) Software maintenance performed to improve the performance, maintainability, or other attributes of a computer program. Contrast with adaptive maintenance, corrective maintenance.

Pharmaceutical alternatives: Drug products that contain the identical therapeutic moiety, or its precursor, but not necessarily in the same amount or dosage form or as the same salt or ester. Each such drug product individually meets either the identical or its own respective compendial or other applicable standard of identity, strength, quality, and purity, including potency and, where applicable, content uniformity, disintegration times and/or dissolution rates.

Pharmaceutical equivalents: Drug products in identical dosage forms that contain identical amounts of the identical active drug ingredient, i.e., the same salt or ester of the same therapeutic moiety, or, in the case of modified release dosage forms that require a reservoir or overage or such forms as prefilled syringes where residual volume may vary, that deliver identical amounts of the active drug ingredient over the identical dosing period; do not necessarily contain the same inactive ingredients; and meet the identical compendial or other applicable standard of identity, strength, quality, and purity, including potency and, where applicable, content uniformity, disintegration times, and/or dissolution rates.

Pharmaceutical product: Any preparation for human or veterinary use that is intended to modify or explore physiologic systems or pathologic states for the benefit of the recipient.

Note: In many countries in Latin America multisource drug products are referred to as "productos similares" and are marketed under an approved new brand proprietary. However, when they are marketed under the non-proprietary name (unbranded), the products are usually known as "generic products." These products cannot be considered interchangeable until appropriate evidence has been submitted to show interchangeability.

Platform: The hardware and software which must be present and functioning for an application program to run (perform) as intended. A platform includes, but is not limited to the operating system or executive software, communication software, microprocessor, network, input/output hardware, any generic software libraries, database management, user interface software, and other similar kinds.

Precision: The closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions.

Predicate rule: Underlying requirements set forth in the Federal Food, Drug, and Cosmetic Act, the PHS Act, and FDA regulations (other than 21 CFR part 11). Regulations governing good clinical practice and human subject protection can be found at 21 CFR parts 50, 56, 312, 511, and 812.

Processed: The final extract (prior to instrumental analysis) of a sample that has been subjected to various manipulations (e.g., extraction, dilution, and concentration).

Project plan: (NIST) A management document describing the approach taken for a project. The plan typically describes work to be done, resources required, methods to be used, the configuration management and quality assurance procedures to be followed, the schedules to be met, the project organization, etc. Project in this context is a generic term. Some projects may also need integration plans, security plans, test plans, quality assurance plans, etc.

Proof of correctness: (NBS) The use of techniques of mathematical logic to infer that a relation between program variables assumed true at program entry implies that another relation between program variables holds at program exit.

Protocol: A document that describes the objective(s), design, methodology, statistical considerations, and organization of a trial. The protocol usually also gives the background and rationale for the trial, but these could be provided in other protocol referenced documents. Throughout the ICH GCP Guideline the term protocol refers to protocol and protocol amendments.

Qualification, installation: (FDA) Establishing confidence that process equipment and ancillary systems are compliant with appropriate codes and approved design intentions, and that manufacturer's recommendations are suitably considered.

Qualification, operational: (FDA) Establishing confidence that process equipment and subsystems are capable of consistently operating within established limits and tolerances.

Qualification, process performance: (FDA) (1) Establishing confidence that the process is effective and reproducible. (2) Establishing confidence through appropriate testing that the finished product produced by a specified process meets all release requirements for functionality and safety.

Quality assurance (**QA**): All those planned and systematic actions that are established to ensure that the trial is performed and the data are generated, documented (recorded), and reported in compliance with Good Clinical Practice (GCP) and the applicable regulatory requirement(s). (1) (ISO) The planned systematic activities necessary to ensure that a component, module, or system conforms to established technical requirements. (2) All actions that are taken to ensure that a development organization delivers products that meet performance requirements and adhere to standards and procedures. (3) The policy, procedures, and systematic actions established in an enterprise for the purpose of providing and maintaining some degree of confidence in data integrity and accuracy throughout the life cycle of the data, which includes input, update, manipulation, and output. (4) (QA) The actions, planned, and performed, to provide confidence that all systems and components that influence the quality of the product are working as expected individually and collectively.

Quality assurance software: (IEEE) (1) A planned and systematic pattern of all actions necessary to provide adequate confidence that an item or product conforms to established technical requirements. (2) A set of activities designed to evaluate the process by which products are developed or manufactured.

Quality assurance unit: Any person or organizational element, except the study director, designated by testing facility management to perform the duties relating to quality assurance of nonclinical laboratory studies.

Quality control (**QC**): The operational techniques and activities undertaken within the quality assurance system to verify that the requirements for quality of the trial-related activities have been fulfilled.

Quality control sample (**QCS**): A spiked sample used to monitor the performance of a bioanalytical method and to assess the integrity and validity of the results of the unknown samples analyzed in an individual batch.

Quantification range: The range of concentration, including upper and lower limit of quantification (ULOQ and LLOQ), that can be reliably and reproducibly quantified with accuracy and precision through the use of a concentration-response relationship.

Randomization: The process of assigning trial subjects to treatment or control groups using an element of chance to determine the assignments in order to reduce bias.

Raw data: Any laboratory worksheets, records, memoranda, notes, or exact copies thereof, that are the result of original observations and activities of a nonclinical laboratory study and are necessary for the reconstruction and evaluation of the report of that study. In the event that exact transcripts of raw data have been prepared (e.g., tapes which have been transcribed verbatim, dated, and verified accurate by signature), the exact copy or exact transcript may be substituted for the original source as raw data. Raw data may include photographs, microfilm, or microfiche copies, computer printouts, magnetic media, including dictated observations, and recorded data from automated instruments.

Recovery: The extraction efficiency of an analytical process, reported as a percentage of the known amount of an analyte carried through the sample extraction and processing steps of the method.

Reference product: The pharmaceutical product with which the "new" product is intended to be interchangeable in clinical practice. The reference product is usually the innovators product for which safety, efficacy, and quality has been documented.

Regulatory authorities: Bodies having the power to regulate. In the ICH GCP guideline the expression Regulatory Authorities include the authorities that review submitted clinical data and those that conduct inspections (see 1.29). These bodies are sometimes referred to as competent authorities.

Regulatory methods: The analytical procedures proposed by the applicant and agreed upon by the Agency to determine whether the drug substance or drug product meets its established specifications. For drug substances and drug products having monographs in the United States Pharmacopeia (USP), the USP analytical methods are considered regulatory by definition.

Relational database: Database organization method that links files together as required. Relationships between files are created by comparing data such as account numbers and names. A relational system can take any two or more files and generate a new file from the records that meet the matching criteria. Routine queries often involve more than one data file, e.g., a customer file and an order file can be linked in order to ask a question that relates to information in both files, such as the names of the customers that purchased a particular product. Contrast with network database, and flat file.

Reproducibility: The precision between two laboratories. It also represents precision of the method under the same operating conditions over a short period of time.

Requirement: (IEEE) (1) A condition or capability needed by a user to solve a problem or achieve an objective. (2) A condition or capability that must be met or possessed by a system or system component to satisfy a contract, standard, specification, or other formally imposed documents. (3) A documented representation of a condition or capability as in (1) or (2).

Retention period: (ISO) The length of time specified for data on a data medium to be preserved.

Retrospective trace: (IEEE) A trace produced from historical data recorded during the execution of a computer program. Note: this differs from an ordinary trace, which is produced cumulatively during program execution.

Revalidation: Relative to software changes, revalidation means validating the change itself, assessing the nature of the change to determine potential ripple effects, and performing the necessary regression testing.

Risk: (IEEE) A measure of the probability and severity of undesired effects. Often taken as the simple product of probability and consequence.

Robustness: The degree to which a software system or component can function correctly in the presence of invalid inputs or stressful environmental conditions.

Routine: (IEEE) A subprogram that is called by other programs and subprograms. Note: This term is defined differently in various programming languages.

Routine inspection: An inspection to determine the compliance of a clinical facility or analytical laboratory with U.S. regulations. Typically there is no prior indication of misconduct, human subject protection problems, or suspect data.

Safety: (Department of Defense, DOD) Freedom from those conditions that can cause death, injury, occupational illness, or damage to or loss of equipment or property, or damage to the environment.

Sample: A generic term encompassing controls, blanks, unknowns, and processed samples.

Selectivity: The ability of the bioanalytical method to measure and differentiate the analytes in the presence of components that may be expected to be present. These could include metabolites, impurities, degradants, or matrix components.

Serious adverse event (SAE) or serious adverse drug reaction (Serious ADR): Any untoward medical occurrence that at any dose: results in death, is life threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity.

Side effect: An unintended alteration of a program's behavior caused by a change in one part of the program, without taking into account the effect the change has on another part of the program.

Software: (ANSI) Programs, procedures, rules, and any associated documentation pertaining to the operation of a system. Contrast with hardware.

Software documentation: (NIST) Technical data or information, including computer listings and printouts, in human readable form, that describe or specify the design or details, explain the capabilities, or provide operating instructions for using the software to obtain desired results from a software system.

Software reliability: (IEEE) (1) The probability that software will not cause the failure of a system for a specified time under specified conditions. The probability is a function of the inputs to and use of the system in the software. The inputs to the system determine whether existing faults, if any, are encountered. (2) The ability of a program to perform its required functions accurately and reproducibly under stated conditions for a specified period of time.

Software safety change analysis: (IEEE) Analysis of the safety-critical design elements affected directly or indirectly by the change to show that the change does not create a new hazard, does not impact on a previously resolved hazard, does not make a currently existing hazard more severe, and does not adversely affect any safety-critical software design element.

Software safety code analysis: (IEEE) Verification that the safety-critical portions of the design are correctly implemented in the code.

Software safety design analysis: (IEEE) Verification that the safety-critical portion of the software design correctly implements the safety-critical requirements and introduces no new hazards.

Software safety requirements analysis: (IEEE) Analysis evaluating software and interface requirements to identify errors and deficiencies that could contribute to a hazard.

Software safety test analysis: (IEEE) Analysis demonstrating that safety requirements have been correctly implemented and that the software functions safely within its specified environment. Tests may include; unit level tests, interface tests, software configuration item testing, system level testing, stress testing, and regression testing.

Software validation: Confirmation by examination and provision of objective evidence that software specifications conform to user needs and intended uses and that the particular requirements implemented through the software can be consistently fulfilled. Design level validation is that portion of the software validation that takes place in parts of the software life cycle before the software is delivered to the end user.

SOPs: Standard operating procedures.

Source code: (1) (IEEE) Computer instructions and data definitions expressed in a form suitable for input to an assembler, compiler, or other translator. (2) The human readable version of the list of instructions (program) that cause a computer to perform a task. Contrast with object code.

Source data: All information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents (original records or certified copies).

Source documents: Original documents, data, and records (e.g., hospital records, clinical, and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate copies, microfiches, photographic negatives, microfilm or magnetic media, X-rays, subject files, and records kept at the pharmacy, at the laboratories and at medico-technical departments involved in the clinical trial).

Specification: (IEEE) A document that specifies, in a complete, precise, verifiable manner, the requirements, design, behavior, or other characteristics of a system or component, and often, the procedures for determining whether these provisions have been satisfied. Contrast with requirement.

Specification, formal: (NIST) (1) A specification written and approved in accordance with established standards. (2) A specification expressed in a requirements specification language. Contrast with requirement.

Specification, functional: (NIST) A specification that documents the functional requirements for a system or system component. It describes what the system or component is to do rather than how it is to be built. Often part of a requirements specification. Contrast with requirement.

Specification, interface: (NIST) A specification that documents the interface requirements for a system or system component. Often part of a requirements specification. Contrast with requirement.

Specification, performance: (IEEE) A document that sets forth the performance characteristics that a system or component must possess. These characteristics typically include speed, accuracy, and memory usage. Often part of a requirements specification. Contrast with requirement.

Specification, product: (IEEE) A document which describes as built version of the software.

Specification, programming: (NIST).

Specification, requirements: (NIST) A specification that documents the requirements of a system or system component. It typically includes functional requirements, performance requirements, interface requirements, design requirements (attributes and constraints), development (coding) standards, etc. Contrast with requirement.

Specimen: Any material derived from a test system for examination or analysis.

Sponsor: An individual, company, institution, or organization which takes responsibility for the initiation, management, and/or financing of a clinical trial.

Sponsor investigator: An individual who both initiates and conducts, alone or with others, a clinical trial, and under whose immediate direction the investigational product is

administered to, dispensed to, or used by a subject. The term does not include any person other than an individual (e.g., it does not include a corporation or an agency). The obligations of a sponsor-investigator include both those of a sponsor and those of an investigator.

Stability: The chemical stability of an analyte in a given matrix under specific conditions for given time intervals.

Standard curve: The relationship between the experimental response value and the analytical concentration (also called calibration curve).

Standard operating procedures (SOPs): Detailed, written instructions to achieve uniformity of the performance of a specific function.

Study completion date: The date the final report is signed by the study director.

Study director: The individual responsible for the overall conduct of a nonclinical laboratory study.

Study initiation date: The date the protocol is signed by the study director.

Subinvestigator: Any individual member of the clinical trial team designated and supervised by the investigator at a trial site to perform critical trial-related procedures and/or to make important trial-related decisions (e.g., associates, residents, and research fellows).

Subject/trial subject: An individual who participates in a clinical trial, either as a recipient of the investigational product(s) or as a control.

Subject identification code: A unique identifier assigned by the investigator to each trial subject to protect the subject's identity and used in lieu of the subject's name when the investigator reports adverse events and/or other trial-related data.

SUPAC: Scale-up and post-approval changes.

System life cycle: The course of developmental changes through which a system passes from its conception to the termination of its use, e.g., the phases and activities associated with the analysis, acquisition, design, development, test, integration, operation, maintenance, and modification of a system.

Test: (IEEE) An activity in which a system or component is executed under specified conditions, the results are observed or recorded and an evaluation is made of some aspect of the system or component.

Test article: Any food additive, color additive, drug, biologic product, electronic product, medical device for human use, or any other article subject to regulation under the act or under sections 351 and 354–360F of the Public Health Service Act. A control article is the test article used as a reference.

Test system: Any animal, plant, microorganism, or subparts thereof to which the test or control article is administered or added for study. Test system also includes appropriate groups or components of the system not treated with the test or control articles.

Testability: (IEEE) (1) The degree to which a system or component facilitates the establishment of test criteria and the performance of tests to determine whether those criteria have been met. (2) The degree to which a requirement is stated in terms that permit establishment of test criteria and performance of tests to determine whether those criteria have been met.

Testing, boundary value: A testing technique using input values at, just below, and just above, the defined limits of an input domain; and with input values causing outputs to be at, just below, and just above, the defined limits of an output domain.

Testing, compatibility: The process of determining the ability of two or more systems to exchange information. In a situation where the developed software replaces an already working program, an investigation should be conducted to assess possible comparability problems between the new software and other programs or systems.

Testing facility: A person who actually conducts a nonclinical laboratory study, i.e., actually uses the test article in a test system. Testing facility includes any establishment required to register under Section 510 of the act that conducts nonclinical laboratory studies and any consulting laboratory described in Section 704 of the act that conducts such studies. Testing facility encompasses only those operational units that are being or have been used to conduct nonclinical laboratory studies.

Therapeutic equivalents: Drug products are considered to be therapeutic equivalents only if they are pharmaceutical equivalents and if they can be expected to have the same clinical effect and safety profile when administered to patients under the conditions specified in the labeling. The FDA classifies as therapeutically equivalent those products that meet the following general criteria: (1) they are approved as safe and effective; (2) they are pharmaceutical equivalents in that they (*i*) contain identical amounts of the same active drug ingredient in the same dosage form and route of administration, (*ii*) meet compendial or other applicable standards of strength, quality, purity, and identity; (3) they are bioequivalent in that (*i*) they do not present a known or potential bioequivalence problem, and they meet an acceptable in vitro standard, or (*ii*) if they do present such a known or potential problem, they are shown to meet an appropriate bioequivalence standard; (4) they are adequately labeled; (5) they are manufactured in compliance with Current Good Manufacturing Practice regulations. The FDA considers drug products to be therapeutically equivalent if they meet the criteria outlined above, even though they may differ in certain other characteristics such as shape, scoring configuration, release mechanisms, packaging, excipients (including colors, flavors, and preservatives), expiration date/time and minor aspects of labeling (e.g., the presence of specific pharmacokinetic information) and storage conditions. When such differences are important in the care of a particular patient, it may be appropriate for the prescribing physician to require that a particular brand be dispensed as a medical necessity. With this limitation, however, FDA believes that products classified as therapeutically equivalent can be substituted with the full expectation that the substituted product will produce the same clinical effect and safety profile as the prescribed product.

Trial site: The location(s) where trial-related activities are actually conducted.

Trojan horse: A method of attacking a computer system, typically by providing a useful program which contains code intended to compromise a computer system by secretly providing for unauthorized access, the unauthorized collection of privileged system or user data, the unauthorized reading or altering of files, the performance of unintended and unexpected functions, or the malicious destruction of software and hardware.

Unambiguous: (1) Not having two or more possible meanings. (2) Not susceptible to different interpretations. (3) Not obscure, not vague. (4) Clear, definite, and certain.

Unexpected adverse drug reaction: An adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g., Investigator's Brochure for an unapproved investigational product or package insert/summary of product characteristics for an approved product) (see the ICH Guideline for Clinical Safety Data Management: Definitions and Standards for Expedited Reporting).

Unknown: A biologic sample that is the subject of the analysis.

Upper limit of quantification (ULOQ): The highest amount of an analyte in a sample that can be quantitatively determined with precision and accuracy.

User: (ANSI) Any person, organization, or functional unit that uses the services of an information processing system.

USP: The current edition of the United States Pharmacopeia and its supplements.

Validate: To prove to be valid.

Validation, process: (FDA) Establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality characteristics.

Validation, prospective: (FDA) Validation is conducted prior to the distribution of either a new product, or product made under a revised manufacturing process, where the revisions may affect the product's characteristics.

Validation, retrospective: (FDA) (1) Validation of a process for a product already in distribution based upon accumulated production, testing, and control data. (2) Retrospective validation can also be useful to augment initial premarket prospective validation for new products or changed processes. Test data is useful only if the methods and results are adequately specific. Whenever test data are used to demonstrate conformance to specifications, it is important that the test methodology be qualified to assure that the test results are objective and accurate.

Validation, software: (NBS) Determination of the correctness of the final program or software produced from a development project with respect to the user needs and requirements. Validation is usually accomplished by verifying each stage of the software development life cycle.

Validation, verification, and testing: (NIST) Used as an entity to define a procedure of review, analysis, and testing throughout the software life cycle to discover errors, determine functionality, and ensure the production of quality software.

Validation protocol: (FDA) A written plan stating how validation will be conducted, including test parameters, product characteristics, production equipment, and decision points on what constitutes acceptable test results.

Variable: A name, label, quantity, or data item whose value may be changed many times during processing. Contrast with constant.

Vendor: A person or an organization that provides software and/or hardware and/or firmware and/or documentation to the user for a fee or in exchange for services. Such a firm could be a medical device manufacturer.

Verifiable: Something that can be proved or confirmed by examination or investigation.

Vulnerable subjects: Individuals whose willingness to volunteer in a clinical trial may be unduly influenced by the expectation, whether justified or not, of benefits associated with participation, or of a retaliatory response from senior members of a hierarchy in case of refusal to participate. Examples are members of a group with a hierarchical structure, such as medical, pharmacy, dental, and nursing students, subordinate hospital and laboratory personnel, employees of the pharmaceutical industry, members of the armed forces, and persons kept in detention. Other vulnerable subjects include patients with incurable diseases, persons in nursing homes, unemployed or impoverished persons, patients in emergency situations, ethnic minority groups, homeless persons, nomads, refugees, minors, and those incapable of giving consent.

Well-being (of the trial subjects): The physical and mental integrity of the subjects participating in a clinical trial.

WHO: World Health Organization.

Appendix II: Bioequivalence Testing Literature

2006

A multivariate test for population bioequivalence
Chervoneva I, Hyslop T, Hauck WW. Stat Med 2006 Jun 30. In this article, we propose a multivariate generalization of the criteria for testing univariate population bioequivalence. Recently, a number of approaches for testing multivariate equivalence have appeared in the literature. Most of them consider a multivariate equivalence region, which implies simultaneous comparison of means in each dimension. In contrast, our proposal combines a comparison of means and a comparison of variances into a single aggregate criterion, using the trace of the covariance matrix as a scalar measure of the total variability. We use a confidence interval approach to multivariate population bioequivalence testing, similar to the univariate case. Two versions of the modified large-sample confidence interval for the linearized multivariate criterion are constructed. In a simulation study, we evaluate the empirical coverage of these confidence intervals and rejection rates of the corresponding tests in finite samples. The proposed methodology is illustrated with an example of testing equivalence of the spray pattern of nasal sprays.

Assessment of the bioequivalence of a generic cyclosporine A by a randomized controlled trial in stable renal recipients
Hibberd AD, Trevillian PR, Roger SD, Wlodarczyk JH, Stein AM, Bohringer EG, Milson-Hawke SM. Transplantation 2006 Mar 15; 81(5):711–7. BACKGROUND: The aim of this study was to determine the bioequivalence of Cysporin, a generic cyclosporine A, compared with Neoral in stable renal transplant recipients. METHODS: Study design consisted of an open label, two-way crossover, randomized controlled trial of Cysporin versus Neoral in stable renal transplant recipients. In all, 33 patients were enrolled; 31 were randomized; and 28 were evaluable. AUC_{0-12} were done on days 14 and 28; C(0) and C(2) were done on days 0, 7, 21, and 35. Dose conversion was 1:1. Outcome measures for serum cyclosporin A concentrations expressed as the mean $\pm$SD were AUC_{0-12} (μg hr/L), C_{max} (μg/L), C(2) (μg/L), T_{max} (hr), and $T_{1/2}$ (hr). Mean and 90% CI of the ratio Cysporin/Neoral of log-transformed data were calculated using a general linear model. RESULTS: The main pharmacokinetic features were: AUC_{0-12}: Cysporin 3495 $\pm$ 1319, Neoral 3853 $\pm$ 1378 ($p < 0.05$); C_{max}: Cysporin 755 $\pm$ 301, Neoral 881 $\pm$ 368 ($p < 0.05$); C(2): Cysporin 613 $\pm$ 235, Neoral 672 $\pm$ 255 ($p > 0.05$); T_{max}: Cysporin 1.9 $\pm$ 0.8, Neoral 1.4 $\pm$ 0.6 ($p < 0.005$); and $T_{1/2}$: Cysporin 8.8 $\pm$ 4.3, Neoral 8.7 $\pm$ 6.2 ($p > 0.05$). Estimated ratios of Cysporin/Neoral were: AUC0.93 (90% CI 0.88–0.98; $p < 0.05$), C_{max} 0.88 (90% CI 0.80–0.97; $p < 0.05$), and T_{max} 1.32 (90% CI 1.14–1.53; $p < 0.005$). CONCLUSIONS: Both the extent and the rate of absorption of Cysporin are significantly less than those of Neoral. The 90% CI for the ratios of Cysporin/Neoral for AUC and C_{max} lie within 0.80 to 1.25. Hence, in this clinical context, Cysporin is pharmacologically bioequivalent with Neoral. This study illustrates the importance of testing bioequivalence of generic cyclosporine A products in transplant recipients not in healthy volunteers.

Bioequivalence in development of antiepileptic drugs
Sommerville KW. Epilepsy Res 2006 Jan; 68(1):82–5. Bioequivalence is an important component of the development of antiepileptic drugs. Development of new formulations after the original testing of any drug requires demonstration that the compounds are therapeutically equivalent and additional efficacy studies may not be required. Extended-release formulations may reduce toxicity with a lower maximum blood concentration

(C_{max}) and improve efficacy with a higher minimum blood concentration (C_{min}). Obtaining an equivalent area under the curve while slowing the gastrointestinal transit and avoiding food effects and dose dumping among a population with epilepsy with individual variability requires extensive engineering of the formulation. The development of extended release divalproex (Depakote ER) is used as an example of the challenges of this phase of drug development. Other routes of administration discussed are rectal preparations, nasal formulations, and intravenous infusions. These newer formulations may offer better patient care and more efficient development.

Bioequivalence of a novel high-dose oral formulation of alpha-dihydroergocryptine
de Mey C, Stamenova P, Daskalov M, Orozova M, Staikov I, Vlahov V, Wangemann M. Arzneimittelforschung 2006; 56(3):205–11. The plasma pharmacokinetics of α-dihydroergocryptine (DHEC, CAS 14271-05-7) were investigated in 24 patients with Parkinson disease after the administration of repeated oral doses of 40 mg DHEC twice daily by means of a novel 40 mg DHEC tablet (Almirid 40 mg test *T*) and an established 20 mg DHEC tablet (Almirid 20 mg reference *R*). The trial was conducted according to a randomized, controlled, open, within-subject crossover design; steady state was established by means of a stepwise up-titration from 5 to 40 mg b.i.d. from days D01 to D19; investigational treatments (40 mg DHEC b.i.d. by means of formulation *R* and *T*) were administered on days D20 and D21 according to a randomized, period-balanced within-subject crossover; treatment with DHEC was down-titrated in stepwise fashion from days D22 to D34. Morning doses of 2×20 mg DHEC (reference) yielded a fast and relatively short-lasting peak with a geometric mean C_{max} of 2157 pg/mL (CV: 0.978) after a median t_{max} of 1.00 hours. C_{min} throughout the first 12 hours was on average 189 pg/mL (CV: 0.908). There was a quite distinct diurnal effect: evening doses of 2×20 mg DHEC (treatment *R*), yielded a relatively lower exposure with geometric mean C_{max}, C_{av}, and C_{min}-values of 800 pg/mL (CV: 0.870), 389 pg/mL (0.813), and 177 pg/mL (CV: 0.942), respectively. In contrast, there was relatively little within-subject distinction between the two formulations: for the day profile after the morning dose, the estimated ratios of the true means (Pr:*R*) for C_{max}, C_{min}, and C_{av} were 1.18 (90% CI: 0.96–1.43, CV_m: 0.394), 0.96 (90% CI: 0.86–1.09, CV_m: 0.230), and 1.06 (90% CI: 0.93–1.21, CV_m: 0.254), respectively; for the night profile after the evening dose, the estimated ratio of the true means (μ_T:μ_R) for C_{max}, C_{min}, and C_{av} were 1.11 (90% CI: 0.91–1.35, CV_m: 0.395), 1.07 (90% CI: 0.95–1.20, CV_m: 0.232), and 1.07 (90% CI: 0.95–1.20, CV_m: 0.220). In view of important medical–ethical constraints not to expose an unreasonably high number of subjects, these findings could be accepted as a sufficient demonstration of bioequivalence.

Bioequivalence testing of a new tablet formulation of generic fluoxetine
Jovanovic D, Kilibarda V, Dordevic S, Jovanovic M, Jovic-Stosic J, Srdic D, Knezevic T. Eur J Drug Metab Pharmacokinet 2006 Jan–Mar; 31(1):35–40. The pharmacokinetics and relative bioavailability of fluoxetine capsules (reference) and tablets (test) were compared in 24 healthy subjects of both sexes after a single 20 mg oral dose of fluoxetine (as a hydrochloride salt). A randomized, crossover design with a two-week washout period between each dose was applied. Serum samples, obtained before dosing and at various appropriate time points up to 192 hours, were analyzed for fluoxetine and norfluoxetine content by a simple, accurate, and precise HPLC method. ANOVA, power analysis, 90% confidence intervals (CI), and two one-sided tests were used for the statistical analysis of pharmacokinetic parameters. The tolerability of the preparations was good. The respective point estimates of the ratios of the geometric means of log C_{max} and log $AUC_{0-\infty}$ of fluoxetine were 0.912 and 0.935 with 90% of 0.838 to 0.992 and 0.857 to 1.020. The corresponding point estimates of norfluoxetine were 0.952 (90% CI=0.843–1.075) and 0.904 (90% CI=0.807–1.013), respectively. Since both 90% CI for the $AUC_{0-\infty}$ and C_{max} geometric mean ratios of fluoxetine and norfluoxetine were included in the 80% to 125% interval proposed by the Food and Drug Administration, the test drug (fluoxetine tablets) was considered bioequivalent to the reference one (Prozac capsules) according to both the rate and the extent of absorption.

Biowaiver monographs for immediate release solid oral dosage forms: amitriptyline hydrochloride

Manzo RH, Olivera ME, Amidon GL, Shah VP, Dressman JB, Barends DM. J Pharm Sci 2006 May; 95(5):966–73. Literature data relevant to the decision to allow a waiver of in vivo bioequivalence (BE) testing for the approval of immediate release (IR) solid oral dosage forms containing amitriptyline hydrochloride are reviewed. Its therapeutic uses, pharmacokinetic properties, the possibility of excipient interactions, and reported BE/bioavailability problems are also taken into consideration. Literature data indicate that amitriptyline hydrochloride is a highly permeable active pharmaceutical ingredient. Data on the solubility according to the current Biopharmaceutics Classification System (BCS) were not fully available and consequently amitriptyline hydrochloride could not be definitively assigned to either BCS Class I or BCS Class II. But all evidence taken together, a biowaiver can currently be recommended provided that IR tablets are formulated with excipients used in existing approved products and that the dissolution meets the criteria defined in the Guidances.

Biowaiver monographs for immediate release solid oral dosage forms: cimetidine

Jantratid E, Prakongpan S, Dressman JB, Amidon GL, Junginger HE, Midha KK, Barends DM. J Pharm Sci 2006 May; 95(5):974–84. Literature data relevant to the decision to allow a waiver of in vivo bioequivalence (BE) testing for the approval of immediate release (IR) solid oral dosage forms containing cimetidine are reviewed. According to the current Biopharmaceutics Classification System (BCS), cimetidine would be assigned to Class III. Cimetidine's therapeutic use and therapeutic index, its pharmacokinetic properties, data related to the possibility of excipient interactions, and reported BE/bioavailability problems were also taken into consideration. On the basis of the overall evidence, a biowaiver can be recommended for cimetidine IR products, provided that the test product contains only those excipients reported in this paper in their usual amounts, and that the test and the comparator drug products both are "rapidly dissolving" as per BCS.

Effects of deaeration methods on dissolution testing in aqueous media: a study using a total dissolved gas pressure meter

Gao Z, Moore TW, Doub WH, Westenberger BJ, Buhse LF. J Pharm Sci 2006 Jul; 95(7):1606–13. Dissolution testing is a critical method for the determination of pharmaceutical product quality and bioequivalence. For some products, dissolved gases in the dissolution medium affect dissolution results thus requiring degassing of the medium prior to use. In this study, we use a total dissolved gas and oxygen meter to measure both oxygen and total gases in dissolution media before and after application of a variety of deaeration methods. Dissolution testing results using a 10 mg Prednisone tablet (NCDA #2) are compared with the percent saturation of oxygen and total gases found in the medium. Reaeration of the medium during different stirring rates was also measured. This study confirms that measurement of total gases and not just oxygen in the medium is necessary to assess adequacy for dissolution testing. For those deaeration techniques that are performed at room temperature, the percent saturation of the total dissolved gases must be well below 100% to prevent outgassing once medium is brought to dissolution test method temperature, typically 37°C. J Pharm Sci 2006; 95:1606–13.

In vitro disintegration and dissolution and in vivo bioequivalence of two alendronate once-weekly formulations

Almeida S, Almeida A, Filipe A, Penedo C, Rocha A, Lainesse A, Vallee F. Arzneimittel-forschung 2006; 56(2):84–9. Bioequivalence of two tablet formulations of 70 mg alendronate (CAS 121268-17-5) was assessed in a single-dose, open-label, randomized, fasted state crossover trial, with a washout period of 21 days, in 80 healthy subjects. Urine samples were collected up to +36 hours post-dosing and the concentrations of alendronic acid were assessed using a high-performance liquid chromatographic method with pre-derivatization and fluorescence detection (HPLC/FL) method. The 90% confidence intervals (90% CI) obtained for $A_e(0–36)$ (cumulative urinary excretion) and R_{max} (maximum rate of urinary excretion) were

98.67% to 118.99% and 102.22% to 122.46%, respectively. The intra-subject coefficient of variation was between 32% and 35% for both parameters. No relevant tolerability problems were detected. Both formulations can be considered bioequivalent. In vitro testing was performed to confirm the adequacy of the quality control conditions and no significant differences were detected neither in the disintegration test nor in the dissolution tests conducted in HCl 0.1 N and H_2O and thus in these conditions, the lack of statistically significant differences in vitro was accompanied by in vivo bioequivalence.

2005

A comparison of four different methods for outlier detection in bioequivalence studies
Ramsay T, Elkum N. J Biopharm Stat 2005; 15(1):43–52. Bioequivalence studies, required by law whenever a new formulation of an existing drug product is introduced to the market, are designed to test whether the bioavailability, defined as the rate and extent to which a substance reaches systemic circulation, is equivalent for each of two or more formulations. Detection and treatment of outlying data in bioequivalence studies are practically important, because inclusion or deletion of potential outlying data may lead to a different conclusion concerning bioequivalence. A review of the literature reveals that four different methods have been proposed for detecting outliers in bioavailability/bioequivalence studies. We present the results of an extensive computer simulation testing the small sample performance of these four testing methods, the results of which indicate that one of these, the estimates distance test, is substantially more powerful than the alternatives.

Assessment of equivalence using a concordance correlation coefficient in a repeated measurements design
Quiroz J. J Biopharm Stat 2005; 15(6):913–28. Some assay validation studies are conducted to assess agreement between repeated, paired continuous data measured on the same subject with different measurement systems. The goal of these studies is to show that there is an acceptable level of agreement between the measurement systems. Equivalence testing is a reasonable approach in assay validation. In this article, we use an equivalence-testing criterion based on a decomposition of a concordance correlation coefficient proposed by Lin. Using a variance components approach, we develop bounds for conducting statistical tests using the proposed equivalence criterion. We conduct a simulation study to assess the performance of the bounds. The criteria are the ability to maintain the stated test size and the simulated power of the tests using these bounds. Bounds that perform well for small sample size are preferred. We present a computational example to demonstrate the methods described in the article.

Assessment of the bioequivalence of two nelfinavir tablet formulations under fed and fasted conditions in healthy subjects
Kaeser B, Charoin JE, Gerber M, Oxley P, Birnboeck H, Saiedabadi N, Banken L. Int J Clin Pharmacol Ther 2005 Mar; 43(3):154–62. OBJECTIVES: This study was designed to assess the bioequivalence between the commercial 250 mg nelfinavir tablet and the new 625 mg nelfinavir tablet (Roche) which was developed to reduce the daily pill burden for patients from 10 to 4 tablets in a nelfinavir 1250 mg twice daily regimen. METHODS: A total of 52 healthy male subjects were enrolled in this randomized four-period crossover study to receive single oral doses of 1250 mg nelfinavir administered as five commercial 250 mg tablets (reference formulation) and as two new 625 mg tablets (test formulation). Each of the two formulations was taken after an overnight fast and immediately after intake of a standard breakfast (820 kcal) on separate occasions. Blood samples were collected pre-dose and at appropriate intervals after drug administration. Plasma concentrations of nelfinavir and its main metabolite M8 were assayed by a validated LC–MS/MS assay and the pharmacokinetics of nelfinavir and M8 were derived using standard non-compartmental analysis. RESULTS: The primary parameters for bioequivalence testing were the logarithmically transformed $AUC_{0-\infty}$ and C_{max} of nelfinavir taken from 50 subjects who completed all four treatments. Bioequivalence was accepted if the 90% confidence interval (CI) was contained entirely in the equivalence region (80%, 125%). In the fed state, this criterion was met for AUC (effect ratio = 95%; CI = 87%, 103%) and C_{max} (effect

ratio = 101%; CI = 94%, 109%) and bioequivalence of the two treatments could be concluded. In the fasted state, AUC clearly failed to meet the bioequivalence criteria (effect ratio = 73%; CI = 59%, 90%) and C_{max} was borderline outside the lower acceptance region (effect ratio = 97%; CI = 79.6%, 118%). Therefore, bioequivalence could not be concluded under fasted condition. Food increased the systemic exposure to nelfinavir (as reflected by comparison of the logarithmically transformed $AUC_{0-\infty}$ values under fed and fasted conditions) by six- and eight-fold after dosing with the 250 and the 625 mg tablet, respectively. CONCLUSIONS: Bioequivalence of the new 625 mg nelfinavir tablet relative to the commercial 250 mg tablet, at a dose of 1250 mg, was confirmed in the fed state but not under fasted conditions. As nelfinavir is recommended to be taken with food, the new tablet is well suited to decrease the daily pill burden for patients on a nelfinavir twice daily regimen and to enhance patient's compliance and adherence.

Bioequivalence assessment of two enteric-coated aspirin brands, Nu-seals and Loprin, after a single oral dose of 150 mg in healthy male adults

Bukhari NI, Zafar A, Shamsi WR, Bashir MA, Mirza AA. Therapie 2005 Mar–Apr; 60(2):167–73. AIM: The bioequivalence of aspirin from two enteric-coated brands, Nu-seals and Loprin, identified as the reference (*R*) and test (*T*) products, respectively, was assessed. METHODS: A two-period randomized crossover design with a washout interval of 15 days was used in this study. The study results were determined in 16 healthy volunteers, all males with ages ranging from 19 to 28 (23.33 ± 3.74) years and body weight of 52 to 92 (65.89 ± 11.39) kg. After oral ingestion of 150 mg of the either brand with 200 mL of water, serial blood samples were obtained over a period of 24 hours. Plasma, harvested from blood was analyzed for the concentration of salicylic acid, a deacetylated metabolite of aspirin, by a validated high performance liquid chromatography method. Pharmacokinetic parameters were determined for both formulations by an interactive computer-assisted PK II procedure. A general linear model for repeated measures and 90% confidence intervals (CI) was employed to assess the sequence of treatment effects and to exclude differences between the parameters due to the product and period of administration, respectively. RESULTS: The observed 90% CI ratios (Loprin/Nu-seals) for peak concentration, time to reach the peak and area under the plasma–concentration–time curve from 0 to ∞ of 1.03, 1.08; 1.04, 1.05; and 1.01, 1.15, respectively, were within the bioequivalence range (0.80, 1.25) stipulated by the U.S. Food and Drug Administration. CONCLUSION: On the basis of the findings, the test (Loprin) and reference drug (Nu-seals) were deemed bioequivalent.

Bioequivalence in vitro evaluation of some antibacterial generic dosage forms

Semde R, Ouedraogo HW, Guissou IP, Amighi K. J Pharm Belg 2005; 60(2):51–5. In this work, bioequivalence between generic and corresponding original brand-name dosage forms of some antibacterial drugs, frequently prescribed in developing countries, have been examined using in vitro dissolution testing. For this purpose, tablet or hard capsule formulations of five active substances (amoxycillin, ampicillin, co-trimoxazole (sulfamethoxazole/trimethoprim), metronidazole, and penicillin V) have been retained. For each active substance, batch samples of three generic and one test formulations have been submitted to the pharmaceutical quality control and dissolution testing. Results obtained have shown that all samples examined met the specifications of quality edited by the pharmacopeias. On the other hand, interchangeability between generic and corresponding test formulations should be possible since their dissolution profiles are superposables enough.

The bioequivalence of highly variable drugs and drug products

Midha KK, Rawson MJ, Hubbard JW. Int J Clin Pharmacol Ther 2005 Oct; 43(10):485–98. "Highly variable drugs" have been defined as those drugs for which the within-subject variability (WSV) equals or exceeds 30% of the maximum concentration (C_{max}) and/or the area under the concentration versus time curve (AUC). Despite the fact that highly variable drugs are generally safe with flat dose–response curves, the bioequivalence of their formulations is a problem because the high variability means that a large number of subjects are required to give adequate statistical power. Highly variable drug products are poor quality formulations where high within-formulation variability (e.g., tablet to tablet variability) poses a

problem rather than high innate WSV of the drug itself. A further problem caused by high variability is that a subset of the population may respond differently to the two formulations producing a significant subject×formulation interaction. Practical examples are shown using replicate designs. The methods proposed to deal with the problems posed by highly variable drugs include: (*i*) drug regulatory jurisdiction states that the 90% confidence interval (90% CI) around the test to reference geometric mean ratio (GMR) is required to fit with bioequivalence acceptance limits of 0.8to 1.25 for both C_{max} and AUC. The WSV for single point estimation of C_{max} is often greater than that for AUC. Therefore, one strategy is not to require a 90% CI for C_{max} of drugs that do not exhibit a toxicity associated with C_{max} and merely require the GMR to fall within the acceptance limits. (*ii*) To arbitrarily broaden the bioequivalence acceptance limits. For example, to permit a sponsor to justify the use of wider limits, e.g., the 90% CI around the GMR of C_{max} values might be required to fit within acceptance limits of 0.75to 1.33 or even 0.70 to 1.42. (*iii*) A more systematic approach would be to broaden the acceptance limits by scaling to either the residual variance from a two-period design or to the WSV of the reference product in a replicate design. Subsequent evaluations of scaling procedures have demonstrated that smaller numbers of subjects are required for bioequivalence studies on formulations of highly variable drugs. A disadvantage of scaling is that the method is less sensitive to differences between the means compared with unscaled treatment, such that the GMR may prove to be unacceptably low or high. This possibility has let to a suggestion that the GMR must fall within the acceptance limits of 0.8 to 1.25 in scaled treatments. (*iv*) A similar method is to scale the metric rather than the acceptance limits. This method was proposed by the U.S. Food and Drug Administration in the context of Individual bioequivalence, but may also be applied (5) to average bioequivalence. (6) To carry out bioequivalence studies at steady state whenever a multiple dose regimen is ethically acceptable for healthy volunteers. This solution is based on the observation that high variability in a single-dose study tends to be dampened at steady state, thus increasing statistical power. Drug regulators have not favored this approach on the grounds that bioequivalence testing should be based on the most discriminating test possible. (7) Finally, the use of metabolite data has been proposed since in many (but by no means all) cases, metabolite is less highly variable than that of the parent drug. This subject remains controversial except when the administered substance is a prodrug which converted by metabolism into the active drug.

Bioequivalence of two recombinant granulocyte colony-stimulating factor formulations in healthy male volunteers

Hernandez-Bernal F, Garcia-Garcia I, Gonzalez-Delgado CA, Valenzuela-Silva C, Soto-Hernandez R, Duconge J, Cervantes-Llano M, Blanco-Garces E, Rodriguez V, Garcia-Vega Y, Bello-Rivero I, Olivera-Ruano L, Lopez-Saura P. Biopharm Drug Dispos 2005 May; 26(4):151–9.

To evaluate the equivalence of the pharmacokinetic, pharmacodynamic, and safety properties of two recombinant G-CSF formulations in healthy male volunteers, a standard two-way randomized crossover double-blind study, with a three week washout period, was conducted. A single 300 μg G-CSF dose was administered subcutaneously. Hebervital (Heber Biotec, Havana, formulation A) and Neupogen (Hoffmann-La Roche S.A, formulation B) were compared. Twenty-four healthy male volunteers were included. The serum G-CSF level was measured by enzyme immunoassay during the first 36 hours after administration. Absolute neutrophils (ANC), white blood cells (WBC), and CD34+ cells counts were the pharmacodynamic variables measured up to 120 hours. Other clinical and laboratory determinations were used as safety criteria. The pharmacokinetic parameters for formulation A and B were very close to each other (i.e., AUC: 235.9 vs. 270.0 ng h/mL, C_{max}: 29.2 vs. 33.4 ng/mL, T_{max}: 4.2 vs. 4.7 hours, half-life: 3.2 vs. 2.8 hours, CL: 260.9 vs. 277.2 mL/hr, $V(d)$: 1.2 vs. 1.1 L, and MRT: 7.58 vs. 7.38 hours). The confidence intervals for the means ratio of all these parameters were within or very close to the 0.8 to 1.25 acceptance range. The pharmacodynamics showed high similarity since ANC and WBC had the same profiles for both products and no differences were detected for the estimated parameters. The CD34+ cells count increments were evident for both formulations in a similar way as well. The treatments were well tolerated. Registered adverse events were similar; back/spine pain was the most frequent. According to the overall

results, these formulations could be considered as clinically comparable.

Bioequivalence study of fluoxetine hydrochloride in healthy volunteers
Keller T, Cambon N, Genevray M, Crivelli F, Crivelli M, Dal BL, Mazzucchelli P, Ismaili S, Marzo A. Arzneimittelforschung 2005; 55(9):491–7. Fluoxetine hydrochloride (CAS 59333-67-4) is a selective serotonin reuptake inhibitor widely used as antidepressant drug. The aim of the present trial was to assess the bioequivalence of a new formulation of the drug (test formulation) when compared with a reference product from the Swiss market. Both drugs were available as 20 mg dispersible tablets. The trial was performed according to a two-period, two-sequence, balanced, randomized, single-dose design with a washout phase of at least 56 days. The two formulations were tested in 30 male healthy volunteers. A specific highly sensitive bioassay in tandem mass spectrometry allowed to set the limit of quantification to 100 pg/mL for fluoxetine and norfluoxetine. Average t_{max} was 5.4 hours for fluoxetine and 71 to 80 hours for norfluoxetine. The peak concentration was on average 14 ng/mL for fluoxetine and 10.5 ng/mL for norfluoxetine. Half-life was on average 48 to 50 hours for fluoxetine and 130 to 138 hours for norfluoxetine. AUC_∞ for fluoxetine and norfluoxetine were on average 790 and 2800 ng/mL h, respectively. All these figures demonstrate that plasma concentration–time profiles of fluoxetine and norfluoxetine are quite different. Applied statistical tests, suggested by operating guidelines, demonstrated bioequivalence of the test formulation and the reference formulation. The conclusion on bioequivalence was based on both fluoxetine and norfluoxetine results. The 90% confidence intervals for C_{max}, AUC_t, and AUC_∞ (fluoxetine and norfluoxetine) were within the acceptance range (0.80–1.25) and t_{max}, processed with a non-parametric test, did not show any statistically significant difference between test and reference formulation. Safety and tolerability proved to be similarly good with both test and reference formulation. In conclusion, the present trial has demonstrated bioequivalence of the test and the reference formulation, both consisting of fluoxetine hydrochloride dispersible tablets.

Bioequivalence study of two different coated tablet formulations of finasteride in healthy volunteers
Almeida A, Almeida S, Filipe A, Gagnon S, Mirapeix A, Girard B, Tanguay M. Arzneimittelforschung 2005; 55(4):218–22. This study was conducted in order to assess the bioequivalence of two different coated tablet formulations containing 5 mg finasteride (CAS 98319-26-7). Twenty-six healthy volunteers were enrolled in an open, randomized, crossover single-dose study with 2 periods×2 sequences and a minimum washout period of seven days. Plasma samples were obtained over 24 hours (at baseline, +0.5,+1,+1.5,+2,+2.5,+3, 3.5,+4,+4.5,+5,+6,+8,+10,+12,+16, and +24 hours after administration). Finasteride levels were determined by high-pressure liquid chromatography with tandem mass detection, HPLC–MS/MS (limit of quantification 0.50 ng/mL). Pharmacokinetic parameters used for bioequivalence assessment (AUC_{last} and C_{max} were main evaluation criteria, however, AUC_∞ was also analyzed) were determined from the finasteride concentration data using non-compartmental analysis. The 90% confidence intervals (obtained by ANOVA) were 86.31 to 98.69 for C_{max}, 95.40 to 104.88 for AUC_{last}, and 96.20 to 105.81 for AUC_∞, i.e., they were all within the predefined ranges. Therefore, it may be concluded that the evaluated formulations are bioequivalent in terms of rate and extent of absorption.

Bioequivalence study of two different tablet formulations of carvedilol in healthy volunteers
Portoles A, Filipe A, Almeida S, Terleira A, Vallee F, Vargas E. Arzneimittelforschung 2005; 55(4):212–7. OBJECTIVE: The aim of this study was to compare the extent and rate of absorption of two different carvedilol (CAS 72956-09-3) tablet formulations: 25 mg tablets, as the test formulation and the reference innovator product (25 mg tablets). METHODS: This study was designed as a single-dose, open-label, randomized, with a two-period and two-sequence crossover design, with blind determination of drug plasma concentration and a minimum of seven-day washout period. Twenty-four healthy volunteers of both sexes were randomly assigned to treatment sequences. Carvedilol concentrations were determined in plasma samples obtained over a 24-hour interval: baseline (pre-administration) and at 14 different

times within the 24 hours after administration. The analytical method, which used HPLC coupled with a MS/MS detector, was duly validated and the analytical assay was performed in compliance with Good Laboratory Practice (GLP). The limit of quantification was 0.50 ng/mL. Pharmacokinetic parameters representing the extent and/or rate of absorption (AUC_∞, AUC_{last}, and C_{max}) were obtained. As secondary objective, the tolerability of both formulations was also evaluated. RESULTS: The geometric mean of the test/reference formulations individual percent ratio was 98.14% for AUC_∞, 98.44% for AUC_{last}, and 98.39% for C_{max}. The 90% CI for the geometric mean of the individual ratio test/references formulations was 95.13% to 101.24% for AUC_∞, 95.23% to 101.76% for AUC_{last}, and 88.26% to 109.67% for C_{max}. CONCLUSIONS: The 90% CI values obtained for AUC_∞, AUC_{last}, and C_{max} are within the interval proposed by the EMEA/CPMP and the FDA as bioequivalence acceptance criteria, and consequently it can be concluded that the test formulation is bioequivalent with the reference formulation both in terms of rate and extent of absorption after single-dose administration. The results from a previous pilot study allowed an optimal design for this trial.

Bioequivalence trials with the incomplete 3×3 crossover design
Lim NK, Park SG, Stanek E. In. Biomed J 2005 Oct; 47(5):635–43. In bioequivalence trials, one often considers two or more generic products with the original one. The 3×3 crossover design can be adopted to evaluate the two generic candidates with a brand name drug, rather than conducting two separate 2×2 crossover trials. Dropouts, however, are more likely to occur due to various administrative reasons when we consider a higher order crossover design. A modified method, which was originally given by Chow and Shao (1997), is extended to compare two generic products with a reference in the incomplete 3×3 crossover design. A simulation study and discussion are also presented.

Biowaiver monographs for immediate release solid oral dosage forms: ranitidine hydrochloride
Kortejarvi H, Yliperttula M, Dressman JB, Junginger HE, Midha KK, Shah VP, Barends DM. J Pharm Sci 2005 Aug; 94(8):1617–25. Literature and experimental data relevant to the decision to allow a waiver of in vivo bioequivalence testing for the approval of immediate release (IR) solid oral dosage forms containing ranitidine hydrochloride are reviewed. According to the current Biopharmaceutics Classification System, ranitidine hydrochloride should be assigned to Class III. However, based on its therapeutic and therapeutic index, pharmacokinetic properties, and data related to the possibility of excipient interactions, a biowaiver can be recommended for IR solid oral dosage forms that are rapidly dissolving and contain only those excipients as reported in this study.

Comparative bioavailability/bioequivalence of two different sertraline formulations: a randomized, 2-period×2-sequence, crossover clinical trial in healthy volunteers
Almeida S, Portoles A, Terleira A, Filipe A, Cea E, Caturla MC. Arzneimittelforschung 2005; 55(4):191–7. An open-label, randomized, crossover single-dose study, using 2 periods×2 sequences, with a minimum washout period of four weeks, was conducted in order to assess the comparative bioavailability of two formulations of sertraline hydrochloride (CAS 79617-96-2) 100 mg tablets. Plasma samples were obtained at intake (baseline) and at +1,+2,+3,+4,+5,+6,+7,+8,+9,+12,+24,+48,+72, and +96 hours post-administration. Sertraline plasma concentrations were determined by high pressure liquid chromatography with tandem mass detection (HPLC–MS/MS) and the lower limit of quantification was set at 100.15 pg/mL. Pharmacokinetic parameters used for bioequivalence assessment (AUC_{last}, AUC_∞, and C_{max}) were determined by non-compartmental analysis. Classical 90% confidence intervals (90% CI) were calculated for the overall sample, and for males and females separately, and gender effects were investigated using an appropriate model. The results showed that overall classical 90% CI were 84.55% to 100.32% for C_{max} 86.96% to 98.68% for AUC_{last}, and 86.79 to 98.93 for AUC_∞, i.e., they were all within the predefined ranges for bioequivalence acceptance. Separate gender analysis showed very similar results for males and females when analyzed independently, and no gender effects were detected in bioequivalence analysis ($p > 0.05$). Therefore, it may be concluded that the evaluated formulations are bioequivalent in terms of rate and extent of absorption.

Comparative bioavailability of two formulations of levofloxacin and effect of sex on bioequivalence analysis. Data from a randomized, 2×2 crossover trial in healthy volunteers

Almeida S, Filipe A, Almeida A, Wong H, Caparros N, Tanguay M. Arzneimittelforschung 2005; 55(7):414–9. Bioequivalence of levofloxacin (CAS 100986-85-4) 500 mg tablets was assessed in a single-dose, open, randomized, crossover trial, with a minimum washout period of seven days. Serum samples were obtained over 36 hours (at baseline, 0.250, 0.500, 0.750, 1.00, 1.25, 1.50, 1.75, 2.00, 2.33, 2.67, 3.00, 4.00, 6.00, 8.00, 12.0, 16.0, 24.0, and 36.0 hours post-dose). Levofloxacin serum concentration levels were determined by high-pressure liquid chromatography with fluorescence detection (limit of quantification 98.31 ng/mL). The 90% confidence intervals (90% Cls; obtained by ANOVA using ln-transformed data) for overall bioequivalence analysis were 99.09% to 115.26% for C_{max}, 99.41% to 105.60% for AUC_{last}, and 98.68% to 104.93% for AUC_{∞}, i.e., all within the predefined ranges. Within-gender analysis also produced 90% CIs within the predefined ranges. The use of gender-related model effects showed that sex was a significant factor for AUC_{last} and AUC_{∞}, however, when parameters were normalized by body weight adjusted dose, none of the tested model effects were significant. Comparison between male and female body weight showed significant differences. Therefore, it may be concluded that the evaluated formulations are bioequivalent in terms of rate and extent of absorption and that possible differences between male and female pharmacokinetic parameters may be related to differences in body weight.

Comparing the concentration curves directly in a pharmacokinetics, bioavailability/bioequivalence study

Liao JJ. Stat Med 2005 Mar 30; 24(6):883–91. In a traditional pharmacokinetics (PK), bioavailability/bioequivalence study, the same number of time points and sampling times are used for each subject. Often, an indirect inference is then made on some PK parameters, such as area under the plasma concentration curve, maximum plasma concentration (C_{max}), time to maximum plasma concentration (T_{max}), or half-life. However, since these PK parameters are summarized from repeated measurements, a lot of information can be lost. The indirect inferences on some PK parameters are not always accurate. Taking the repeated measurements of the concentration curve into consideration, a functional linear model has been developed to compare concentration curves directly instead of the PK parameters. Considering the nature of repeated measurements, a multiple testing procedure is proposed to assess the equality of two concentration curves. A real dataset is used to demonstrate the proposed procedure.

Comparison of two recombinant erythropoietin formulations in patients with anemia due to end-stage renal disease on hemodialysis: a parallel, randomized, double-blind study

Perez-Oliva JF, Casanova-Gonzalez M, Garcia-Garcia I, Porrero-Martin PJ, Valenzuela-Silva CM, Hernandez-Montero T, Lagarde-Ampudia M, Casanova-Kutsareva Y, Avila-Albuerne Y, Vargas-Batista A, Bobillo-Lopez H, Herrera-Valdes R, Lopez-Saura PA, Bioequivalence Study of Erythropoietin Group. BMC Nephrol 2005 May 23; 6(1):5. BACKGROUND: Recombinant human erythropoietin (EPO) is used for the treatment of last stage renal anemia. A new EPO preparation was obtained in Cuba in order to make this treatment fully nationally available. The aim of this study was to compare the pharmacokinetic, pharmacodynamic, and safety properties of two recombinant EPO formulations in patients with anemia due to end-stage renal disease on hemodialysis. METHODS: A parallel, randomized, double-blind study was performed. A single 100 IU/kg EPO dose was administered subcutaneously. Heberitro (Heber Biotec, Havana, formulation A), a newly developed product and Eprex (CILAG AG, Switzerland, formulation B), as reference treatment were compared. Thirty-four patients with anemia due to end-stage renal disease on hemodialysis were included. Patients had not received EPO previously. Serum EPO level was measured by enzyme immunoassay during 120 hours after administration. Clinical and laboratory variables were determined as pharmacodynamic and safety criteria until 216 hours. RESULTS: Both groups of patients were similar regarding all demographic and baseline characteristics. EPO kinetics profiles were similar for both formulations; the pharmacokinetic parameters were very close (i.e., AUC: 4667 vs. 4918 mIU h/mL;

C_{max}: 119.1 vs. 119.7 mIU/mL; T_{max}: 13.9 vs. 18.1 hours; half-life: 20.0 vs. 22.5 hours for formulations A and B, respectively). The 90% confidence intervals for the ratio between both products regarding these metrics were close to the 0.8 to 1.25 range, considered necessary for bioequivalence. Differences did not reach 20% in any case and were not determined by a formulation effect, but probably by a patients' variability effect. Concerning pharmacodynamic features, a high similitude in reticulocyte counts increments until 216 hours and the percentage decrease in serum iron until 120 hours was observed. There were no differences between formulations regarding the adverse events and their intensity. The more frequent events were pain at injection site (35.3%) and hypertension (29%). Additionally, further treatment of the patients with the study product yielded satisfactory increases in hemoglobin and hematocrit values. CONCLUSION: The formulations are comparable. The newly developed product should be acceptable for long-term application.

Determination of rifampicin bioequivalence in a three-drug FDC by WHO and Indian protocols: effect of sampling schedule and size

Agrawal S, Kaur KJ, Singh I, Bhade SR, Kaul CL, Panchagnula R. Int J Tuberc Lung Dis 2005 Jan; 9(1):75–80. SETTING: To promote the quality assurance of fixed-dose combination (FDC) formulations, the World Health Organization (WHO) has prepared a convenient simplified protocol for the determination of rifampicin (RMP) bioequivalence. During the development of this protocol, it was proved that sampling time up to eight hours can determine the rate and extent of RMP absorption. However, this protocol utilizes 20 volunteers in contrast to other local regulatory requirements of a minimum of 12 volunteers. The different sample sizes utilized in these protocols may affect the sensitivity of the bioequivalence outcome. OBJECTIVE: To determine the effect of sampling size and schedule on RMP bioequivalence when two different protocols are used. DESIGN: A bioequivalence trial was conducted with a study design of 20 volunteers and 24 hours sampling time, which fulfils the requirements of both the WHO and Indian regulatory protocols. Pharmacokinetic and statistical analysis was done by stepwise reduction in sample size and schedule. RESULT: Bioequivalence limits of RMP were unaffected by a reduced sample size of 12 volunteers and eight hours sampling time. CONCLUSION: Minimizing sample size after validation for borderline and poor quality FDC formulations can further reduce the cost of conducting bioequivalence trials.

Evaluation of the bioequivalence and pharmacokinetics of two formulations of rizatriptan after single oral administration in healthy volunteers

Chen J, Jiang WM, Xie YL, Jin L, Mei N, Liang XG. Arzneimittelforschung 2005; 55(7):355–8. The pharmacokinetic parameters of two oral formulations of rizatriptan (CAS 144034-80-0, a capsule preparation as test and rizatriptan tablet as reference), given at a single dose of 10 mg each, were compared in an open-label, randomized, single oral dose, two-period crossover design in 20 healthy volunteers under fasting conditions. Plasma concentrations of rizatriptan were measured by a validated HPLC assay. The parametric 90% confidence intervals of the geometric mean values of the test/reference ratios were 91.9% to 101.9% (point estimate: 97.3%) for $AUC_{0-\infty}$, 93.0% to 102.2% (point estimate: 96.5%) for AUC_{0-t}, 90.1% to 100.0% (point estimate: 95.4%) for C_{max}, being within the acceptance criteria for bioequivalence (80–125%). T_{max} values were analyzed by the nonparametric Wilcoxon test and the difference was not statistically significant. Therefore, it is concluded that the test and reference rizatriptan formulations are bioequivalent with regard to both the extent and the rate of absorption.

Geometric mean ratio-dependent scaled bioequivalence limits with leveling-off properties

Karalis V, Macheras P, Symillides M. Eur J Pharm Sci 2005 Sep; 26(1):54–61. In this study, novel approaches for the design of bioequivalence (BE) limits are developed. The new BE limits scale with intrasubject variability but only until a geometric mean ratio (GMR)-dependent plateau value and combine the classic (0.80–1.25) and expanded (0.70–1.43) BE limits into a single criterion. Plots of the extreme GMR values accepted as a function of coefficient of variation (CV) have a convex shape, similar to the classic unscaled 0.80 to 1.25 limits. The performance of the novel approaches in comparison to the classic unscaled 0.80 to 1.25 limits as well as the two

expanded BE limits, i.e., 0.70 to 1.43 and 0.75 to 1.33 was assessed using simulated data. Two-period crossover BE investigations with 12, 24, or 36 subjects were simulated with assumptions of CV 10, 20, 30, or 40%. At low CV values, the performance of the novel BE limits is almost identical to the 0.80 to 1.25 criterion. On the contrary, the expanded BE limits are very permissive even at high GMR values. For high CV% values (30 and 40%), the new BE limits show a much greater probability of declaring BE when GMR = 1 in comparison to the classic 0.80 to 1.25 limits. In addition, when the drug products differ more than 25%, the new BE limits show much lower percentage of acceptance than the expanded 0.70 to 1.43 limits. One of the major advantages of the new BE limits is their gradual expansion with variability until a GMR-dependent plateau value. Finally, the continuity and leveling-off properties of the new BE limits make them suitable for the assessment of BE studies, irrespective of the level of variability encountered.

In vitro and in vivo equivalence studies of alendronate monosodium tablets
Roldan EJ, Quattrocchi O, Zanetti D, Piccinni E, Tessler J, Caballero LE, Lloret AP. Arzneimittelforschung 2005; 55(2):93–101. OBJECTIVE: The aim of this study was to test the bioequivalence of two alendronate tablets (CAS 121268-17-5; Marvil 10 and Marvil 70 as test formulations, in short "test"; reference formulation, in short "reference") in vitro and in vivo in healthy adult male subjects and to describe a mode for researching the bisphosphonate oral formulation pharmaceutical quality. METHODS: Two dissolution tests with 10- and 70-mg alendronate tablets, a preliminary clinical test with 10-mg tablets ($n = 10$) and a bioequivalence study with 70-mg tablets ($n = 23$) were performed. Clinical studies were single-dose, open, crossover, randomized, including a four-week washout period. Alendronate was assessed by HPLC in urine after 6 (UE6) and 24 (UE24) hours post-intake. In all the experiments, the reference was the one that had proved efficacy and safety in international regulatory clinical trials. RESULTS: The dissolution test showed a comparable release profile between reference and test, of both, the 10 and 70 mg tablet, the difference (f_1) and similarity (f_2) factors being within the acceptance values. The clinical trials showed great variability in urinary recovery, from one-third the average figure up to two to three-fold. The amount recovered with the 70 mg tablet was 11- to 15-fold higher than with the 10 mg tablets, suggesting higher (test/reference) was found to be 72% to 122% for UE24, and when analyzed in individuals with apparent steady bone metabolism during the washout period ($n = 19$), it was 86% to 137%. Both margins are considered acceptable in view of the particular kinetic and dynamic features of bisphosphonates, their very high inter- and intra-individual variability, extremely low absorption, time-changeable bone compartment, high margin of safety, and long-term achievable therapeutic benefits. CONCLUSION: Test is bioequivalent to reference.

LC–MS determination and bioavailability study of imidapril hydrochloride after the oral administration of imidapril tablets in human volunteers
Yun JH, Myung JH, Kim HJ, Lee S, Park JS, Kim W, Lee EH, Moon CJ, Hwang SJ. Arch Pharm Res 2005 Apr; 28(4):463–8. The purpose of the present study was to develop a standard protocol for imidapril hydrochloride bioequivalence testing. For this reason, a specific LC–MS method was developed and validated for the determination of imidapril in human plasma. A solid-phase extraction cartridge, Sep-pak C18, was used to extract imidapril and ramipril (an internal standard) from deproteinized plasma. The compounds were separated using a XTerra MS C18 column (3.5 μm, 2.1 × 150 mm) and acetonitrile–0.1% formic acid (67:33, v/v) adjusted to pH 2.4 by 2 mmol/L ammonium formic acid, as mobile phase at 0.3 mL/min. Imidapril was detected as *m/z* 406 at a retention time of ca. 2.3 min, and ramipril as *m/z* 417 at ca. 3.6 min. The described method showed acceptable specificity, linearity from 0.5 to 100 ng/mL, precision (expressed as a relative standard deviation of less than 15%), accuracy, and stability. The plasma concentration versus time curves of eight healthy male volunteers administered a single dose of imidapril (10 mg), gave an $AUC_{12hours}$ of imidapril of 121.48 ± 35.81 ng/mL h, and C_{max} and T_{max} values of 32.59 ± 9.76 ng/mL and 1.75 ± 0.27 hours. The developed method should be useful for the determination of imidapril in plasma with sufficient sensitivity and specificity in bioequivalence study.

Minimum sample size and sampling time requirements for assessment of rifampicin bioequivalence from FDC formulations

Agrawal S, Kaur KJ, Singh I, Bhade S, Kaul CL, Panchagnula R. Int J Tuberc Lung Dis 2005 Nov; 9(11):1273–80. SETTING: The WHO- and IUATLD-recommended protocol for rifampicin (RMP) bioequivalence utilizes 20 to 22 volunteers and eight hours, whereas the requirement of other regulatory authorities is 12 volunteers with a 24-hour sampling schedule. Differing sampling size and time requirements may change the outcome of RMP bioequivalence. OBJECTIVE: To determine the minimal sample size and time required to assess RMP bioequivalence from fixed-dose combination formulations. DESIGN: Bioequivalence studies were conducted that fulfilled the criteria of the WHO and Indian regulatory protocols. From earlier studies, retrospective pharmacokinetic evaluation, power of the test, and bioequivalence limits were also calculated using 8 to 22 volunteers and sampling points of 8 to 24 hours. Pharmacokinetic and statistical evaluations from three representative studies showing low, moderate, and high intra-subject variability are given to determine minimum requirements for RMP bioequivalence. RESULT: It was found that a sampling schedule up to eight hours was sufficient to compare the absorption process of RMP. There was no influence of reduced sample size on bioequivalence estimates of RMP that showed low or moderate variability. However, in a study showing higher variation, a sample size of 14 to 16 subjects was found to be optimal. CONCLUSION: It is possible to reduce the sample size requirement for determination of RMP bioequivalence using the WHO protocol.

New questions regarding bioequivalence of levothyroxine preparations: a clinician's response

Green WL. AAPS J 2005 Mar 30; 7(1):E54–8. A recent decision by the Food and Drug Administration (FDA) to declare various brands of levothyroxine bioequivalent has provoked objections from several physicians' organizations. These organizations assert that the method of testing bioequivalence is flawed, and that indiscriminate switching among preparations could lead to serious instances of undertreatment and overtreatment of hypothyroid patients. In this review, we first list common indications for thyroid hormone administration, distinguishing its use as replacement therapy in hypothyroidism from its use to suppress thyrotropin (TSH) secretion in cases of thyroid cancer, nodules, and goiter. The dangers associated with changing to a preparation with different bioavailability are summarized, noting the particular danger of giving a more active preparation to a patient receiving TSH-suppressive doses of levothyroxine. However, these dangers are part of a larger problem: there are data showing that large numbers of patients are already receiving an improper dosage of levothyroxine, as judged from measurements of serum TSH. The recent history of FDA actions concerning levothyroxine bioequivalence and the arguments of those in disagreement are summarized. The immediate response to these problems should be better education of both patients and physicians. It is also recommended that there will be further discussion of the problems in determining bioequivalence, and that consideration be given to more accurate and clinically relevant methods. Such methods should include assessment of the changes in TSH induced by each preparation in athyrotic patients.

Oral dosage form performance tests: new dissolution approaches

Hauck WW, Foster T, Sheinin E, Cecil T, Brown W, Marques M, Williams RL. Pharm Res 2005 Feb; 22(2):182–7. The performance test is one of a series of tests that compose the specification in a United States Pharmacopeia (USP) dosage form monograph. For an orally administered, nonsolution dosage form, it is usually satisfied by either a dissolution or a disintegration procedure. Dissolution acceptance criteria are usually set in private negotiations between an applicant and a regulatory agency. With information about this private agreement and other information provided in a sponsor's Request for Revision to USP, the USP's Council of Experts elaborates a public dosage form monograph. Based on the relationship between the regulatory decisions and the Request for Revision, the USP dissolution procedure links to a regulatory judgment about bioavailability and bioequivalence and, ultimately, to a judgment about safety and efficacy. The current dissolution procedure and acceptance criteria are perceived as having worked well over the years and are generally accepted. This article discusses new approaches

that merit consideration. These approaches focus on (*i*) explicit use of hypothesis testing, (*ii*) use of parametric tolerance intervals, (*iii*) improved ways to set dissolution acceptance criteria, and (*iv*) a more flexible protocol to assess conformity. Application of the proposed approaches may better assess, manage, and communicate both manufacturer and consumer risk for dissolution testing.

Pharmacokinetic profiling and bioequivalence assessment of two marketed brands of nevirapine tablets in healthy Indian volunteers

Narang VS, Lulla A, Malhotra G, Purandare S. Arzneimittelforschung 2005; 55(10):598–603. Nevirapine (CAS 129618-40-2), a non-nucleoside reverse transcriptase inhibitor, has been effectively used for treatment of HIV-infected patients. A randomized, two-way, crossover study was conducted in 24 fasting, healthy, Indian male subjects to compare plasma pharmacokinetic profile, and single-dose tolerability of a new nevirapine tablet formulation (test, *T*) with that of a reference (*R*) tablet. Each volunteer received *T* and *R* formulations separated by at least 19 days of drug free washout period. Plasma concentrations of nevirapine, determined up to 288 hours post-dose by a sensitive and validated HPLC assay, were utilized to assess pharmacokinetic parameters, such as the maximum observed plasma concentration (C_{max}), time to C_{max} (t_{max}), and area under plasma concentration curve (AUC_∞). The primary plasma pharmacokinetic parameters of anti-retroviral substances, C_{max} and AUC_∞, were comparable for either of the formulations. Oral absorption of nevirapine was almost complete within five hours. Geometric mean ratios (% reference) of AUC_∞ and C_{max} and their 90% confidence intervals were 96.9 [93.69–100.24] and 100.8 [94.61–107.4], respectively. As the 90% confidence intervals of the geometric mean ratio were entirely within 80% to 125% for log-transformed parameters, the two formulations were considered bioequivalent in the extent and rate of absorption. Both formulations exhibited similar tolerability under fasting conditions.

Statistical aspects of bioequivalence testing between two medicinal products

Zintzaras E. Eur J Drug Metab Pharmacokinet 2005 Jan–Jun; 30(1–2):41–6. A generic drug product (test product) is bioequivalent to an innovator product (reference product) when their bioavailabilities in the same molar dose are similar. Bioavailability is expressed by pharmacokinetic parameters such as the area under plasma concentration–time curve (AUC), the maximum plasma concentration (C_{max}), and the time of maximum plasma concentration (t_{max}). The assessment of bioequivalence is carried out by in vivo bioequivalence studies. This paper examines and appraises design issues for performing a bioequivalence study: the use of crossover, parallel, replicated, and add-on designs; and the determination of sample size. In addition, it presents the valid statistical approaches for proving bioequivalence: average bioequivalence on transformed and untransformed data; parametric and non-parametric analyses; moment-based individual bioequivalence; and direct curve comparison metrics.

Switchability of Neoral and Equoral according to Food and Drug Administration rules and regulations

Masri MA, Haberal M, Rizvi A, Stephan A, Bilgin N, Naqvi A, Barbari A, Kamel G, Zafar N, Emiroglu R, Colak T, Manzoor K, Matha V, Kamarad V, Rost M, Rizk S, Hazime A, Perlik F. Transplant Proc 2005 Sep; 37(7):2988–93. According to the U.S. Food and Drug Administration (FDA), if a drug product contains a drug substance that is chemically identical and is delivered to the site of action at the same rate and extent as another drug product, then it is equivalent and can be substituted (switchable) for that drug product. Methods used to define bioequivalence as stated by the FDA rules (FDA 21 CFR 320, 24) are (*i*) pharmacokinetic (PK) studies in healthy volunteers, (*ii*) comparative clinical trials, and (*iii*) pharmacodynamic (PD) studies (bioactivity). We evaluated the switchability of Equoral (IVAX-U.S.A.) with Neoral (Novartis, Switzerland) using all FDA rules. In a single oral dose, we undertook a comparative bioavailability study of Equoral (IVAX, U.S.A.), Neoral (Novartis, USA), and Neoral (Novartis, U.K.). The PKs of Equoral and Neoral were determined with blood levels at 0, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, 16, 24, 30, 36, 42, and 48 hours. The area under curve (AUC), AUC extrapolated to infinity ($AUC_{0-\infty}$), rate of absorption (T_{max}), extent of absorption (C_{max}), half time ($t_{1/2}$) of Equoral and Neoral were all within the 90% confidence interval of 80% to 125%

boundaries. A comparative multinational multicenter clinical trial in stable renal transplant patients included 70 patients (22 women and 48 men) of mean age of 33 years (range: 26–43) was performed in Turkey, Lebanon, and Pakistan. In this study, the ratios of LSM and the 90% confidence intervals for the nontransformed/parameters (AUC_{0-t}, AUC_{∞}, T_{max}, and C_{max}) of Equoral and Neoral SGC were 98% and 95%, respectively, which are within the 80% to 125% FDA acceptance range. For immunosuppressive drugs, the site of action is the lymphocyte and the measurable response is the decrease in lymphocyte count caused by the relative concentration of the drug in the lymphocyte. In a controlled switch, fixed-dose study, both Equoral and Neoral achieved the same concentration in the lymphocytes and caused the same degree of lymphocyte count reduction. The results of the testing (bioavailability–bioequivalence, clinical studies, and pharmacodynamic–bioactivity) required by FDA for interchangeability ("switchability") of immunosuppressive agents suggest that Neoral and Equoral are switchable.

Tests for equivalence based on odds ratio for matched-pair design

Liu JP, Fan HY, Ma MC. J Biopharm Stat 2005; 15(6):889–901. Currently, methods for evaluation of equivalence under a matched-pair design use either difference in proportions or relative risk as measures of risk association. However, these measures of association are only for cross-sectional studies or prospective investigations, such as clinical trials and they cannot be applied to retrospective research such as case–control studies. As a result, under a matched-pair design, we propose the use of the conditional odds ratio for assessment of equivalence in both prospective and retrospective research. We suggest the use of the asymptotic confidence interval of the conditional odds ratio for evaluation of equivalence. In addition, a score test based on the restricted maximum likelihood estimator is derived to test the hypothesis of equivalence under a matched-pair design. On the other hand, a sample size formula is also provided. A simulation study was conducted to empirically investigate the size and power of the proposed procedures. Simulation results show that the score test not only adequately controls the Type I error, but it can also provide sufficient power. A numerical example illustrates the proposed methods.

2004

Bioavailability of divalproex extended-release formulation relative to the divalproex delayed-release formulation

Dutta S, Zhang Y. Biopharm Drug Dispos 2004 Nov; 25(8):345–52. Divalproex sodium extended-release tablet (divalproex-ER) is a novel formulation of the conventional divalproex sodium delayed-release tablet (divalproex). In five multiple-dose studies in healthy subjects ($n=82$) and epilepsy patients ($n=86$), the estimates of divalproex-ER/divalproex ratios for steady-state 24 hours valproic acid area under the curve (AUC) central values, maximum concentration (C_{max}) central values, and minimum concentration (C_{min}) means had ranges of 0.77 to 0.97, 0.71 to 0.87, and 0.78 to 1.03, respectively. These studies used different divalproex regimens (two, three, or four times daily) and meal conditions (fasting, low, medium, and high calorie meals). Divalproex-ER was administered once daily. A meta-analysis of divalproex-ER/divalproex relative bioavailability across five studies under different meal conditions and divalproex dosing frequencies was performed. This meta-estimate of relative bioavailability was used to provide dosing recommendations for conversion of patients from divalproex to divalproex-ER. The estimated AUC, C_{max}, and C_{min} divalproex-ER/divalproex ratios (95% confidence interval) were 0.89 (0.85–0.94), 0.79 (0.74–0.84), and 0.96 (0.90–1.02), respectively. The food and divalproex regimen had no effect on the relative bioavailability. While switching from divalproex to divalproex-ER, the divalproex-ER daily dose may have to be increased by an average of 12% (calculated as 1.0/0.89) to achieve comparable plasma exposure. Since the divalproex-ER dosage strengths (250 and 500 mg) are not 12% higher than the divalproex dosage strengths (125, 250, and 500 mg), an 8% to 20% higher divalproex-ER daily dose should be considered for conversion from divalproex to divalproex-ER.

Bioequivalence evaluation of two rabeprazole enteric coated formulations in healthy Chinese volunteers

Chen J, Jiang WM, Gao XL, Jiang X, Zhang QZ, Zheng ZH. Eur J Drug Metab Pharmacokinet 2004 Apr–Jun; 29(2):103–6. A bioequivalence study of two rabeprazole enteric-coated formulations was carried out in 20 healthy Chinese volunteers according to a single-dose, two-sequence, crossover randomized design. The two formulations were administered in two treatment days, separated by a washout period of seven days. Blood samples were collected at specified time intervals over 10 hours post-dosing. Plasma samples were separated and assayed for rabeprazole using a selective and sensitive HPLC method with UV detection. The pharmacokinetic parameters AUC_{0-t}, AUC_{max}, C_{max}, t_{max}, $t_{1/2}$, and MRT were determined from plasma concentration–time profile of both formulations. ANOVA and two one-sided *t*-test procedures showed no significant difference in log-transformed C_{max}, AUC_{0-t} $AUC_{0-\infty}$, while the 90% confidence interval of the ratio of the geometric means of their values were also used to assess bioequivalence between the two formulations. The results of this study indicated that the two rabeprazole formulations can be considered to be bioequivalent.

Bioequivalence studies on bisphosphonates: the example of alendronate

Lainesse A, Ozalp Y, Wong H, Alpan RS. Arzneimittelforschung 2004 Sep; 54(9a):569–72. The study was designed to evaluate the bioequivalence of two formulations of alendronate (CAS 121268-17-5, Osalen 10 mg tablets, in the following referred to as "test" vs. the originator product, in the following referred to as "reference") in 89 healthy male and female volunteers, who were administered four 10 mg alendronate tablets under fasting conditions. The trial was performed according to an open, randomized, crossover design with a washout period of 14 days in one study center. Urine samples were taken up to 36 hours post-dose, and the concentrations of alendronate were determined by HPLC/Fl method. The mean A_e(0–36) were 102.89 ± 57.52 and 96.23 ± 60.81 μg for the test and reference formulations, respectively, while the mean R_{max} were 36.15 ± 21.07 and 35.36 ± 22.88 μg/h, respectively. The test and reference tablets T_{max} were 0.592 ± 0.858 and 0.583 ± 0.858 hours, respectively. No significant differences of pharmacokinetic parameters between the two studied formulations were found. The 90% confidence interval for the primary target parameters, intra-individual ratios of A_e(0–36) and R_{max} of alendronate were between 1.01 and 1.17 for A_e(0–36) and between 0.96 and 1.11 for R_{max}, and thus within the acceptance range for bioequivalence trials. In the light of the present study, it can be concluded that alendronate test tablets are bioequivalent to the reference formulation.

Bioequivalence study of levothyroxine tablets compared to reference tablets and an oral solution

Koytchev R, Lauschner R. Arzneimittelforschung 2004; 54(10):680–4. The study was designed to evaluate the bioequivalence of three levothyroxine sodium (CAS 51-48-9) formulations, i.e., a test and a reference tablet and an oral solution. A bioequivalence study was carried out in 25 healthy volunteers, who were administered a single dose of 600 μg levothyroxine in the form of the test formulation (levothyroxine sodium tablets 200 μg; Eferox), the originator product, and an oral solution. The trial was performed in one study center according to an open, randomized, three-way crossover design with washout periods of 35 days between administration. Blood samples were taken up to 48 hours post-dose, the plasma was separated and the concentrations of levothyroxine and triiodothyronine were determined by radioimmunoassay with I125 labeling method. The levothyroxine mean C_{max} were 112.0 ± 17.3, 113.4 ± 18.5, and 111.3 ± 15.1 ng/mL; while the mean AUC_{0-24} were 2263.7 ± 332.8, 2307.3 ± 351.3, and 2286.1 ± 331.0 ng h/mL for the test and the reference tablets as well as for the oral solution, respectively. No significant differences were found of principal pharmacokinetic parameters between the studied formulations. The 90% confidence interval for the primary target parameters, intra-individual ratios of AUC_{0-24} and C_{max} of levothyroxine were within the acceptance ranges for bioequivalence trials, i.e., AUC_{0-24} 0.954 to 1.016 and 0.966 to 1.011 as well as C_{max} 0.948 to 1.027 and 0.968 to 1.032 for test tablets versus reference tablets and the oral solution, respectively. Similar results were observed for triiodothyronine. In the light of the present study, it can be concluded that the levothyroxine test tablet is bioequivalent to the reference formulation in

respect of extent and rate of absorption. The results of the present trial confirm the findings of a previous study, performed under steady-state conditions with Eferox tablets 100 μg in patients without thyroid function.

Bridging bioequivalence studies
Liu JP. J Biopharm Stat 2004 Nov; 14(4):857–67. In some new regions, an innovative drug of the original region was not marketed. However, after the patent of the innovative drug is expired, a generic copy of the innovative drug from the original region was introduced and approved for marketing in the new region. Another generic copy manufactured by the local sponsor of the new region is seeking for approval in the new region. Despite unavailability of the innovative drug, the regulatory authority of the new region still wants to approve the local generic copy based on assessment of bioequivalence between the local generic drug and the innovative drug. Following the bridging concept suggested by the ICH E5 guidance, we propose a method to evaluate average bioequivalence between the generic copy of the new region and the innovative drug of the original region using the generic copy of the original region as the bridging reference formulation. Sample size required by the bioequivalence study in the new region is also provided. Numerical examples illustrate the proposed method.

Characterizing biological products and assessing comparability following manufacturing changes
Chirino AJ, Mire-Sluis A. Nat Biotechnol 2004 Nov; 22(11):1383–91. Changes in production methods of a biological product may necessitate an assessment of comparability to ensure that these manufacturing changes have not affected the safety, identity, purity, or efficacy of the product. Depending on the nature of the protein or the change, this assessment consists of a hierarchy of sequential tests in analytical testing, preclinical animal studies, and clinical studies. Differences in analytical test results between pre- and post-change products may require functional testing to establish the biological or clinical significance of the observed difference. An underlying principle of comparability is that under certain conditions, protein products may be considered comparable on the basis of analytical testing results alone. However, the ability to compare biological materials is solely dependent on the tests used, since no single analytical method is able to compare every aspect of protein structure or function. The advantages and disadvantages of any given method depend on the protein property being characterized.

Drug delivery to the nasal cavity: in vitro and in vivo assessment
Newman SP, Pitcairn GR, Dalby RN. Crit Rev Ther Drug Carrier Syst 2004;21(1):21–66. Drugs are given intranasally for both local and systemic applications, and the use of the intranasal route is predicted to rise dramatically in the next 10 years. Nasal drug delivery may be assessed by a variety of means, but high reliance is often placed upon in vitro testing methodology (emitted dose, droplet or particle size distribution, spray pattern, and plume geometry). Spray pattern and plume geometry define the shape of the expanding aerosol cloud, while droplet size determines the likelihood of deposition within the nasal cavity by inertial impaction. Current Food and Drug Administration guidance recommends these methods as a means of documenting bioavailability (BA) and bioequivalence (BE) for topically acting solution formulations, because they can be performed reproducibly and are more discriminating among products. Nasal drug delivery in vivo may be determined by several radionuclide imaging methods: the two-dimensional imaging technique of gamma scintigraphy has been used most widely, but the three-dimensional method of positron emission tomography (PET) is being used increasingly often. In some situations a good in vitro/in vivo correlation (IVIVC) exists; for instance, negligible penetration into the lungs has been demonstrated in the case of nasal pump sprays delivering large droplets, while a clear difference may be shown in intranasal deposition between two aerosols with markedly different size distributions. However, recent studies have shown a poorer IVIVC for two similar nasal pump sprays, where significant differences in in vitro parameters were not reflected in differences in nasal deposition in vivo. It is suggested that radionuclide imaging data may have an important role to play as an adjunct to in vitro testing in BA and BE assessments and may provide a clearer understanding of the changes in in vitro parameters

that are important for predicting differences in in vivo performance. Copyright 2004 Begell House, Inc.

Experimental design and statistical methods for classical and bioequivalence hypothesis testing with an application to dairy nutrition studies
Tempelman RJ. J Anim Sci 2004;82 E-Suppl.:E162–172. Genetically modified (GM) corn hybrids have been recently compared against their isogenic reference counterparts in order to establish proof of safety as feedstuffs for dairy cattle. Most such studies have been based on the classical hypothesis test, whereby the null hypothesis is that of equivalence. Because the null hypothesis cannot be accepted, bioequivalence-testing procedures in which the alternative hypothesis is specified to be the equivalence hypothesis are proposed for these trials. Given a Type I error rate of 5%, this procedure is simply based on determining whether the 90% confidence interval on the GM versus reference hybrid mean difference falls between two limits defining equivalence. Classical and bioequivalence power of test are determined for 4×4 Latin squares and double-reversal designs, the latter of which are ideally suited to bioequivalence studies. Although sufficient power likely exists for classical hypothesis testing in recent GM versus reference hybrid studies, the same may not be true for bioequivalence testing depending on the equivalence limits chosen. The utility of observed or retrospective power to provide indirect evidence of bioequivalence is also criticized. Design and analysis issues pertain to Latin square and crossover studies in dairy nutrition studies are further reviewed. It is recommended that future studies should place greater emphasis on the use of confidence intervals relative to *p*-values to unify inference in both classical and bioequivalence-testing frameworks.

Interpretation and optimization of the dissolution specifications for a modified release product with an in vivo/in vitro correlation (IVIVC)
Hayes S, Dunne A, Smart T, Davis J. J Pharm Sci 2004 Mar; 93(3):571–81. This article considers the in vivo significance attached to in vitro dissolution testing. Almost invariably, the in vitro dissolution test is interpreted in terms of bioequivalence. The literature that describes methods for setting in vitro dissolution specifications is reviewed. The most common interpretation of these specifications is a deterministic one, i.e., those batches passing the dissolution specifications would be bioequivalent with the reference if tested in vivo and those failing the dissolution specifications would not be bioequivalent if tested in vivo. Due to random variation, the deterministic interpretation is not appropriate. Instead, we need to consider the conditional probability that a batch that has passed the in vitro dissolution test would demonstrate bioequivalence if tested in vivo, and that a batch known to have failed the in vitro dissolution test would demonstrate bioinequivalence if tested in vivo. One way to estimate these probabilities is by means of a simulated experiment in which the production and testing (in vivo and in vitro) of a large number of batches is computer simulated. Such a simulation can only be performed if the relationship between the in vitro dissolution characteristics and the in vivo performance of the product has been modeled. These models are generally referred to as in vivo/in vitro correlations. The results of one such experiment are described. The above-mentioned conditional probabilities are shown to depend on the choice of dissolution specifications. This result leads to the notion of optimal dissolution specifications. However, both of the conditional probabilities cannot be maximized simultaneously. The probability of making a correct decision on the basis of the in vitro dissolution test is introduced as a possible optimality criterion. This probability is a linear combination of the two conditional probabilities of interest. Using this criterion, the optimal dissolution specifications can be found by searching over the multidimensional space defined by the half width of each interval used in the specifications to find the combination that maximizes this probability. This process is demonstrated using the Nelder–Mead search routine. The choice of dissolution specifications has profound implications for the routine production of the product because if the specifications were very narrow the probability of a batch passing would be low, resulting in a low hit rate. The same computer program used to perform the simulation experiment can be used to estimate the hit rate. Furthermore, it can be used to explore the magnitude of changes required in the parameters describing the test product (particularly variability) to increase a low hit rate

to an acceptable level. Copyright 2004 Wiley-Liss, Inc. and the American Pharmacists Association.

LC–MS determination and bioavailability study of loperamide hydrochloride after oral administration of loperamide capsule in human volunteers

Yu JH, Kim HJ, Lee S, Hwang SJ, Kim W, Moon CJ. J Pharm Biomed Anal 2004 Oct 29;36(2):421–7. The purpose of the present study was to develop a standard protocol for loperamide hydrochloride bioequivalence testing. For this purpose, a simple rapid and selective LC–MS method utilizing a single quadrupole mass spectrometer was developed and validated for the determination of loperamide hydrochloride in human plasma, and we followed this with a bioavailability study. Methyl *tert*-butylether was used to extract loperamide hydrochloride and ketoconazole [internal standard (IS)] from an alkaline plasma sample. LC separation was performed on a Zorbax RX C18 column (5 μm, 2.1 mm×150 mm) using acetonitrile:water:formic acid [50:50:0.1 (v/v)] as a mobile phase. The retention times of loperamide hydrochloride and IS were 1.2 and 0.8 min, respectively. Quadrupole MS detection was by monitoring at *m/z* 477 ($M+1$) corresponding to loperamide hydrochloride and at *m/z* 531 ($M+1$) for IS. The described assay method showed acceptable precision, accuracy, linearity, stability, and specificity. The bioavailability of loperamide hydrochloride was evaluated in eight healthy male volunteers. The following pharmacokinetic parameters were elucidated after administering a single dose of four 2 mg capsules of loperamide: the area under the plasma concentration versus time curve from time 0 to 72 hours (AUC_{72hr}) 19.26±7.79 ng h/mL; peak plasma concentration (C_{max}) 1.18±0.37 ng/mL; time to C_{max} (T_{max}) 5.38±0.74 hours; and elimination half-life ($t_{1/2}$) 11.35±2.06 hours. The developed method was successfully used to study the bioavailability of a low dose (8 mg) of loperamide hydrochloride.

Novel scaled average bioequivalence limits based on GMR and variability considerations

Karalis V, Symillides M, Macheras P. Pharm Res 2004 Oct; 21(10):1933–42. PURPOSE: (*i*) To develop novel approaches for the construction of bioequivalence (BE) limits incorporating both the intrasubject variability and the geometric mean ratio (GMR) and (*ii*) to assess the performance of the novel approaches in comparison to several scaled BE procedures and the classic unscaled average BE. METHODS: Plots of the BE limits or the extreme GMR values accepted as a function of the coefficient of variation (CV) were constructed for published and the developed scaled procedures. Two-period crossover BE investigations with 12, 24, or 36 subjects were simulated with assumptions of a CV 10, 20, 30, or 40%. The decline in the percentage of accepted studies was recorded as the true GMR for the two formulations was raised from 1.00 to 1.50. Acceptance of BE was evaluated by published and the developed scaled procedures, and, for comparison, by the unscaled average BE. RESULTS: Two GMR-dependent BE limits are proposed for the evaluation of average BE: (*i*) BELscG1 with Ln(Upper, Lower BE limit)=±[(5-4GMR)0.496*s*+Ln(1.25)] and (*ii*) BELscG2 with Ln(Upper, Lower BE limit)=±[(3−2GMR)(0.496*s*+Ln(1.25))], where *s* is the square root of the intrasubject variance. The range of BE limits becomes narrower as GMR values deviate from unity, and increases with variability. The two new approaches exhibit the highest statistical power at low CV values. At high levels of variability, BELscG1 and BELscG2 show high statistical power, as well as the lowest percentages of acceptance among the scaled methods when GMR=1.25. The latter becomes more obvious when a large number of subjects are incorporated in the studies. CONCLUSIONS: The GMR and CV estimates of the BE study can be used in conjunction with the GMR versus CV plot for the assessment of average BE. The new approaches, BELscG1 and BELscG2, appear to be highly effective at all levels of variation investigated.

The Precautionary Principle and statistical approaches to uncertainty

Keiding N, Budtz-Jorgensen E. Int J Occup Med Environ Health 2004;17(1):147–51. The central challenge from the Precautionary Principle to statistical methodology is to help delineate (preferably quantitatively) the possibility that some exposure is hazardous, even in cases where this is not established beyond reasonable doubt. The classical approach to hypothesis testing is unhelpful, because lack of significance can be due either to uninformative data or to genuine

lack of effect (the Type II error problem). Its inversion, bioequivalence testing, might sometimes be a model for the Precautionary Principle in its ability to "prove the null hypothesis". Current procedures for setting safe exposure levels are essentially derived from these classical statistical ideas, and we outline how uncertainties in the exposure and response measurements affect the no observed adverse effect level, the Benchmark approach and the "Hockey Stick" model. A particular problem concerns model uncertainty: usually these procedures assume that the class of models describing dose/response is known with certainty; this assumption is, however, often violated, perhaps particularly often when epidemiological data form the source of the risk assessment, and regulatory authorities have occasionally resorted to some average based on competing models. The recent methodology of the Bayesian model averaging might be a systematic version of this, but is this an arena for the Precautionary Principle to come into play?

Prednisolone: limited sampling strategies for estimating pharmacokinetic parameters
Suarez-Kurtz G, Estrela Rde C, Salvadori MC. Ther Drug Monit 2004 Feb; 26(1):16–22. To develop limited-sampling strategy (LSS) models for estimating prednisolone's area under plasma concentration versus time curve ($AUC_{0-\infty}$), its maximum concentration in plasma (C_{max}), and total clearance (CL/F). Healthy subjects ($n=24$), enrolled in a bioequivalence study, received 20 mg PO of the prodrug prednisone as reference and test tablets, and plasma prednisolone concentrations ($n=576$) were measured by a validated HPLC assay. A linear regression analysis of $AUC_{0-\infty}$, C_{max}, CL/F, and log(CL/F) against the plasma prednisolone concentrations for the reference formulation was carried out to develop LSS models to estimate these parameters. The LSS models were validated on the test formulation datasets and on simulated sets generated by the software ADAPT II. LSS models based on a single [1.5 hours for C_{max} and 7 hours for $AUC_{0-\infty}$, CL/F, and log(CL/F)] plasma sample, accurately estimated ($R^2=0.84$–0.97, mean bias $<1\%$, and mean precision $<10\%$) these pharmacokinetic parameters. Validation tests indicated that the most informative single-point LSS models developed for the reference formulation provide precise estimates ($R^2>0.83$, mean bias $<3\%$, and mean precision $<10\%$) of the corresponding pharmacokinetic parameters for the test formulation. LSS models based on the two most informative sampling points (1.5 and 7 hours) were required for accurate estimates ($R^2>0.87$, mean bias $<6\%$, and mean precision $<8\%$) of prednisolone's C_{max}, $AUC_{0-\infty}$, CL/F, and log(CL/F) for the simulated datasets. Finally, bioequivalence assessment of the prednisone formulations, based on LSS-derived $AUC_{0-\infty}$ and C_{max} values provided results identical to those obtained using the original values for these parameters. One- and two-point LSS models provided accurate estimates of prednisolone's C_{max}, $AUC_{0-\infty}$, and CL/F, following single-oral dose of prednisone, and allowed correct assessment of bioequivalence between two prednisone formulations.

2003

Active-control clinical trials to establish equivalence or noninferiority: methodological and statistical concepts linked to quality
Gomberg-Maitland M, Frison L, Halperin JL. Am Heart J 2003 Sep; 146(3):398–403. The randomized, double-blind, placebo-controlled trial is the optimum method for clinical evaluation of new treatments, as assessed by clinicians and statisticians. However, if a known standard of therapy exists, it may be difficult to prove that a new therapy is superior. Equivalence and noninferiority clinical trial designs are now frequently utilized in clinical medical research. This article reviews the statistical differences between superiority, equivalence, and noninferiority design schemes, which pose specific ethical questions and have important implications for interpretation and clinical application of trial results. A guideline is proposed as a standard approach for reporting to facilitate qualitative assessment of the methodology of these trials.

Assessing individual bioequivalence using the structural equation model
Carrasco JL, Jover L. Stat Med 2003 Mar 30;22(6):901–12. The structural equation model (SEM) is introduced as a useful approach for assessing individual bioequivalence. SEM parameters

are estimated using a partial likelihood analysis and the hypotheses of individual bioequivalence is evaluated in a disaggregate way, testing separately the hypothesis concerning SEM parameters, and assessing the overall hypothesis of individual bioequivalence using the intersection–union principle. Limits of bioequivalence for SEM parameters are proposed and a power analysis is carried out.

Assessing individual bioequivalence with high-order crossover designs: a unified procedure

Hsuan FC, Reeve R. Stat Med 2003 Sep 30;22(18):2847–60. The U.S. FDA's newly issued guidance on bioequivalence recommends the use of individual bioequivalence (IBE) for highly variable drugs and possibly for modified release dosage forms. The recommended approach to the analysis is to follow the methodology of Hyslop, Hsuan, and Holder (HHH), based on a linear mixed model. A limitation of the HHH method is that it works only for uniform designs, such as RTRT/TRTR. In this paper, we present an alternative approach based on a multivariate model. The multivariate model is shown to be a strict superset of the linear mixed model and can successfully model data where the mixed model fails. Our multivariate approach coincides with the HHH method where the HHH method applies, but generalizes to any high-order crossover design, such as the Balaam design, RTR/TRT, and TRSS/RSTT/STRR. We present numerical examples to demonstrate the proposed method, and examine its properties with a simulation study.

Assessing noninferiority of a new treatment in a three-arm clinical trial including a placebo

Pigeot I, Schafer J, Rohmel J, Hauschke D. Stat Med 2003 Mar 30;22(6):883–99. In noninferiority trials, where noninferiority of a new experimental drug compared to an active control has to be shown, it may be advisable to use an additional placebo group for internal validation if ethically justifiable. The focus of this paper is on such designs. Assuming normality and homogeneity of variances, we will derive a statistical test procedure that turns out to be equivalent to the assessment based on Fieller's confidence interval. Based on the power function of this test, sample size calculations are carried out to achieve a given power. Additionally, the optimal allocation of the total sample size is derived. As an alternative to this parametric procedure, the bootstrap percentile interval is discussed and finally compared with Fieller's confidence interval in a study on mildly asthmatic patients.

Assessment of the bioequivalence of two oxcarbazepine oral suspensions versus a film-coated tablet in healthy subjects

Flesch G, Tudor D, Denouel J, Bonner J, Camisasca R. Int J Clin Pharmacol Ther 2003 Jul; 41(7):299–308. Oxcarbazepine (trileptal) oral suspension has been reformulated and a study was performed to compare the bioavailability after single doses and at steady state of the current and former oral suspension versus the marketed film-coated tablets and to compare the bioavailability of the current and former oral suspension. The results support the switch from the former oral suspension to the current oral suspension and also from both oral suspensions to the film-coated tablet and vice versa. The study was an open-label, single-center, three-way crossover trial. Each treatment period consisted of a single dose of 600 mg oxcarbazepine on day 1, 600 mg oxcarbazepine b.i.d. repeated administration from day 4 up to including day 7, and a final dose of 600 mg oxcarbazepine administered on the morning of day 8. Blood samples were taken on days 1, 7, and 8 (pre-dose). Plasma concentrations of the main metabolite of oxcarbazepine were determined using a validated HPLC assay. The two oral suspensions were compared with the film-coated tablet as reference formulation under fasted conditions. Also the current oral suspension was compared with the former oral suspension. These comparisons were made using data following single-dose administration and under steady-state conditions. Plasma AUC for single dose and $AUC_{0-12hours}$ at steady state and plasma C_{max}, log-transformed (natural base) were used for the assessment of bioequivalence. The 90% confidence interval (CI) approach was used for testing bioequivalence. Bioequivalence was accepted if CI was contained within the region (0.8%, 1.25%) At steady state, both the former and the current oral suspensions showed bioequivalence with the film-coated tablet with respect to AUC and C_{max}. The current oral suspension was also bioequivalent when compared with the former oral

suspension with respect to AUC and C_{max}. After single dose, the former oral suspension was bioequivalent when compared with the film-coated tablet with respect to both AUC and C_{max}. However, the current oral suspension was bioequivalent to both the film-coated tablet and the former oral suspension with respect to AUC but not to C_{max}.

Bioequivalence and other unresolved issues in generic drug substitution
Meredith P. Clin Ther 2003 Nov; 25(11):2875–90. BACKGROUND: Substitution of generic drugs for brand-name products is highly controversial and often met with suspicion by healthcare providers and patients. Historically, the debate has focused on the issue of bioequivalence, and clinical practice has identified a number of drug classes for which generic substitution should be approached with caution. Current bioequivalence requirements are based on a measure of average bioequivalence; however, there are fears that use of this measure may be inappropriate in the case of a drug with a narrow or wide therapeutic range or high intrasubject or intersubject variability. Under these circumstances, measures of individual and population bioequivalence are proposed to be more accurate than measures of average bioequivalence. OBJECTIVE: This paper addresses issues of bioequivalence and other concerns with generic drug substitution. METHODS: The author conducted a MEDLINE search of the English-language literature containing the key terms generic, multisource, quality, and brand and published between 1973 and 2003. The names of branded pharmaceuticals whose patents had recently expired (e.g., Ventolin HFA, Adalat, Capoten, Tagamet HB 200, and Valium) also were used to search for articles on generic substitution. Reference lists of relevant articles were also searched. Bioequivalence issues are presented together with more general concerns over generic drug substitution, such as consumer perception of risk, differences in product and packaging appearance, and differences in excipients. RESULTS: The literature reviewed act to highlight a number of different drug categories and patient subpopulations for which generic substitution can still prove to be problematic. CONCLUSION: The author recommended that healthcare providers continue to exercise caution in the consideration of generic drug substitution under certain circumstances.

Bioequivalence of azathioprine products
Baker DE. Rev Gastroenterol Disord 2003 Fall;3(4):219–23. All azathioprine oral tablets are considered bioequivalent by the Food and Drug Administration based on traditional testing. However, since these tests were conducted, it has been determined that some patients have a deficiency of the enzyme most responsible for the metabolism of 6-mercaptopurine-thiopurine methyltransferase (TPMT). Azathioprine is rapidly converted to 6-mercaptopurine, its active metabolite. So it is possible that differences in TPMT activity may influence the bioequivalence of azathioprine products among individuals, especially those patients deficient in TPMT enzyme activity. However, this possibility has not been evaluated.

Clinical development of an everolimus pediatric formulation: relative bioavailability, food effect, and steady-state pharmacokinetics
Kovarik JM, Noe A, Berthier S, McMahon L, Langholff WK, Marion AS, Hoyer PF, Ettenger R, Rordorf C. J Clin Pharmacol 2003 Feb; 43(2):141–7. The immunosuppressant everolimus used in organ transplantation is formulated as a conventional tablet for adults and a dispersible tablet that can be administered in water for pediatric use. As part of the pediatric clinical development program, the relative bioavailability and food effect for the dispersible tablet were evaluated in healthy adult subjects as a prelude to characterizing the steady-state pharmacokinetics in pediatric kidney allograft recipients. In a randomized, open-label, three-way crossover study, 24 healthy adults received single 1.5-mg oral doses of everolimus as (*i*) six 0.25-mg dispersible tablets in water, (*ii*) two 0.75-mg conventional tablets, and (*iii*) six 0.25-mg dispersible tablets in water after a high-fat breakfast. C_{max} and AUC were evaluated by standard bioequivalence testing to determine relative bioavailability and to quantify the effect of food. In a multicenter open-label efficacy/safety trial, pediatric renal allograft recipients received 0.8 mg/m^2 (maximum 1.5 mg) b.i.d. everolimus as dispersible tablets in water. Serial trough concentrations over the first week and a steady-state pharmacokinetic profile on day 7 posttransplant were collected in 19 patients ranging from ages 2 to 16 years.

The bioavailability of everolimus from the dispersible tablet was 10% lower relative to the conventional tablet, with a ratio (90% confidence interval) of 0.90 (0.76–1.07). After a high-fat meal, t_{max} was delayed by a median 2.5 hours, and C_{max} was reduced by 50%. Overall absorption, however, was not affected by food inasmuch as the fed/fasting AUC ratio was 0.99 (0.83–1.17). In pediatric patients, steady state was reached between days 3 and 5. The corresponding steady-state parameters were as follows: C_{min}, 4.4 ± 1.7 ng/mL; C_{max}, 13.6 ± 4.2 ng/mL; and AUC, 87 ± 27 ng hr/mL. Steady-state concentration–time profiles in pediatric transplant patients receiving the dispersible tablet were comparable to those of adult patients receiving the conventional tablet when both were dosed to yield similar trough concentrations. If a pediatric patient is converted from the everolimus dispersible tablet to the conventional tablet, this should be based on a 1:1 milligram switch with subsequent therapeutic drug monitoring to further individualize the dose as needed. The dispersible tablet formulation should be taken consistently either with or without food to minimize fluctuations in exposure over time.

Comparative bioavailability study of two formulations of risperidone available in the Chilean market

Gaete LE, Solis J, Venegas P, Carrillo MJ, Schatloff O, Saavedra I. Rev Med Chil 2003 May; 131(5):527–34. BACKGROUND: Bioavailability of a particular drug can vary according to the formulation used. Therefore, studies of comparative bioavailability of different formulations of a same drug are worthwhile. AIM: To compare the bioavailability of two risperidone formulations available in the Chilean market. MATERIAL AND METHODS: The bioavailability of a local risperidone formulation (Spiron) was compared with the original formulation of the drug (Risperdal) in 12 healthy volunteers, aged 19 ± 1 years. A single dose of 3 mg was given orally, using a randomized double-blind protocol in two periods. Fifteen blood samples were obtained at regular intervals, until 24 hours after drug administration. Risperidone plasma levels were measured by high-pressure liquid chromatography. Pharmacokinetic parameters were calculated using a computer program that is independent of compartmental analysis. RESULTS: The area under the curve of plasma concentration versus time, from 0 to ∞ ($ABC_{0-\infty}$) and from 0 to 24 hours (ABC_{0-24}), early exposure (ABC from 0 to maximal time) and maximal plasma concentrations were significantly lower for Spiron. Half-life time and time to achieve the maximal concentration were similar for the two formulations. CONCLUSIONS: According to bioequivalence tests suggested by the U.S. Food and Drug Administration (FDA) (90% confidence interval for the difference of log-transformed mean pharmacokinetic parameters), the formulations Risperdal and Spiron, cannot be considered interchangeable.

Examining outlying subjects and outlying records in bioequivalence trials

Wang W, Chow SC. J Biopharm Stat 2003 Feb; 13(1):43–56. The problem of detecting outliers in bioequivalence trials is considered. We formulate the problem as a hypothesis-testing problem under a mean-shift model and propose a test procedure based on the likelihood function. The test statistic has two components: one is to detect whether a specific pharmacokinetic measurement of a subject for certain formulation/drug product is an outlying value and the other is to test whether a subject as a whole is an outlying subject (with unusual high or low bioavailability for all formulations/drug products). Under normality assumption, the proposed procedure is most powerful. The small sample distribution of the proposed test statistic is derived. A numerical example illustrates the use of the procedure. The proposed test is then compared in a simulation study against the Hotelling T2 test, recommended by Liu and Weng (1991) for the use of outlier detection in bioequivalence studies. The results from the simulation study show that the proposed test is more powerful than the Hotelling T2 test.

Harmonization of testing drugs for bioequivalence: problems and possible solutions

Zherdev VP, Kolyvanov GB, Litvin AA, Sariev AK. Eksp Klin Farmakol 2003 Mar–Apr; 66(2):60–4. Problems encountered in the testing for bioequivalence of reproduced drugs (generics) are discussed in the parts incompletely resolved in domestic methodological recommendations. There are special cases when such drugs significantly vary in

concentration and dosage, contain endogenous substances, exhibit intensive metabolism with a genetically polymorphous component, belong to "long-lived" compounds, and are intended for local administration. Also mentioned are problems related to insufficient sensitivity of analytical methods and some ethical aspects of investigations.

In vitro bioequivalence testing
Chow SC, Shao J, Wang H. Stat Med 2003 Jan 15;22(1):55–68. A statistical test is proposed for in vitro bioequivalence testing between drug products such as nasal aerosols and nasal sprays. The proposed test generalizes the one recommended in the FDA 1999 guidance to the situation where replicated observations obtained from each sampled canister or bottle of the drug product are available. The technique developed by Hyslop, Hsuan, and Holder is used so that the proposed test is asymptotically accurate. The type I error probability and power of the proposed test are investigated through a simulation study. A method for determining the required sample size to achieve a desired power is also proposed. A numerical example is given for illustration.

Lack of bioequivalence of gatifloxacin when coadministered with calcium-fortified orange juice in healthy volunteers
Wallace AW, Victory JM, Amsden GW. J Clin Pharmacol 2003 Jan; 43(1):92–6. Previous work has demonstrated that the chelation interaction seen with ciprofloxacin when it is coadministered with antacids also happens when it is coadministered with calcium-fortified foods. This study was conducted to study whether this was a drug-specific finding or whether the interaction occurs with other members of the fluoroquinolone class of drugs. Sixteen healthy volunteers received single 400-mg oral doses of gatifloxacin with 12 oz each of water, nonfortified orange juice, and calcium-fortified orange juice, and had plasma samples drawn for assay over the subsequent 48 hours. Results demonstrated significant increases in total oral clearance (15%) and volume of distribution (13%) along with a matching significant decrease (12%) in exposure (AUC) when gatifloxacin was taken with the fortified juice. Although not statistically significant, peak concentrations decreased by 15% and reached (t_{max}) approximately 38% later when gatifloxacin was coadministered with the calcium-fortified juice. Bioavailability testing indicated that although the 90% confidence intervals for the ratio of the geometric means of the calcium-fortified juice and water arms' AUC stayed within the range of 80% to 125%, those for C_{max} did not. This study demonstrated a chelation or adsorption interaction between the fortified juice and gatifloxacin, which reached regulatory significance. As a result, clinicians may wish to instruct patients to take gatifloxacin either with nonfortified foods or on an empty stomach.

Nonparametric estimators of a monotonic dose–response curve and bootstrap confidence intervals
Dilleen M, Heimann G, Hirsch I. Stat Med 2003 Mar 30;22(6):869–82. In this paper, we consider study designs that include a placebo and an active control group as well as several dose groups of a new drug. A monotonically increasing dose–response function is assumed, and the objective is to estimate a dose with equivalent response to the active control group, including a confidence interval for this dose. We present different nonparametric methods to estimate the monotonic dose–response curve. These are derived from the isotonic regression estimator, a nonnegative least-squares estimator, and a bias-adjusted nonnegative least-squares estimator using linear interpolation. The different confidence intervals are based upon an approach described by Korn and upon two different bootstrap approaches. One of these bootstrap approaches is standard, and the second ensures that resampling is done from empiric distributions that comply with the order restrictions imposed. In our simulations, we did not find any differences between the two bootstrap methods, and both clearly outperform Korn's confidence intervals. The nonnegative least-squares estimator yields biased results for moderate sample sizes. The bias adjustment for this estimator works well, even for small and moderate sample sizes, and surprisingly outperforms the isotonic regression method in certain situations.

Proving noninferiority or equivalence of two treatments with dichotomous endpoints using exact methods

Chan IS. Stat Methods Med Res 2003 Jan; 12(1):37–58. Since the early work of R.A. Fisher, exact methods have been recognized as important tools in data analysis because they provide valid statistical inference even with small sample sizes, or with sparse or skewed data. With the recent advance of computational power and the availability of commercial software packages, exact methods have gained substantial popularity over the past two decades. However, most of these exact methods have been devoted to testing classical null hypotheses of no differences, and until recently little was known about exact methods dealing with noninferiority or equivalence hypotheses. The presence of nuisance parameters in testing noninferiority/equivalence hypotheses presents a special challenge for exact methods because of the intense computational requirement. In this paper, we review exact methods available for proving noninferiority or equivalence of two treatments with a dichotomous endpoint. First, we present the general methodology for conducting exact tests for noninferiority or equivalence; we then discuss several unconditional and conditional methods available for constructing hypothesis tests and confidence intervals based on three commonly used measures, namely, the difference, relative risk, and odds ratio of two independent proportions or rates. Finally, we illustrate with several examples the application of these exact methods in analyzing and planning noninferiority or equivalence trials.

Relative bioavailability study of 20-mg enalapril tablets in healthy male volunteers

Lohitnavy O, Lohitnavy M, Polnok S, Taytiwat P. J Med Assoc Thai 2003 Oct; 86(10):947–52. The pharmacokinetic and relative bioavailability studies of 20-mg enalapril tablets, the test product manufactured by Biolab, Thailand when compared with the reference product (Merck Sharp & Dohme, U.S.A.) was conducted in 14 healthy Thai male volunteers following a single-dose, two-period, crossover design. Each subject received 20-mg enalapril tablets of both formulations with a one-week washout period. Plasma samples collected over a 24-hour period after administration were analyzed by LC–MS/MS. Pharmacokinetic parameters were determined by using non-compartmental analysis. Regarding bioequivalence testing, the 90% confidence intervals of C_{max} and $AUC_{0-\infty}$ ratios (test/reference) of enalapril were 101.8% to 134.9% and 105.9% to 121.4% and those of enalaprilat were 104.2% to 122.3% and 104.5% to 118.1%. Based on the European bioequivalence guideline, the 90% confidence intervals of C_{max} and $AUC_{0-\infty}$ ratios of both parent and metabolite forms were within the acceptable ranges of 70% to 143% and 80% to 125%, respectively. It was concluded that the test formulation was bioequivalent to the reference formulation and both formulations can be used interchangeably in clinical practice.

A replicate study design for testing bioequivalence: a case study on two desmopressin nasal spray preparations

Joukhadar C, Schenk B, Kaehler ST, Kollenz CJ, Bauer P, Muller M, Eichler HG. Eur J Clin Pharmacol 2003 Nov; 59(8–9):631–6. Epub 2003 Oct 17. OBJECTIVE: The present study was carried out to test bioequivalence between two different desmopressin nasal spray preparations. Due to the high variability of plasma pharmacokinetics of intranasally administered peptides like desmopressin, appropriate study designs are required to assess bioequivalence. Therefore, a single-dose, replicate study design was used to evaluate bioequivalence of two desmopressin nasal sprays. SUBJECTS AND METHODS: Thirty-two healthy male volunteers were enrolled in the study and were randomly assigned to receive the test- and reference drug on two occasions in a four-period two-sequence crossover study design. Subjects received a single dose of 20 μg (10 μg per nostril) of desmopressin-acetate per study day separated by washout periods of at least one week. Desmopressin blood concentrations were measured serially over a 14-hour period using a validated radioimmunoassay method. Statistical analysis was initially performed using a complicated mixed-analysis model testing for individual bioequivalence according to recommendations by the Food and Drug Administration. This approach, however, failed to converge with all defined main pharmacokinetic parameters and, thus, a traditional mixed analysis of variance

analysis based on population averages was definitely used for testing bioequivalence between study drugs. The procedure of selecting an appropriate statistical analysis for a replicate study design was predefined in the study protocol. RESULTS: The 90% confidence intervals (CI) were calculated for the area under the time–concentration curve (AUC), maximum concentration (C_{max}), and the time to reach C_{max} (t_{max}) of test/reference drug ratios for a bioequivalence range from 0.80 to 1.25. The mean test/reference drug ratios were completely within the 90% CIs with values of 1.041 (CI: 0.892–1.216), 1.021 (CI: 0.913–1.140), and 1.068 (CI: 0.914–1.249) for $AUC_{0-14hours}$, C_{max}, and t_{max}, respectively. CONCLUSION: The rate and the extent of intranasal desmopressin absorption are identical for both study preparations. Thus, the desmopressin test preparation met all equivalence criteria and thereby was proven bioequivalent with a marketed reference nasal desmopressin spray.

Sample size considerations for establishing clinical bioequivalence of allergen formulations
Rabin RL, Slater JE, Lachenbruch P, Pastor RW. Arb Paul Ehrlich Inst Bundesamt Sera Impfstoffe Frankf A M 2003;(94):24–33. Bioequivalence of formulations must be established by proving that the differences between the formulations are within a specified interval according to equation (1), the Interval Hypothesis. Explicit estimates of sample size determined from equation (8) and listed in Table 1 are qualitatively larger than those that would be determined from equation (2), the Hypothesis of No Difference. equation (8) was derived from the TOST procedure; other valid methods should yield comparable results. In any context, this discussion has illustrated that the failure to demonstrate a difference is not sufficient to demonstrate equivalence, and that a properly powered equivalence study of allergen formulations will generally demand many more than four study subjects.

A simple formula for sample size calculation in equivalence studies
Zhang P. J Biopharm Stat 2003 Aug; 13(3):529–38. Bioequivalence and clinical equivalence can be claimed based on the two one-sided test approach or the confidence interval approach. Consequently, the power function of the equivalence test can be derived from either noncentral *t*-distribution or central *t*-distribution. The sample size is then determined from the power function either by numerical method or by closed formulas. In this paper, we propose a simple formula for sample size calculation based on central *t*-distribution. The proposed formula has better properties than those currently available and it can be easily applied in all equivalence studies.

A simple method to estimate sample sizes for safety equivalence studies using inverse sampling
Moore N, Tubert-Bitter P, Fourrier A, Begaud B. J Clin Epidemiol 2003 May; 56(5):433–5. Safety equivalence studies may be required to demonstrate that a new procedure or process is at least as safe as a previous one. They usually involve low or very low outcome rates that are often not precisely determined, making patient-based sample sizing uncertain. Using a reverse sampling approach, a method is derived from standard equations to estimate the number of events that need to be observed to demonstrate equivalence using the confidence interval approach. For instance, for a one-sided (nonsuperiority) hypothesis, 5% α risk, and 80% power, almost 100 events need to be observed in each study arm to demonstrate equivalence within 30%, or 250 events for 20% equivalence. The number of patients to be included can be derived directly from expected event rates.

2002

Assuring quality and performance of sustained and controlled release parenterals: workshop report
Burgess DJ, Hussain AS, Ingallinera TS, Chen ML. AAPS PharmSci 2002;4(2):E7. This is a summary report of the American Association of Pharmaceutical Scientists, the Food and Drug Administration, and the U.S. Pharmacopoeia cosponsored workshop on "Assuring Quality and Performance of Sustained and Controlled Release Parenterals." Experts from the pharmaceutical industry, the regulatory authorities, and academia participated in this workshop to

review, discuss, and debate formulation, processing, and manufacture of sustained and controlled release parenterals and identify critical process parameters and their control. Areas were identified where research is needed to understand the performance of these drug delivery systems and to assist in the development of appropriate testing procedures. Recommendations were made for future workshops, meetings, and working groups in this area.

Bioequivalence of ticlopidine hydrochloride administered in single dose to healthy volunteers

Marzo A, Dal Bo L, Rusca A, Zini P. Pharmacol Res 2002 Nov; 46(5):401–7. Ticlopidine hydrochloride (CAS 55142-85-3) is an inhibitor of platelet aggregation used in the management and prevention of thromboembolic disorders. A new formulation of ticlopidine hydrochloride (test) was compared with the reference Tiklid, available in the market, to assess their bioequivalence and to register the new formulation as a generic according to the abbreviated new drug application procedure. Twenty-four healthy male volunteers were treated with the two formulations (one tablet containing 250 mg of active ingredient) according to a single-dose, balanced, crossover, double-blind design with a washout between the two study periods. Plasma concentration of ticlopidine was assayed in timed samples over a 24-hour period with a well-validated HPLC method with UV detection, which allowed 5 ng/mL to be assayed as the lowest quantifiable concentration. The double-blind key was disclosed only after having completed the assay of unknown samples. From plasma concentrations, C_{max}, t_{max}, AUC_{0-t}, $AUC_{0-\infty}$, and $t_{1/2}$ were evaluated through noncompartmental pharmacokinetic analysis. C_{max} and AUCs were $\log_{10}$-transformed and statistically processed using crossover ANOVA. No statistically significant formulation, period, or sequence effect was encountered. Ninety percent confidence intervals of C_{max} and AUCs were comprised in the stipulated 0.80 to 1.25 range. Similarly, Schuirmann's test led to statistically significant degrees on both the sides explored. Time to peak, t_{max}, processed with the nonparametric Kruskal–Wallis test, did not show any statistically significant degree. According to the guidelines operating in Europe, the test formulation of ticlopidine hydrochloride can be declared bioequivalent with the reference, both formulations in 250 mg tablets.

Bioequivalence study of enalapril tablets in healthy Thai male volunteers

Lohitnavy O, Lohitnavy M, Taytiwat P, Polnok S; J Med Assoc Thai 2002 Jun; 85(6):716–21. The bioequivalence study of 5-mg enalapril tablets, Enaril (Biolab, Thailand) compared to Renitec (Merck Sharp & Dohme, U.S.A.) was conducted in 14 healthy Thai male volunteers following a single-dose, two-period, crossover design. Each subject received four 5-mg enalapril tablets of both formulations with a one-week washout period. Plasma samples collected over a 24-hour period after administration were analyzed by LC/MS/MS. Pharmacokinetic parameters were determined using noncompartmental analysis. Regarding bioequivalence testing, the 90% confidence intervals of C_{max} and $AUC_{0-\infty}$ ratios (Enaril/Renitec) of enalapril were 86.3% to 126.1% and 93.0% to 118.5% and those of enalaprilat were 86.4% to 124.1% and 90.3% to 116.8%. Based on the European bioequivalence guideline, the 90% confidence interval of C_{max} and $AUC_{0-\infty}$ ratios of both parent and metabolite forms were within acceptable ranges of 70% to 143% and 80% to 125%, respectively. It was concluded that Enaril 5-mg tablet was bioequivalent to Renitec 5-mg tablet.

Bioequivalence testing for locally acting gastrointestinal products: what role for gamma scintigraphy?

Wilding I. J Clin Pharmacol 2002 Nov; 42(11):1200–10. Bioequivalence testing for locally acting gastrointestinal products is a challenging issue for both the pharmaceutical industry and the global regulatory authorities. It is widely accepted that for medicinal products not intended to be delivered into the systemic circulation, pharmacokinetic bioavailability cannot be used. However, it is becoming increasingly accepted that local availability may be assessed, where appropriate, by approaches that qualitatively reflect the presence of the active substance at the site of action. These methods must be specifically chosen for that combination of active substance and route of drug delivery. This paper argues for the use of gamma scintigraphy as a validated measure of local availability and bioequivalence for topically acting products administered to the gastrointestinal tract by the oral and rectal route.

Biopharmaceutics classification system: the scientific basis for biowaiver extensions
Yu LX, Amidon GL, Polli JE, Zhao H, Mehta MU, Conner DP, Shah VP, Lesko LJ, Chen ML, Lee VH, Hussain AS. Pharm Res 2002 Jul; 19(7):921–5. The current BSC guidance issued by the FDA allows for biowaivers based on conservative criteria. Possible new criteria and class boundaries are proposed for additional biowaivers based on the underlying physiology of the gastrointestinal tract. The proposed changes in new class boundaries for solubility and permeability are as follows: (*i*) narrow the required solubility pH range from 1.0–7.5 to 1.0–6.8 and (*ii*) reduce the high permeability requirement from 90% to 85%. The following new criterion and potential biowaiver extension require more research: (*i*) define a new intermediate permeability class boundary and (*ii*) allow biowaivers for highly soluble and intermediately permeable drugs in IR solid oral dosage forms with no less than 85% dissolved in 15 minutes in all physiologically relevant dissolution media, provided these IR products contain only known excipients that do not affect the oral drug absorption. The following areas require more extensive research: (*i*) increase the dose volume for solubility classification to 500 mL; (*ii*) include bile salt in the solubility measurement; (*iii*) use the intrinsic dissolution method for solubility classification; (*iv*) define an intermediate solubility class for BCS Class II drugs; and (*v*) include surfactants in in vitro dissolution testing.

Comment in: Stat Med 2005 Mar 15;24(5):817–8. A practical approach for comparing means of two groups without equal variance assumption
Wang H, Chow SC. Stat Med 2002 Oct 30;21(20):3137–51. In this paper, we consider two groups of i.i.d. normally distributed random variables ($N(\mu_x, \sigma_x^2)$) and ($N(\mu_y, \sigma_y^2)$) without assuming equal variance ($\sigma_x^2 = \sigma_y^2$). We propose a simple method for constructing confidence bounds based on Howe's approximation I. Its applications in parallel clinical trial (testing H_0: $\mu(x) - \mu(y) = 0$ vs. H_1: $\mu x - \mu(y) < 0$) and parallel bioequivalence trial (testing H_0:mid R:$\mu(x) - \mu(y)$mid R: δ vs. H_1:mid R:$\mu(x) - \mu(y)$mid R: $< \delta$) are studied. Sample size calculation formulae for both cases are derived. Their performances are evaluated by simulation. Our study shows that the proposed procedure can control type I error satisfactorily compared with Cochran–Cox's and Satterthwaite's approximations while maintaining a relatively high power. The proposed approach is not only simple for constructing the confidence limit but also provides a simple and accurate formula for sample size calculation.

Effect of food on the pharmacokinetics of (−) and (+) dOTC when administered as an oral racemate
Smith PF, Forrest A, Adams JM, Ballow CH. J Clin Pharmacol 2002 Jun; 42(6):658–61. The objective of this study was to evaluate the effect of food on the pharmacokinetics of racemic dOTC, a nucleoside analog reverse transcriptase inhibitor, in adult male volunteers. Twelve healthy adult male subjects were enrolled in a randomized, open-label, single-dose crossover study. All were nonsmoking, within 15% of ideal body weight, and between 18 and 50 years of age. Subjects received single oral doses of 800 mg racemic dOTC, in random order, either fed or fasted. The meal given to fed subjects was the standard Food and Drug Administration high-fat breakfast, and all subjects completed both study periods. Sixteen plasma samples for pharmacokinetic assessments were collected for 72 hours following dosing and assayed for (−) and (+) dOTC concentrations. Area under the plasma concentration–time curve (AUC), maximum observed plasma concentration (C_{max}), and time to maximum concentration (t_m) were determined for each enantiomer by standard noncompartmental techniques. Statistical hypothesis testing was by Wilcoxon signed rank, and the two one-sided tests procedure was used to determine bioequivalence between the fed and fasted study periods. The only effect of coadministration of racemic dOTC with food was a delay in time-to-peak concentration (t_{max}) between 0.6 and 0.7 hours for both (−) and (+) dOTC stereoisomers ($p \leq 0.02$). Neither AUC ($p \geq 0.10$) nor C_{max} ($p \geq 0.35$) differed significantly between the fed and fasted study periods for either (−) or (+) dOTC. Both AUC and C_{max} were equivalent between the fed and fasted study periods. It was concluded that there is no clinically significant effect of a high-fat meal on the pharmacokinetics of either (−) or (+) dOTC when administered orally as a racemic mixture.

Equivalence testing of salbutamol dry powder inhalers: in vitro impaction results versus in vivo efficacy

Weda M, Zanen P, de Boer AH, Gjaltema D, Ajaoud A, Barends DM, Frijlink HW. Int J Pharm 2002 Dec 5;249(1–2):247–55. The aim of the study was to evaluate several impactors for in vitro equivalence testing of salbutamol with respect to efficacy and to define in vitro equivalence limits validated with in vivo efficacy data. The four impactors described in Supplement 2000 of the European Pharmacopoeia were used: Glass Impinger (GI), Metal Impinger (MI), Multistage Liquid Impinger (MSLI) and Andersen Cascade Impactor (ACI). Three salbutamol dry powder formulations with different fine particle doses (FPDs) were prepared and the aerodynamic particle size distribution was measured. The recovery was also determined for each impactor. The same three preparations were administered to 12 asthmatic patients in a randomized, placebo-controlled, four-way crossover study. Cumulative doses from 50 to 400 μg were given. The FEV(1) was measured at baseline and 15 minutes after each dose. The in vitro results showed large differences between the FPDs of the three preparations with all impactors, whereas only small differences were observed between the four impactors. Since the recoveries of the MI and GI were low, in vitro equivalence testing should only be performed with the MSLI or ACI. The in vivo measurements did not show significant differences in efficacy between the three active preparations, even at the most discriminatory dose of 50 μg. It is concluded that in case there are no relevant differences between delivered dose, inhalation device, and excipients, for salbutamol dry powder inhalers equivalence can be assumed when the 90% confidence interval for the FPD ratio of the test and reference products is within 0.50 to 1.20, and each of the two products has an FPD (particles <6 μm) of at least 10 μg.

Estimation of the effect of food on the disposition of oral 5-fluorouracil in combination with eniluracil

Shepard DR, Mani S, Kastrissios H, Learned-Coughlin S, Smith D, Ertel P, Magnum S, Janisch L, Fleming GF, Schilsky RL, Ratain MJ. Cancer Chemother Pharmacol 2002 May; 49(5):398–402. Epub 2002 Feb 23. AIMS: To determine the effect of food on the pharmacokinetics of 5-fluoruracil (5-FU) taken orally with eniluracil and to compare the performance of different pharmacokinetic analysis methods in the detection a potential food–drug interaction. METHODS: In a randomized, open-label, two-way crossover study, 12 patients received eniluracil (50 mg, orally) on days 1 and 2 and 5-FU (20 mg/m^2, orally) on day 2 following either a two-hour fast or 20 minutes after a standard meal. Treatments were separated by seven days. Timed blood samples were collected during the first two treatment periods and 5-FU concentrations determined by GC/MS. Data were analyzed and pharmacokinetic parameter estimates were obtained using a noncompartmental, two-stage, and population analysis methods. RESULTS: In fasted individuals, the clearance/bioavailability of 5-FU was estimated to be 5.6 L/hr. The mean absorption lag time was 0.24 hours and was followed by rapid absorption of 5-FU. Administration of 5-FU and eniluracil with food resulted in a decrease in the 5-FU absorption rate constant by 90%. As a result, the peak plasma concentration (C_{max}) of 5-FU was decreased by 21% and the time to C_{max} was increased 2.9-fold. Clearance of 5-FU, relative bioavailability, and area under the plasma concentration versus time curve (AUC) remained unchanged with coadministration of food. Similar results were obtained using all three data analysis methods. CONCLUSIONS: Administration of food with oral 5-FU and eniluracil slowed absorption of 5-FU and decreased 5-FU C_{max}, but did not affect AUC. Further investigation of the incorporation of population pharmacokinetic approaches in food effect studies is warranted.

Evaluation of the bioequivalence of tablets and capsules containing the novel anticancer agent R115777 (Zarnestra) in patients with advanced solid tumors

Crul M, de Klerk GJ, Swart M, Weiner L, Palmer PA, Bol CJ, Beijnen JH, Schellens JH. Eur J Drug Metab Pharmacokinet 2002 Jan–Mar; 27(1):61–5. R115777 (Zamestra) is a novel anticancer agent, currently undergoing phase III clinical testing. An open, crossover trial was performed in 24 patients with solid tumors to compare the bioavailability of a new tablet formulation with the standard capsule formulation. Both dosage forms were administered once daily in doses of

300 or 400 mg. Patients received R115777 as a capsule on day 1 and as a tablet on day 2, or vice versa. Blood samples were drawn up to 24 hours after drug intake and R115777 levels were measured using a validated high-performance liquid chromatography method. The following pharmacokinetic parameters were determined and compared for the two formulations: time to maximal plasma concentration (T_{max}), half-life ($t_{1/2}$), maximal plasma concentration (C_{max}), and area under the curve at 24 hours ($AUC_{24hours}$). For the latter two parameters, 90% classical confidence intervals of the ratio tablet/capsule were calculated after a log-transformation, using an analysis of variance. For $t_{1/2}$ and T_{max}, no statistically significant differences were found between tablet and capsule. The point estimates of the ratio's of the log-normalized C_{max} and $AUC_{24hours}$ were 0.94 and 0.92, respectively, and the 90% confidence intervals were 0.81 to 1.09 and 0.83 to 1.03, which is within the critical range for bioequivalence of 0.80 to 1.25. In conclusion, the established pharmacokinetic parameters demonstrate that the capsule and tablet formulations of R115777 are interchangeable.

Fluctuation in therapeutic control associated with interchange of prednisolone tablet formulations: assessment of bioequivalence by dissolution test

Konishi H, Kanemoto K, Ikuno Y, Minouchi T, Inoue T, Hodohara K, Fujiyama Y, Yamaji A; Yakugaku Zasshi. 2002 Oct; 122(10):813–7. A 47-year-old woman received combination therapy with prednisolone (PSL), danazol, cepharanthin, ascorbic acid, and cimetidine for the treatment of idiopathic thrombocytopenic purpura. The platelet count was well controlled for over one year. Then the PSL tablet formulation was altered from Tablet A to Tablet B with the same treatment regimen, but the platelet counts fell drastically thereafter. However, the platelet counts recovered by changing the PSL tablet formulation back from Tablet B to Tablet A. In vitro dissolution testing was undertaken to assess bioequivalence between Tablets A and B. PSL in Tablet B was released more slowly compared with that in Tablet A regardless of the medium pH conditions, and the difference in the release rate between the two tablet formulations increased with increasing medium pH value. The difference exceeded the allowance limit (15%) for judgment of bioequivalence under conditions above pH 4, indicating that Tablets A and B might be nonbioequivalent. The intragastric pH of the patient was probably raised due to coadministration of cimetidine. Therefore, the present results suggest that the disparity in the immunosuppressive effects between the two PSL tablet formulations was attributable to the difference in their dissolution behavior in the gastrointestinal tract. We consider that it is better to avoid interchanging PSL tablet formulations in clinical practice.

In vitro and in vivo equivalence of two oral atenolol tablet formulations

Cuadrado A, Rodriguez Gascon A, Hernandez RM, Castilla AM, de la Maza A, Lopez de Ocariz A, Calvo B, Pedraz JL. Arzneimittelforschung 2002;52(5):371–8. A randomized, crossover, open study of bioequivalence between two different atenolol (CAS 29122-68-7) tablet formulations is presented. An in vitro comparative study between the two formulations was also performed. Both products meet the United States Pharmacopeia (USP) 23 specification. The values of similarity factor (f_2) and difference factor (f_1) obtained ensure sameness or equivalence of the two dissolution curves. Twenty-four healthy volunteers (male/female) participated in the bioequivalence study. Each treatment was given as a single 100-mg tablet following an overnight fast. Atenolol concentrations in plasma were determined up to 30 hours after treatment by HPLC. The pharmacokinetic parameters $AUC_{0-\infty}$, C_{max}, and $C_{max}/AUC_{0-\infty}$ were tested for bioequivalence after logarithmic transformation of data and ratios of t_{max} were evaluated nonparametrically. The parametric analysis revealed the following test/reference ratios and their 90% confidence intervals (90% CI): 1.06 (0.99–1.13) for AUC, 1.07 (0.97–1.18) for C_{max}, and 0.99 (0.94–1.07) for $C_{max}/AUC_{0-\infty}$. The 90% CI for t_{max} was 0.91 to 1.23. All parameters showed bioequivalence between both formulations. A discrete fall in both systolic blood pressure (SBP) and diastolic blood pressure (DBP) was observed after the drug administration. The fall extent (approximately 11 mmHg in supine position) and the time course of both parameters after the drug administration were similar for both formulations. Minimal values for SBP and DBP were achieved at six hours after the drug administration for both formulations. Heart rates were also reduced after the administration of both formulations

of atenolol in a similar extent (12 b.p.m.) and following a similar time profile (i.e., maximal reductions were observed between one and three hours after the drug administration). It can be concluded that both formulations are equivalent in vitro and in vivo.

Nevirapine quantification in human plasma by high-performance liquid chromatography coupled to electrospray tandem mass spectrometry. Application to bioequivalence study

Laurito TL, Santagada V, Caliendo G, Oliveira CH, Barrientos-Astigarraga RE, De Nucci G. J Mass Spectrom 2002 Apr; 37(4):434–41. A rapid, sensitive, and specific method to quantify nevirapine in human plasma using dibenzepine as the internal standard (IS) was developed and validated. The method employed a liquid–liquid extraction. The analyte and the IS were chromatographed on a C18 analytical column (150×4.6 mm i.d. 4 μm) and analyzed by tandem mass spectrometry in the multiple reaction monitoring mode. The method had a chromatographic run time of 5.0 minutes and a linear calibration curve over the range 10 to 5000 ng/mL ($r^2 > 0.9970$). The between-run precision, based on the relative standard deviation for replicate quality controls was 1.3% (30 ng/mL), 2.8% (300 ng/mL), and 3.6% (3000 ng/mL). The between-run accuracy was 4.0%, 7.0%, and 6.2% for the above-mentioned concentrations, respectively. This method was employed in a bioequivalence study of two nevirapine tablet formulations (Nevirapina from Far-Manguinhos, Brazil, as a test formulation, and Viramune from Boehringer Ingelheim do Brasil Quimica e Farmaceutica, as a reference formulation) in 25 healthy volunteers of both sexes who received a single 200-mg dose of each formulation. The study was conducted using an open, randomized, two-period crossover design with a three-week washout interval. The 90% confidence interval (CI) of the individual ratio geometric mean for Nevirapina/Viramune was 96.4% to 104.5% for AUC_{0-last}, 91.4% to 105.1% for $AUC_{0-\infty}$, and 95.3% to 111.6% for C_{max} (AUC = area under the curve; C_{max} = peak plasma concentration). Since both 90% CI for AUC_{0-last} and $AUC_{0-\infty}$ and C_{max} were included in the 80% to 125% interval proposed by the U.S. Food and Drug Administration, Nevirapina was considered bioequivalent to Viramune according to both the rate and extent of absorption.

A note on sample size calculation for mean comparisons based on noncentral *t*-statistics

Chow SC, Shao J, Wang H. J Biopharm Stat 2002 Nov; 12(4):441–56. One-sample and two-sample *t*-tests are commonly used in analyzing data from clinical trials in comparing mean responses from two drug products. During the planning stage of a clinical study, a crucial step is the sample size calculation, i.e., the determination of the number of subjects (patients) needed to achieve a desired power (e.g., 80%) for detecting a clinically meaningful difference in the mean drug responses. Based on noncentral *t*-distributions, we derive some sample size calculation formulas for testing equality, testing therapeutic noninferiority/superiority, and testing therapeutic equivalence, under the popular one-sample design, two-sample parallel design, and two-sample crossover design. Useful tables are constructed and some examples are given for illustration.

A note on statistical methods for assessing therapeutic equivalence

Chow SC, Shao J. Control Clin Trials 2002 Oct; 23(5):515–20. The two one-sided tests procedure and the confidence interval approach are two commonly used statistical approaches for testing therapeutic equivalence or assessing bioequivalence. However, some confusion arises. For example, what is the difference between the two approaches, given the fact that in some cases the two approaches produce the same test? Should we use level 1-alpha or 1-2alpha when applying the confidence interval approach? When different confidence intervals are available, which confidence interval should be used? The purpose of this paper is to clarify this confusion. It is shown that the approach of using 1-alpha confidence intervals produces level alpha tests, but the sizes of these tests may be smaller than alpha, and that the use of 1-2alpha confidence intervals generally does not ensure that the corresponding test be of level alpha, although there are exceptional cases. The sizes of several tests obtained using different confidence intervals are also evaluated.

On statistical power for average bioequivalence testing under replicated crossover designs

Wan H, Chow SC. J Biopharm Stat 2002 Aug; 12(3):295–309. In its recent guidance on bioequivalence, the U.S. Food and Drug Administration (FDA) recommends that a two-sequence, four-period (2×4) replicated crossover design be used for assessment of population and individual bioequivalence [FDA. Guidance for Industry on Statistical Approaches to Establishing Bioequivalence; Center for Drug Evaluation and Research, Food and Drug Administration: Rockville, MD, 2001]. The recommended replicated crossover design not only allows estimates of both the intersubject and the intrasubject variabilities and the variability due to subject-by-formulation interaction, but also provides an assessment of average bioequivalence (ABE). In this article, power function for assessment of ABE under a general replicated crossover design (i.e., a 2×2 m replicated crossover design) based on the traditional analysis of variance model and the mixed effects model as suggested by the FDA are studied. It is found that the power of a 2×2 m replicated crossover design depends upon the variability due to subject-by-formulation interaction and the number of replicates. Based on the derived power function, formula for sample size calculation for assessment of ABE under a 2×2 m replicated crossover design is also provided.

Oxcarbazepine final market image tablet formulation bioequivalence study after single administration and at steady state in healthy subjects

Flesch G, Tudor D, Souppart C, D'Souza J, Hossain M. Int J Clin Pharmacol Ther 2002 Nov; 40(11):524–32. A final market image (FMI) tablet formulation of oxcarbazepine was compared with the marketed formulation [current market formulation (CMF)] and with the clinical trial formulation (CTF) tablet used during clinical efficacy and safety studies. The goal of the study was to compare the bioavailability after single doses and at steady state of the FMI versus CMF and CTF as well. Additionally, the effect of food was evaluated on the final market formulation. The study was an open-label, single-center, four-way crossover trial. Each treatment period consisted of a single dose of 600 mg OXC on day 1. From day 4 up to including day 7, 600 mg b.i.d. were administered. A final dose of 600 mg was administered in the morning on day 8. Blood samples were taken on day 1 before and on day 7 (pre-dose) and on day 8 (morning dose). Plasma concentrations of MHD (the main metabolite of OXC) were determined by using a validated HPLC assay. FMI as test formulation was compared with the CMF and CTF as reference formulations. FMI under fed conditions was also compared with FMI under fasting conditions. These comparisons were made using data following single-dose administration and steady-state conditions. Plasma AUC for single dose or $AUC_{0-12hours}$ for steady state, and plasma C_{max}, log-transformed (natural base), were used for the assessment of bioequivalence. The 90% confidence interval (CI) approach was used for testing bioequivalence. Bioequivalence was accepted if the CI was contained within the region (0.8%, 1.25%). At steady state under fed conditions, tested formulation (FMI) was bioequivalent to CTF and with the reference marketed formulation (CMF) with regard to AUC and C_{max}. After single dose under fed conditions, FMI and CTF were bioequivalent with regard to AUC and C_{max}, and FMI and CMF were equivalent with regard to AUC but not C_{max}. Food had no effect on the bioavailability of the FMI. These results clearly support the switch from the CMF to the final market image tablet in the countries where Trileptal is/was already registered.

Randomized crossover studies of the bioequivalence of two fenofibrate formulations after administration of a single oral dose in healthy volunteers

Sonet B, Vanderbist F, Streel B, Houin G. Arzneimittelforschung 2002;52(3):200–4. Bioequivalence of a newly developed semisolid formulation (Lidose) of fenofibrate (CAS 49562-28-9), and a reference, micronized formulation of fenofibrate was investigated in two randomized, open-label clinical studies with a crossover design. Both studies involved two distinct groups of 24 healthy volunteers. Doses of 67 and 200 mg, respectively, were used in studies 1 and 2. On day 1, a single oral dose was administered to all subjects, using one of the two formulations to be compared. Single oral dosing with the other formulation occurred after a washout period of at least eight days. Blood samples were taken after each dosing for measurement of plasma fenofibric acid concentrations by high-performance liquid chromatography combined with

fluorescence detection, and plasma pharmacokinetic parameters were determined. No statistically significant differences were noted for C_{max}, T_{max}, AUC_{0-t}, and AUC0-variation of between subjects treated with the new formulation and those receiving the reference formulation. Side effects were mild and not significantly different between the two fenofibrate preparations. These two studies based on validated methods demonstrate that the new and the reference fenofibrate formulations are bioequivalent when administered at the two doses studied.

Relationship between sample size and the definition of equivalence in noninferiority drug studies

Millar JA, Burke V. J Clin Pharm Ther 2002 Oct; 27(5):329–33. Statistical testing of clinical trial data leads to acceptance of a hypothesis if a test of the opposite (null) hypothesis (H_0) fails to reach a critical probability value. The usual aim is to demonstrate that a new treatment is superior to a comparator, whence H_0 is that the two treatments are the same. By contrast, in studies designed to show that a new treatment is equivalent to an existing therapy, the same principle is satisfied by an amended null hypothesis, such that the treatments differ by more than a defined amount. This reversal entails subtle but important logical and practical problems that affect particularly the calculation of sample size. The choice of the limits used to define equivalence is critical to the calculation of sample size in a manner not previously discussed, and in the interpretation of data in relation to the probability of Type I and Type II errors. Investigators, regulatory bodies, and institutional ethics committees must ensure that the range of values chosen to indicate equivalence is clinically appropriate and be aware of the effect of this decision on possible errors in accepting or rejecting H_0.

Size isn't everything

Hampton JR. Stat Med 2002 Oct 15;21(19):2807–14. Clinical trials now often involve thousands of patients, and statisticians emphasize the importance of trial size in ensuring that "correct" answers are obtained. However, when a good treatment appears for a disease that was hitherto untreatable—for example, oranges for scurvy or streptomycin for tuberculosis—only a small trial is needed. Large trials are needed only to demonstrate small effects. The meta-analysis of small trials is often misleading, and may hide undesirable effects of individual drugs. The concept of equivalence between treatments is important, and while a statistically adequate equivalence trial may have to be very large, many clinicians will question the need for extreme statistical propriety. Clinical trials often do not reflect "real-world" practice, and the clinical relevance of a trial is more important than its size.

2001

A bioavailability/bioequivalence study of two oral lansoprazole formulations after single administration to healthy volunteers

Jovanovic D, Kilibarda V, Maksimovic M. Pharmazie 2001 Oct; 56(10):800–2. Two oral lansoprazole formulations, containing encapsulated microgranules, Lasoprol (test formulation) and Lanzor (reference), were administered to 12 healthy volunteers of both sexes in a single dose of 30 mg lansoprazole to investigate their comparative bioavailability. No statistically significant differences, at the probability level of 90%, were observed neither for the maximal serum concentrations (1.12:1.22 μg/mL) nor for the area under the concentration–time curves (5.01:5.77 μg/mL hr), the parameter to which the inhibition of acid secretion induced by lansoprazole is directly related. The similar holds true for the value of time to reach the maximal concentration of lansoprazole in serum, although this parameter was previously described as less sensitive in comparative bioavailability studies. The terminal elimination half-lives were 4.56 hours for Lasoprol and 4.57 hours for the reference formulation. The results indicate the bioequivalence and good tolerability of both lansoprazole formulations. The overall pharmacokinetic profile of the drug was comparable with the data previously reported by other investigators.

Bioequivalence of different prednisolone tablet formulations

Luippold G, Benohr P, Schneider S, Marto M, Muhlbauer B. Arzneimittelforschung 2001;51(8):638–42. The relative bioavailability of different prednisolone (CAS 50-24-8) tablet formulations (Prednisolone Ferring 2, 5, and 20 mg) was investigated in comparison to a reference formulation. The study was performed in a GCP/ICH-conform manner using a randomized crossover design in 13 healthy volunteers. With respect to the pharmacokinetic parameters C_{max} (maximal prednisolone concentration), $AUC_{0-12hours}$ (area under the concentration–time curve until 12 hours after drug intake), $AUC_{0-\infty}$ (area under the concentration–time curve until infinity), and $t_{1/2}$ (elimination half-life time), 10×2 mg prednisolone tablets did not show any relevant differences as compared to the reference (1×20 mg) meaning that the 90% confidence intervals were within the given 0.80–1.25 limits for the decision of bioequivalence. Although not statistically significant, t_{max} (time to reach the maximal prednisolone plasma concentration) was 11 minutes shorter regarding the test preparation as compared to the reference. The pharmacokinetic parameters of 4×5 prednisolone tablets were also well in accordance with the reference. The most important parameters C_{max}, AUC, and $t_{1/2}$ were within the defined limits for the acceptance of bioequivalence and, in addition, t_{max} did not show any significant differences. The 20-mg prednisolone tablet formulation showed almost identical parameters of C_{max}, AUC, $t_{1/2}$, and t_{max} in comparison to the reference substance. Taken together, the results of the bioavailability parameters indicate the bioequivalence of the three prednisolone test preparations as compared to the reference.

Estimated coefficient of variation values for sample size planning in bioequivalence studies

Yuen KH, Wong JW, Yap SP, Billa N. Int J Clin Pharmacol Ther 2001 Jan; 39(1):37–40. OBJECTIVE: The aim of the present communication is to provide information regarding the intrasubject coefficient of variation obtained from 30 bioequivalence studies covering 16 drugs which can be used for estimation of sample size. Additionally, an attempt was also made to estimate the test power of each of the studies conducted. METHODS: The intrasubject coefficient of variation was estimated from the residual mean square error obtained from analysis of variance of the parameters $AUC_{0-\infty}$, C_{max} and $C_{max}/AUC_{0-\infty}$ after logarithmic transformation. The test power in the analyses of the above parameters was subsequently estimated using nomograms provided by Diletti et al. [1991]. RESULTS AND CONCLUSION: Thirty products covering 16 drugs were studied in which 22 were immediate-release (including one dispersible tablet) and 8 sustained-release formulations. The intrasubject coefficient of variation for the parameter $AUC_{0-\infty}$ was smaller than C_{max}, and hence considerably more studies were able to attain a power of $>80\%$ using 12 volunteers for the $AUC_{0-\infty}$, when compared with the C_{max}. However, the variability in the C_{max} could be reduced by using the parameter $C_{max}/AUC_{0-\infty}$, and thus, provide a more realistic estimation of sample size, since the latter reflects only the rate of absorption and not both the rate and extent as in the case of C_{max} [Endrenyi et al. 1991].

Individual bioequivalence revisited

Chen ML, Lesko LJ. Clin Pharmacokinet 2001;40(10):701–6. For decades, the establishment of bioequivalence has generally relied on the comparison of population averages between the test and reference formulations. In the early 1990s, individual bioequivalence was proposed to ensure that an individual could be switched from the reference product to the test product with unchanged efficacy and safety. Since 1997, the U.S. Food and Drug Administration (FDA) has published three guidance documents on the proposed criterion and statistical methodology for the individual bioequivalence approach. From a scientific standpoint, the individual bioequivalence criterion appears to offer several advantages for some drug products compared with the average criterion. It allows comparison of intraindividual variances, scaling the bioequivalence criterion to the reference variability and detection of an important subject-by-formulation interaction if it exists. Based on these considerations, the FDA has recently recommended replicate study designs for modified release dosage forms and highly variable drug products. The new criterion also promotes inclusion of a heterogeneous population of volunteers in bioequivalence studies. Despite all the advantages of the individual

bioequivalence approach, questions remain on the optimal use of replicate study designs and the proposed criterion for evaluation of bioequivalence between formulations. In the finalized guidance documents, therefore, the FDA maintains the average bioequivalence criterion while allowing other criteria under certain circumstances. Collection and analysis of bioequivalence data from replicate study designs may permit further assessment and resolution of these questions.

Limits of 80% to 125% for AUC and 70% to 143% for C_{max}. What is the impact on bioequivalence studies?

Hauck WW, Parekh A, Lesko LJ, Chen ML, Williams RL. Int J Clin Pharmacol Ther 2001 Aug; 39(8):350–5. OBJECTIVE: The U.S. Food and Drug Administration (FDA) currently uses bioequivalence (BE) limits for fasting BE studies that are based on the 90% confidence interval for the ratio of difference of the test and reference products C_{max} and AUC falling within 80% to 125%. The FDA has also proposed that BE limits be used similarly for AUC and C_{max} measurements from fed BE studies. In some cases, regulatory agencies have considered a wider BE limit for C_{max}, because of the typically higher variability of C_{max} compared to AUC. We investigated the consequences of changing from an 80%/125% limit for both pharmacokinetic measures to one that uses a limit of 80%/125% for AUC and 70%/143% for C_{max}. METHODS: We computed the sample sizes required for BE studies using 80%/125% for AUC and 70%/143% for C_{max} as BE limits. We also determined the range of the ratios of C_{max} and AUC values in a study that could meet the 70%/143% and 80%/125% BE limits. RESULTS: The sample size for the study, to have adequate power with 80%/125% for AUC and 70%/143% for C_{max}, will be determined primarily by the intrasubject variability of AUC, though with a substantial proportion of studies (about one-third) still determined by the variability of C_{max}. The ratio of mean C_{max} values that can pass a wider 70%/143% BE limit could easily be as high as 128%. CONCLUSION: Without further scientific or clinical rationale, we find it difficult to justify widening the bioequivalence limit for C_{max} to 70%/143% for either fasting or fed BE studies.

Non-bioequivalence of various trademarks of enrofloxacin and Baytril in cows

Sumano LH, Ocampo CL, Gutierrez OL. Dtsch Tierarztl Wochenschr 2001 Jul; 108(7):311–4. Including Baytril, in various parts of the world, many commercial preparations of enrofloxacin for parenteral administration are being employed for the treatment of bacterial diseases in cows. To optimize clinical responses and to minimize development of bacterial resistance to this agent, the copied pharmaceutical preparations must comply with some key pharmacokinetic features when bioequivalence studies are performed. To assess whether or not there was bioequivalence among nine commercial preparations of enrofloxacin and the original one, a controlled pharmacokinetic study was carried out. These were done utilizing the microbiological agar-diffusion test as quantitative/qualitative analytical method. A non-compartmental model defined kinetic variables. Results for Baytril revealed that maximal serum concentration (C_{smax}) was only matched by one preparation while area under the curve (AUC) of the serum concentration/activity of enrofloxacin and metabolites in time was not matched by any preparation. Time to C_{smax} (T_{max}), elimination half-life, and shape of the time–serum concentrations of enrofloxacin profiles obtained for the nine generic preparations differ significantly somehow from the corresponding data obtained for the reference enrofloxacin. The need for studies to demonstrate bioequivalence becomes mandatory if similar preparations of enrofloxacin become commercially available. Enrofloxacin should be used selectively and cautiously to limit development of bacterial resistance. Non-bioequivalence of relevant pharmacokinetic values, such as C_{smax} and bioavailability (AUC) would facilitate development of bacterial resistance and limit the useful life span of this antibacterial agent.

Nontraditional study designs to demonstrate average bioequivalence for highly variable drug products

Patterson SD, Zariffa NM, Montague TH, Howland K. Eur J Clin Pharmacol 2001 Nov; 57(9):663–70. OBJECTIVE: To demonstrate average bioequivalence (ABE), the 90% confidence intervals on the ratio of geometric means for area under the concentration–time

curve (AUC) and maximum observed plasma concentration (C_{max}) must lie within 0.80 to 1.25. Demonstration of ABE for highly variable drug products requires large numbers of subjects in a standard, adequately powered, two-period crossover. METHODS: Application of nontraditional study designs can help to meet this hurdle. Study design and analysis for replicate and group sequential-replicate study designs are presented and illustrated using examples. It is demonstrated how to use such approaches to meet the difficult regulatory hurdle of ABE for a highly variable drug product. RESULTS: To illustrate, data are provided from three separate ABE studies for a highly variable drug product at three dosage strengths. In all three studies, a replicate study design was used to compensate for high intrasubject variation. Additionally, for the last study, a group sequential study design was imposed to provide early evidence of conclusive results. CONCLUSION: Replicate designs and group-sequential designs in bioequivalence should be used to demonstrate ABE for highly variable drug products or when uncertain of true intrasubject variability to ensure conclusive study results.

On sample size calculation in bioequivalence trials
Chow SC, Wang H.J Pharmacokinet Pharmacodyn 2001 Apr; 28(2): 155–69. Sample size calculation plays an important role in bioequivalence trials. In practice, a bioequivalence study is usually conducted under a crossover design or a parallel design with raw data or log-transformed data. In this paper, we discuss the differences in sample size calculation between a crossover design and a parallel design with raw data or log-transformed data. Formulas for sample size calculation under a crossover design and a parallel design with raw data or log-transformed data are derived. A brief discussion for the relationship among these formulas is given.

Preliminary bioequivalence testing of two nicardipine HCl sustained-release formulations with in vitro/in vivo correlations
Sorasuchart W, Ayres JW. Eur J Drug Metab Pharmacokinet 2001 Jan–Jun; 26(1–2):1–7. A new nicardipine HCl oral sustained-release dosage form was evaluated for bioequivalence in comparison with a reference product, Cardene SR. Six healthy subjects, fasted overnight, were enrolled in a single-dose, open-label, randomized, and two-way crossover study. Blood samples were collected over a 12-hour period, and nicardipine plasma concentrations analyzed from plasma. Pharmacokinetic parameters, including C_{max}, t_{max}, and AUC, were obtained from drug plasma concentration–time curves and pharmacokinetic analysis conducted using WinNonlin. The two one-sided *t*-test was applied in statistical analysis for comparison of the pharmacokinetic parameters between the two products. There was no convincing evidence that nicardipine HCl test product and Cardene SR were bioequivalent. Amounts of nicardipine HCl release in vivo were mathematically obtained by deconvoluting plasma concentration–time data after oral administration using IV bolus injection data as a reference. Plots of percentages of drug release in vitro against those in vivo illustrated triphasic curves. After the in vitro time scale was corrected and then plotted against in vivo data, plots provided a polynomial relationship (R^2 of 0.9920 and 0.9954). The in vitro/in vivo correlation may be useful in reformulating this particular test formulation to obtain a product with an in vivo release rate identical to Cardene SR.

Regulatory perspectives on in vitro (dissolution)/in vivo (bioavailability) correlations
Uppoor VR. J Control Release 2001 May 14;72(1–3):127–32. In vitro dissolution has been extensively used as a quality control tool for solid oral dosage forms. In several cases, however, it is not known whether one can predict the in vivo performance of these products from in vitro dissolution data. In an effort to minimize unnecessary human testing, investigations of in vitro/in vivo correlations (IVIVC) between in vitro dissolution and in vivo bioavailability are increasingly becoming an integral part of extended release (ER) drug product development. This increased activity in developing IVIVCs indicates the value of IVIVCs to the pharmaceutical industry. Because of the scientific interest and the associated utility of IVIVC as a valuable tool, the U.S. Food and Drug Administration has published a Guidance in September 1997, entitled Extended Release Oral Dosage Forms: Development, Evaluation and Application of IVIVCs. A predictive IVIVC enables in vitro dissolution to serve

as a surrogate for in vivo bioequivalence testing. IVIVCs can be used in place of biostudies that may otherwise be required to demonstrate bioequivalence, when certain preapproval and postapproval changes are made in formulation, equipment, manufacturing process or in the manufacturing site. IVIVC development could lead to improved product quality (more meaningful dissolution specifications) and decreased regulatory burden (reduced biostudy requirements). This article will discuss in detail the FDA Guidance which deals with the development, evaluation methods and criteria, and applications of IVIVCs. From a regulatory point of view, the applications of IVIVC to grant biowaivers and set dissolution specifications for ER oral dosage forms will be presented. Additionally, since the principles of IVIVC are considered to be similar for non-oral dosage forms, the guidance for oral extended release products may be applied for non-oral products as well. While the principles are likely to be the same, it is an interesting challenge to look at appropriate methods for dissolution testing and for development of IVIVCs for products such as injectable depot formulations.

Relative bioavailability of diclofenac after a single administration of a new multiple-unit formulation of enteric-coated pellets

Walter K, von Nieciecki A. Arzneimittelforschung 2001;51(8):643–50. The relative bioavailability of diclofenac (CAS 15307-86-5) was investigated after a single administration of a multiple-unit formulation containing 75 mg diclofenac sodium in enteric-coated pellets (A) in comparison to an enteric-coated tablet with 50 mg diclofenac sodium (B), a capsule containing 140 mg diclofenac resinate (C), and a dispersible tablet containing 46.5 mg diclofenac acid (D). The study was carried out in a four-way crossover design in 16 healthy male volunteers. Serum concentrations of diclofenac were determined with a validated and specific HPLC-method. After dose normalization, a mean relative bioavailability of 99% (B), 142% (C), and 116% (D) was determined for the pellet formulation. According to the corresponding 90% confidence interval, bioequivalence for the extent of bioavailability of the test formulation can be concluded compared to the enteric-coated tablet. In comparison to the formulations C and D, the test formulation showed an increased extent of bioavailability. Further differences in pharmacokinetics were observed for the rate-dependent parameters. For the test formulation, the highest mean maximum serum concentration (1595 ng/mL) was measured with a corresponding t_{max} of 0.8 hours. For the reference formulations, mean peak serum concentrations of 1285 ng/mL after 2.0 hours (B), 370 ng/mL after 1.8 hours (C) and 735 ng/mL after 1.9 hours (D) were observed. Despite the enteric-coating of the pellets, a short lagtime of 0.4 hours was determined for the test formulation. For the other rapid-release formulation (D), the lagtime was of a similar magnitude (0.3 hours), while drug release and absorption from the enteric-coated tablet and the diclofenac resinate capsule were delayed (1.8 and 0.7 hours, respectively). Due to the rapid and high bioavailability of diclofenac, the multiple-unit formulation fulfills the prerequisites for the oral treatment of acute painful conditions when prompt analgesic and anti-inflammatory efficacy is desired.

Sample size determination for equivalence test using rate ratio of sensitivity and specificity in paired sample data

Lui KJ, Cumberland WG. Control Clin Trials 2001 Aug; 22(4):373–89. Before implementing a new diagnostic test, we may wish to study whether this test is noninferior to a reference test with respect to the sensitivity and/or the specificity. This paper discusses sample size determination for one-sided equivalence (or noninferiority) testing of the rate ratio using paired-sample data. Using large sample theory, this paper derives asymptotic sample size formulae for the required number of subjects giving a desired power $100(1-\beta)\%$ at a specified alpha-level. To evaluate the accuracy of these formulae, this paper considers several test statistics and uses Monte Carlo simulation to estimate the corresponding type I error and power with the given resulting sample sizes in a variety of situations. Finally, this paper notes those situations for which the asymptotic sample size formulae developed here are of limited use and suggests a simple empirical adjustment to alleviate this limitation.

United States Food and Drug Administration requirements for approval of generic drug products

Meyer MC. J Clin Psychiatry 2001;62(Suppl. 5):4–9; discussion 23–4. As generic products become more available for the treatment of psychiatric disorders, clinicians must stay abreast of

the U.S. Food and Drug Administration (FDA) requirements for the approval of generic drug products. The FDA declares that pharmaceutical equivalents only are therapeutically equivalent, and pharmacokinetic data are all that is usually required to determine therapeutic equivalence. The rationale behind the overall concept of bioequivalence is that if two pharmaceutical equivalents provide identical plasma concentration–time profiles in humans, there is no evidence to demonstrate that the two identical dosage forms will exhibit a difference in safety and efficacy. This article reviews current terminology used in abbreviated new drug applications for generic products, typical bioequivalence study designs, and FDA bioequivalence guidance for clozapine.

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Assessment of selection bias in estimates of relative bioavailability and intrasubject variability from bioequivalence evaluations

Wang Y. J Biopharm Stat 2000 Aug; 10(3):407–24. The outcome selection of bioequivalence evaluations for abbreviated new drug applications results in bias towards unity of test–reference ratio estimates, and underestimation of intrasubject variability for the test drug product. In this study, the selection bias in the estimates of test–reference ratio and intrasubject variability as function of the true test–reference ratio, intrasubject variability, and sample sizes was derived, and the relationship of the selection bias with the true test–reference ratio, intrasubject variability, and sample sizes was evaluated using the derived functions for the selection bias. It was shown in this study that the selection bias decreases with the true test–reference ratio approaching unity, with decreasing intrasubject variability, or with increasing sample sizes of bioequivalence trials. As demonstrated in this study, the selection bias can reach such high levels that it can result in misleading optimism about interchangeability between the bioinequivalent generic versions of a reference drug product, especially for highly variable drug products. As demonstrated in this study, trial repeating raises the chances for test products of low test–reference ratio to meet the bioequivalence requirements, and as such inflates value of the expected selection bias. The a priori knowledge of intrasubject variability for the reference drug product may be used to infer the intensity of outcome selection, and as such to predict selection bias in test–reference ratio estimates for the test drug product.

Bioavailability investigation of two different oral formulations of methylprednisolone

Geister U, Guserle R, Bungers E, Schaarschmidt D, Doser K. Arzneimittelforschung 2000 Mar; 50(3):286–92. Two different oral methylprednisolone (CAS 83-43-2) formulations [Methylprednisolon-ratiopharm 8 mg tables as test preparation (T) and tablets of a reference preparation (R)] were investigated in 16 healthy volunteers in order to prove bioequivalence between these preparations. A single 8 mg oral dose was given according to a randomized two-way crossover design in the fasted state. Blood samples for determination of methylprednisolone plasma concentrations were collected at predefined time points up to 16 h following drug administration. A washout period of three days separated both treatment periods. Methylprednisolone plasma concentrations were determined by means of a validated HPLC method. Values of 342.53 ng h/mL (test preparation) and 336.61 ng h/mL (reference preparation) for the parameter $AUC_{0-\infty}$ demonstrate a nearly identical extent of drug absorption. Maximum concentrations (C_{max}) of 66.58 and 70.51 ng/mL were achieved for test and reference preparation. The time to reach maximum plasma concentration (t_{max}) was 2.2 h for both preparations. C_{max} and $AUC_{0-\infty}$-values were tested parametrically by the two one-sided *t*-test procedure. Bioequivalence was concluded if the 90% confidence intervals of the T/R-ratios were in the range of 80–125% for $AUC_{0-\infty}$ and 70–143% for C_{max}. Based on the results obtained in this study, bioequivalence between methylprednisolone ratiopharm and the reference preparation was demonstrated.

The bootstrap procedure in individual bioequivalence

Shao J, Chow SC, Wang B. Stat Med 2000 Oct 30;19(20):2741–54. A bootstrap-type hypothesis test procedure for assessing individual (or population) bioequivalence between two drug

formulations is suggested in a draft guidance from the U.S. Food and Drug Administration (FDA). The purpose of this article is to study the unknown properties of this test procedure and propose some improved test procedures. We find that: the FDA's bootstrap computation is not correct; the power of the FDA's test can be very low, the use of the REML method suggested in the draft guidance does not have any advantage over the use of simpler methods such as the moment method, and the method of sample size determination in the draft guidance is inappropriate. We study the size and power of different bootstrap test procedures and suggest a method for sample size determination. It is our hope that this article will draw some attention to further research in this area, and eventually a satisfactory statistical method can be implemented for assessing individual (or population) bioequivalence.

Case studies, practical issues, and observations on population and individual bioequivalence

Zariffa NM, Patterson SD, Boyle D, Hyneck M. Stat Med 2000 Oct 30;19(20):2811–20. The FDA has proposed replacing the 1992 average bioequivalence (ABE) with population and individual bioequivalence (PBE and IBE). This has led to considerable public discussion between regulatory, academic, and industry experts. At the heart of the discussion has been the relatively modest amount of available data to examine the behavior of the PBE and IBE criteria. A retrospective analysis of 22 datasets from 15 replicate cross-over bioequivalence studies has been conducted (n = 12–74). AUC and C_{max} parameters from these studies were analyzed using ABE, PBE, and IBE methods. Out of the 22 datasets for AUC, 19 pass ABE, all pass PBE, and 20 pass IBE. Out of the three datasets that failed ABE, all passed PBE and one passed IBE. The results for C_{max} are more variable. Out of the 16 datasets where ABE is demonstrated, 1 dataset failed both PBE and IBE. Out of the six datasets that failed ABE, two passed both PBE and IBE, three passed PBE but not IBE, and one failed all three criteria. There were five datasets that passed ABE and PBE but not IBE. Additional practical issues involving the behavior of the new criteria and its expected impact on sample size for highly variable drug products will be presented. The characterization of key parameters and their interrelationships will also be discussed with particular emphasis on the subject by formulation term in the IBE criteria. It is concluded that more studies and simulations are desirable before full-scale implementation of PBE and IBE criteria.

Comparative bioavailability of two formulations of azithromycin

Gulati R, Tripathi CD, Chandra D. J Assoc Physicians India 2000 Jun; 48(6):606–8. AIM: To compare the bioequivalence of two brands of azithromycin capsules in healthy male volunteers for regulatory purpose. METHOD: A single oral dose of 500 mg of either test (Panacea Biotec Ltd) or reference (Pfizer India Ltd), preparation of azithromycin was administered to 12 volunteers in double-blind randomized crossover fashion. Serum levels of azithromycin were analyzed using microbiological assay. The pharmacokinetic parameters studied were C_{max}, T_{max}, AUC, $t_{1/2}$, K_e, CL, and MRT. In vitro dissolution tests were conducted for both the preparations and compared with in vivo absorption. RESULTS: The mean peak serum azithromycin concentration of 0.516 ± 0.008 μg/mL was observed at 2.33 ± 0.22 hours with test brand and was similar to that of reference brand with C_{max} of 0.494 ± 0.011 μg/mL at 2.71 ± 0.26 hours. The statistical difference between all the other pharmacokinetic parameters was insignificant. CONCLUSION: Both the brands of azithromycin can be considered to be bioequivalent on the basis of results obtained.

A contract research organization's response to the new FDA guidances for bioequivalence/bioavailability studies for orally administered drug products

Kimanani E, Stypinski D, Curtis G, Stiles M, Heessels P, Logan S, Nelson K, St Germain E, Boswell G. J Clin Pharmacol 2000 Oct; 40(10):1102–8. The new FDA Guidance for Industry BA and BE Studies for Orally Administered Drug Products—General Considerations and Average, Population, and Individual Approaches to Establishing Bioequivalence imply significant changes in the areas of enrollment, cost, ethics, time, entry, validation applications (EVAs), and statistical and pharmacokinetic methods. The changes from three-period to two-period

design for food effect studies, the elimination of most steady state studies, and the analyses of only the active moiety or ingredient are welcome. However, if the current guidances are adopted, additional time will be needed for participants, and more participants will be needed, resulting in higher costs to drug developers. The PK parameters needed to assess BE and the need for replicate designs for drugs with long $t_{1/2}$ are still unclear. Finally, the advantages of the aggregate property of the FDA metric versus the disaggregate criteria are challenged, and four bioequivalence criteria are proposed.

Definition of individual bioequivalence: occasion-to-occasion versus mean switchability
Kimanani EK. Stat Med 2000 Oct 30;19(20):2797–810. Two moment-based scaled definitions of individual bioequivalence are discussed. Based on a mixed effects linear model, their evaluations respectively lead to an unweighted [θ(11)] and a parametric [theta(15)] metric. The two metrics are estimated with respect to study design and two estimation methods. Results show that the two IBE metrics perform equivalently in the fully replicated design. In the semi-replicated design, the definition of theta(11) may not be valid while the evaluation of theta(15) results in a reduction of the weights in the mean difference and switchability components of the metric. Percentage rejection rates in the latter design indicate that theta(11) is more conservative than theta(15). This is because there is an increase of about 15% in the producer risk in theta(11) relative to theta(15) when compared with a 7% increase in the consumer risk in theta(15) relative to theta(11). A further disadvantage of the design is that there is a 33% loss in the subject-by-treatment variance efficiency which is reflected in a similar amount of decreased sensitivity to departures from perfect bioequivalence even when more subjects are used to equalize the number of exposure occasions in the two designs. It is concluded that a mean switchability criterion may be more appropriate from an interpretability perspective, the bootstrap resampling method used to evaluate individual bioequivalence based on theta(11) may need to be bias-corrected and that the semi-replicated design should be used cautiously.

Demonstration of in vivo bioequivalence of a generic albuterol metered-dose inhaler to Ventolin
Stewart BA, Ahrens RC, Carrier S, Frosolono M, Lux C, Han SH, Milavetz G. Chest 2000 Mar; 117(3):714–21. STUDY OBJECTIVE: To use histamine bronchoprovocation and bioassay statistical procedures to evaluate the in vivo bioequivalence of a generic albuterol metered-dose inhaler (MDI). DESIGN: A randomized, double-blind, balanced, crossover design was used to determine the potency of each generic albuterol MDI actuation relative to Ventolin (Glaxo Wellcome; Research Triangle Park, NC) administration. One treatment was administered on each of four study days. A histamine bronchoprovocation procedure was initiated 1.25 h before and 15 minutes after administration of the study treatment. PATIENTS: Twenty-four nonsmoking subjects with mild-to-moderate asthma were studied (18–65 years of age; FEV(1), $>60\%$ of predicted; and provocative concentration of histamine causing a 20% fall in FEV(1) [PC(20)], $<$ or $=8$ mg/mL at screening). INTERVENTIONS: One and four actuations (90 and 360 μg, respectively) of the generic MDI and of Ventolin MDI. Placebo inhalers were used to maintain blinding of inhaler and doses. MEASUREMENTS AND RESULTS: The primary outcome variable was histamine PC(20) measured after study treatment administration. A significant dose–effect relationship was present ($p<0.0001$). Deviation from parallelism of the generic and Ventolin dose–response curves ($p=0.95$) and differences in overall mean response between the two formulations ($p=0.68$) were not significant. Using Finney 2×2 bioassay statistical procedures, we estimated that one actuation of the generic albuterol MDI was equivalent to 1.01 puffs of Ventolin (90% confidence interval, 0.69–1.50). CONCLUSION: The generic albuterol MDI delivers a quantity of albuterol to the β (2)-receptor site in the lung that is the bioequivalent to Ventolin. Further, this study reinforces the validity of this statistical methodology for determining in vivo bioequivalence.

Dissolution testing as a prognostic tool for oral drug absorption: dissolution behavior of glibenclamide
Lobenberg R, Kramer J, Shah VP, Amidon GL, Dressman JB. Pharm Res 2000 Apr; 17(4):439–44. PURPOSE: The dissolution behavior of two commercially available glibenclamide

formulations was tested in various media. The aim of the study was to investigate whether the use of biorelevant dissolution media (BDM) would be advantageous over the use of standard media for predicting the in vivo performance of the two formulations. METHODS: The dissolution tests were performed using USP 23 apparatus 2. Conventional buffers and USP media were compared with two BDM containing different amounts of lecithin and sodium taurocholate. RESULTS: The dissolution of two drug powders was highly dependent on wetting, particle size, pH, and the composition of the medium used. In addition, the dissolution behavior of the two glibenclamide formulations showed differences in all media tested. The dissolution results of the two formulations were compared with those from an in vivo bioequivalence study undertaken by the central quality control laboratory of the German pharmacists (ZL). The bioequivalence criterion set by the ZL requires more than 80% drug release within 10 minutes. Results in FaSSIF, one of the BDMs, met the ZL criterion and this medium was also able to discriminate between the two formulations. This was not the case for the other media tested. CONCLUSIONS: The study indicates that BDM are better able to discriminate between glibenclamide formulations than standard dissolution media.

Evaluation of rifampicin bioequivalence in fixed-dose combinations using the WHO/IUATLD recommended protocol

Panchagnula R, Agrawal S, Kaur KJ, Singh I, Kaul CL. Int J Tuberc Lung Dis 2000 Dec; 4(12):1169–72. For an accurate assessment of rifampicin bioequivalence from fixed-dose combinations (FDCs), and to reduce the time and cost constraints associated with bioequivalence studies, the World Health Organization and the International Union Against Tuberculosis and Lung Disease have developed a simplified screening protocol. This study was undertaken with the objective of testing the applicability of this protocol for all types of FDCs. Data were obtained for volunteers common to three studies, and pharmacokinetic parameters were evaluated by different statistical tests. From the results, it has been demonstrated that the simplified screening protocol is suitable for evaluating the bioequivalence of rifampicin in all the types of FDCs available in the market.

The existence of sequence effect in crossover bioequivalence trials

Zintzaras E. Eur J Drug Metab Pharmacokinet 2000 Jul–Dec; 25(3–4):241–4. A generic drug product (T) to be approved for marketing authorization a bioequivalence trial is required. In the trial the generic product is compared to the innovator product (R) in terms of the pharmacokinetic parameters AUC and C_{max}. The regulatory requirement for bioequivalence is that the 90% confidence intervals for the ratio (T/R) of the generic to innovator product pharmacokinetic parameter averages lies within the limits (80%, 125%). The design of the trial is usually a two-period crossover. This design has the limitation that if the statistical analysis reveals significant sequence effect then the bioequivalence results may be biased and their interpretation is difficult. The sequence effect is confounding with the unequal residual effect and with the formulation by period interaction. Since the existence of the sequence effect questions the quality of the trial, the applicant should provide possible explanations and information on the subjects, the trial conditions, the clinical settings, and the assay methodology. An additional statistical analysis on the data from the first period of the trial may support the bioequivalence. If it is proven that the sequence effect is a true effect then the generic may be approved for marketing authorization.

Generic drugs

Lemye R. Rev Med Brux. 2000 Sep; 21(4):A273–5. When the term of the patent is completed, medicinal products may be copied. Generic products only refer to "essentially similar specialities". It means that bioequivalence must be established. Generic medicines are cheaper than branded products involving a major economical issue. In Belgium, the share of generic medicines is very small if compared with other European countries. It must be pointed out that up to now the saving is not very attractive but this can be changed in a near future. Moreover, the bioequivalence is still in doubt. Substitution is certainly not the good way of solving the problem. A better communication and relationship between doctors and pharmacists and their common research of the most appropriate prescription should be a successful

approach. It should increase the patient's compliance. Actually, the lack of compliance is according to all observers a major issue. Involving both doctors and pharmacists should be the best way of improving quality of care for patient's benefit.

Guidances related to bioavailability and bioequivalence: European industry perspective
Ahr G, Voith B, Kuhlmann J. Eur J Drug Metab Pharmacokinet 2000 Jan–Mar; 25(1):25–7. The investigations of bioavailability and bioequivalence can be classified according to three separate areas of information. Firstly, estimation of bioavailability judged on a drug substance's in vivo characteristics taking into account solubility, polymorphism, stability (especially under the conditions of the GI tract), gut wall permeability, and first pass metabolism. Secondly, evaluation of formulation properties including dissolution profile in the GI tract and its contribution to exposure variability with respect to the desired absorption characteristics. Finally, maintaining quality during the market phase with respect to equivalence to the clinical trial formulations. While in the first two areas, the range of the estimated mean values and the intra- and inter-subject variabilities contain the desired information for proper medical decisions, in the third area the mean values and their confidence limits describe the quality with regard to the formulations of proven efficacy. Guidelines should clearly distinguish between the different areas in their recommendations regarding the intended information, e.g. mean values and/or ranges and confidence intervals. New approaches of granting limited waivers for BE studies [e.g. Biopharmaceutical Classification System (BCS)] should be expanded to consideration of pharmacokinetic properties of drugs (e.g. gastrointestinal metabolism, evidence for an absorption window, magnitude of first-pass effect, half-life) as already partly implemented in the German waiver concept, and further (scientifically) validated to achieve world-wide harmonization (e.g. via ICH).

An individual bioequivalence criterion: regulatory considerations
Chen ML, Patnaik R, Hauck WW, Schuirmann DJ, Hyslop T, Williams R. Stat Med 2000 Oct 30;19(20):2821–42. Over the years, concerns have been raised regarding the appropriateness of using the average bioequivalence approach for evaluation of comparability between formulations. In lieu of average bioequivalence, scientists from academia, industry, and regulatory agencies have spent considerable effort and time in exploring the concepts of population and individual bioequivalence, and developing the statistical methods to assess the bioavailability metrics using these approaches. Recently, the Food and Drug Administration (FDA) has published a preliminary draft guidance entitled "In vivo bioequivalence studies based on population and individual bioequivalence approaches". The concept of prescribability and switchability underscores the difference between the population and individual bioequivalence approaches. The most important consideration for individual bioequivalence, the focus of this paper, rests on the assurance that products deemed bioequivalent can be used interchangeably in the target population (switchability). In addition to the comparison of averages, the individual bioequivalence approach compares within-subject variabilities and assesses subject-by-formulation interaction. The proposed criterion represents substantial departure from the current practice and thus has resulted in extensive public discussion. In contrast to the current average bioequivalence procedure, the proposed individual bioequivalence approach offers flexible equivalence criteria based on the individual therapeutic window and variability of the reference drug product. The proposed criterion rewards manufacture of less variable drug products, allows scaling criteria for highly variable/narrow therapeutic range drugs, and promotes the use of subjects from the general population in bioequivalence studies. The FDA is currently considering various approaches for resolution of issues raised from the public debate on the subject-by-formulation interaction term, statistical methods, and resource implications.

A modified large sample approach in the assessment of population bioequivalence
Quiroz J, Ting N, Wei GC, Burdick RK. J Biopharm Stat 2000 Nov; 10(4):527–44. The U.S. Food and Drug Administration (FDA) requires pharmaceutical companies to show bioequivalence between different formulations or generic companies to show bioequivalence between generic drugs and brand drugs before approval. In a recent FDA guidance on bioequivalence, new

criteria were proposed for assessment of population and individual bioequivalence. In this article, computer simulation is used to compare a modified large sample (MLS) upper bound for the population bioequivalence ratio with the bootstrap upper bound recommended by the FDA. The comparison criteria are the ability to maintain the stated confidence level and the estimated power of tests based on these bounds.

Numerical methods for the evaluation of individual bioequivalence criteria
Kimanani EK, Lavigne J, Potvin D. Stat Med 2000 Oct 30;19(20):2775–95. The evaluation of individual bioequivalence (IBE) by bootstrap resampling using common statistical software, for example SAS, is extremely time consuming. In this article, an estimation procedure that can be implemented in a high level language with the same degree of accuracy as SAS is described. The necessary parameter estimating equations under both least square (LSE) and restricted maximum likelihood (REML) methods are given. The algorithms used to numerically compute these values are outlined and tested, in FORTRAN, on several simulated datasets and shown to reproduce SAS results with at least 10(−3) precision. More importantly, the REML bootstrap algorithm reduces the time taken in SAS by a factor of 20. Secondary results indicate that LSE and REML parameter estimates are similar for mild unbalancedness. PROC MIXED, with unstructured (UN) and compound symmetry heterogeneous (CSH) variance structures give the same results except when the subject-by-treatment interaction variance, σ (2)(*D*), is 0 in which case CSH significantly overestimates sigma(2)(D) and underestimates the within-treatment variances. It is concluded that bootstrap evaluation of IBE is efficiently done using either the LSE or REML algorithm in FORTRAN.

On a reasonable disaggregate criterion of population bioequivalence admitting of resampling-free testing procedures
Wellek S. Stat Med 2000 Oct 30;19(20):2755–67. The aggregate criterion of population bioequivalence (PBE) recommended in the new FDA guidance for in vivo bioavailability studies is criticized for using the same distributional parameter as a reference for scaling the squared distance between the averages and the difference in variability. In order to circumvent this and other difficulties inherent in the approach to PBE having been recommended thus far, we introduce a disaggregate criterion instead which requires of population bioequivalent drug formulations that both the difference in means scaled by the pooled intrasubject standard deviation be sufficiently small in absolute value, and the total variability be not substantially increased under the test as compared with the reference formulation. For testing the first of the statistical hypotheses associated with this combined criterion, an exact optimal procedure based on the ordinary two-sample *t*-statistic is presented. As a test for equivalence with respect to total variability, a modification of Liu and Chow's one-sided test procedure for the assessment of intrasubject variability is recommended. Both subtests are combined by means of the intersection–union principle explained here in general terms, to form an overall test for disaggregate PBE maintaining any specified significance level. The power of the combined procedure is investigated by means of Monte Carlo simulation.

Oral bioavailability of phenobarbital: a comparison of a solution in Myvacet 9-08, a suspension, and a tablet
Yska JP, Essink GW, Bosch FH, Lankhaar G, van Sorge AA. Pharm World Sci 2000 Apr; 22(2):67–71. PURPOSE: A three-way crossover study with seven healthy male volunteers was conducted to determine the relative bioavailability of phenobarbital after single dose administration of 100 mg of phenobarbital as oral solution in Myvacet 9-08, and as a suspension, compared with a 100 mg phenobarbital tablet. MATERIALS AND METHODS: At 4-week intervals, each subject received the solution in Myvacet 9-08, the suspension and the tablet in randomized order. Blood samples were collected for 48 h after each dose for analysis of phenobarbital. From the individual serum concentration-versus-time curves C_{max} and T_{max} were determined and $AUC_{0–48}$ was calculated. RESULTS: All three oral dosage forms of phenobarbital are bioequivalent. No significant differences in T_{max} were observed. CONCLUSION: The oral solution in Myvacet 9-08, and the suspension of phenobarbital proved to be bioequivalent to a tablet.

Pharmacokinetics and bioequivalence testing of generic fluconazole preparations in healthy Thai volunteers

Manorot M, Rojanasthien N, Kumsorn B, Teekachunhatean S. Int J Clin Pharmacol Ther 2000 Jul; 38(7):355–9. AIM: To determine the bioequivalence of two oral formulations of generic fluconazole in 12 healthy Thai volunteers. SUBJECTS, MATERIALS, AND METHODS: The test preparation was Flucozole (Siam Bheasach, Thailand) and the reference was Diflucan (Pfizer Inc.). The two products were administered as 200 mg single oral doses in a two-period crossover design with a 2-week washout period. After drug administration, serial blood samples were collected over a period of 72 hours. Serum fluconazole concentrations were determined by HPLC, and the pharmacokinetic parameters were analyzed by non-compartmental analysis. RESULTS: The time to reach the maximal concentration (T_{max}, hour) of Flucozole (1.18 ± 0.56) was statistically faster than that of Diflulan (1.59 ± 0.54). The 90% confidence intervals of the $AUC_{0-\infty}$ ratio and the C_{max}, ratio μ_T/μ_R for Flucozole/Diflucan were 0.97–1.20 and 1.01–1.26, respectively. These values were within the acceptable bioequivalence intervals of 0.80–1.25 and 0.7–1.43 for the ratio of the average $AUC_{0-\infty}$ and C_{max}, respectively. CONCLUSION: Thus, our study demonstrated the bioequivalence of Flucozole and Diflucan with respect to the rate (C_{max}) and extent of absorption ($AUC_{0-\infty}$).

A practical approach for evaluating population and individual bioequivalence

Gould AL. Stat Med 2000 Oct 30;19(20):2721–40. Pharmacokinetic measurements provided by subjects to each of the two formulations of a drug have a joint distribution that can be characterized by parameters reflecting scale and correlation as well as location. The bioavailability of the formulations can be expressed in terms of the means of the marginal distributions, their means and variances, or the marginal means and variances and the joint correlation. These expressions correspond, respectively, to "average", "population", and "individual" bioequivalence when the joint distribution of the measurements is bivariate normal. Current proposals for assessing the degree of bioequivalence of two formulations are based on statistics that are composites of variance components and squares of expected mean differences from a mixed linear model. There are technical and practical issues associated with these proposals, particularly that they require more complicated designs than the familiar 2×2 crossover. This paper describes an alternative approach that can be applied with standard 2×2 crossover designs, and that provides evaluations of population and individual bioequivalence that should be adequate for all practical clinical purposes. The approach is based on easily computed correlation and regression coefficients whose statistical properties under normality are well known and for which non-parametric and robust alternatives exist when normality cannot be assumed. The approach yields conclusions consistent with those obtained by the current proposals when applied to datasets supplied by the FDA. In the cases where the conclusions do not match, the new approach appears to be more consistent with the data.

Recommendations for bioequivalence testing of cyclosporine generics revisited

Christians U, First MR, Benet LZ. Ther Drug Monit 2000 Jun; 22(3):330–45. The immunosuppressant cyclosporine is generally considered a critical-dose drug. The validity of standard criteria to establish bioequivalence between cyclosporine formulations has recently been challenged. Recommendations included establishment of individual bioequivalence rather than average bioequivalence, establishment of bioequivalence in transplant patients and in subgroups known to be poor absorbers, as well as long-term efficacy and safety studies in transplant patients. However, at the moment, individual bioequivalence is a theoretical concept, the practical benefits of which have not statistically been proven. The proposed patient pharmacodynamic studies can be expected to require an unrealistically high number of subjects to achieve sufficient statistical power. It is well established that the common practice of blood-concentration-guided dosing of cyclosporine efficiently compensates for interindividual and intraindividual variability and allows for safely switching cyclosporine formulations as bioinequivalent as Sandimmune and Neoral. Recent studies comparing the generic cyclosporine formulation SangCya with Neoral, including individual bioequivalence, bioequivalence in transplant patients, and long-term safety after switching from Sandimmune to SangCya, confirmed that it was valid to conclude bioequivalence of

both cyclosporine formulations based on standard average bioequivalence criteria. Present FDA guidelines for approving bioequivalence can be considered adequate and sufficient for generic cyclosporine formulations.

Relative bioavailability of salmon calcitonin given intramuscularly
Chen P, Lai JM, Deng JF, Lu SB, Ku H. Zhonghua Yi Xue Za Zhi (Taipei). 2000 Aug; 63(8):619–27. BACKGROUND: Salmon calcitonin, a polypeptide hormone, is used in the treatment of osteoporosis, hypercalcemia, and Paget's disease. The purpose of this study was to evaluate the pharmacokinetics and relative bioavailability of two salmon calcitonin products, Miacalcic (Novartis Pharmaceuticals, Basle, Switzerland) and Calcinin (Purzer Pharmaceuticals, Taipei, Taiwan). METHODS: This was a randomized, single-dose, crossover study conducted under fasting conditions with a washout period of one week between doses. Ten healthy male subjects were enrolled in this study. Each subject received a 100 IU dose (20 μg; 50 IU/ampule×2) of salmon calcitonin intramuscularly (i.m.) followed by collection of blood samples at specified time intervals. Serum salmon calcitonin concentrations were measured using a validated radioimmunoassay method with a detection limit of 15.0 pg/mL. Values for the area under the serum concentration from time zero to last time and infinity curve (AUC_{0-t} and $AUC_{0-\infty}$), peak concentration (C_{max}), time to peak concentration, terminal first order rate constant, terminal half-life, mean residence time, total clearance divided by absolute bioavailability, onset time, maximal effect, and duration were compared for each product. RESULTS: The 90% confidence intervals for AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} after logarithmic transformation were 93.2% to 113.1%, 97.2% to 114.9% and 84.9% to 108.0%, respectively. CONCLUSIONS: Based on the two one-sided tests procedure, we conclude that Miacalcic and Calcinin are bioequivalent.

Sensitivity of empirical metrics of rate of absorption in bioequivalence studies
Ring A, Tothfalusi L, Endrenyi L, Weiss M. Pharm Res 2000 May; 17(5):583–8. PURPOSE: The sensitivity and effectiveness of indirect metrics proposed for the assessment of comparative absorption rates in bioequivalence studies [C_{max}, T_{max}, partial AUC (AUC_p), feathered slope (SL_f), intercept metric (I)] were originally tested by assuming first-order absorption. The present study re-evaluates their sensitivity performances using the more realistic inverse Gaussian (IG) model characterizing the input process for oral drug administration. METHODS: Simulations were performed for both the first-order and the exponential model (EX) which is determined by only one parameter, the mean absorption time [$MAT = 1/k(a)$], and the IG model, which additionally contains a shape parameter, the relative dispersion of absorption time distribution (CV2A). Kinetic sensitivities (KS) of the indirect metrics were evaluated from bioequivalence trials (error free data) generated with various ratios of the true parameters (MAT and CV2A) of the two formulations. RESULTS: The behavior of the metrics was similar with respect to changes in MAT ratios with both models: KS was low with C_{max}, moderate with SL_f and AUC_p, and high with I and T_{max} following correction for apparent lag time (T_{lag}). Changes of the shape parameter CV2A, however, were not detectable by C_{max}, T_{max}, SL_f, and AUC_p. Changes in both MAT and CV2A were well reflected by I with CV2A-ratio >1. I exhibited approximately full KS also with CV2A-ratio <1 when a correction was first applied for the apparent lag time. CONCLUSIONS: The time profile of absorption rates is insufficiently characterized by only one parameter (MAT). Indirect metrics which are sensitive enough to detect changes in the scale and shape of the input profile could be useful for bioequivalence testing. Among the tested measures, I is particularly promising when a correction is applied for T_{lag}.

Some statistical considerations on the FDA draft guidance for individual bioequivalence
Hsuan FC. Stat Med 2000 Oct 30;19(20):2879–84. In December 1997, the FDA proposed a draft guidance for future in vivo bioequivalence studies. The guidance suggested specific criteria for new drug sponsors to show individual bioequivalence (IBE). The criteria use a mixed-scaling aggregate strategy. It has been generally accepted that, under some particular situations, the proposed criteria would result in a relaxation of the current bioequivalence standard set by the average bioequivalence (ABE) criterion. Here we study the magnitude of this relaxation under three scenarios: when the conditions for an ABE investigation are met, when the drugs are highly variable, and when the experiments are poorly conducted.

The magnitude of relaxation we report here may be surprisingly large to many. For example, when a drug is highly variable (with the intrasubject coefficient of variation reaching 40%), the allowable limit for the ratio of the formulation means could reach 55–180% in an IBE investigation. In comparison, the usual allowable limit in an ABE investigation is 80–125%. Our investigation raises doubts on whether the implied standard of the new proposed IBE criteria would adequately ensure switchability in highly variable drugs.

The U.S. draft guidance regarding population and individual bioequivalence approaches: comments by a research-based pharmaceutical company
Hauschke D, Steinijans VW. Stat Med 2000 Oct 30;19(20):2769–74. Generally, the motivation for switching from average bioequivalence to population and/or individual bioequivalence is well recognized in the light of certain limitations of the concept of average bioequivalence. However, this switch still results in unresolved issues which should be addressed before the regulatory guidance is finalized.

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Are the current bioequivalence standards sufficient for the acceptance of narrow therapeutic index drugs? Utilization of a computer simulated warfarin bioequivalence model
Walker SE, Friesen MH. J Pharm Pharm Sci 1999 Jan–Apr; 2(1):15–22. PURPOSE. The purpose of this computer simulation was to determine the likelihood of two bioequivalent (vs. reference) generic warfarin formulations (with varying bioavailability) passing current bioequivalence criteria against each other at varying bioavailability. METHODS: A bioequivalence simulation program generated 100 warfarin bioequivalence (BE) studies with 24 patients/study. The reference formulation (R) was assigned a bioavailability of 90%. In these simulations, the first generic [G(1)] had a bioavailability that was incrementally decreased from 90%. The second generic [G(2)] had a bioavailability that was incrementally increased from 90%. The bioequivalence testing was performed initially as G(1) versus R, then G(2) versus R, and finally G(2) versus G(1). The tests were performed according to current criteria for therapeutic index drugs. RESULTS: Five thousand four hundred BE studies with a total of 129,600 subjects and 2,462,400 sampling times were simulated. When G(1) versus R was compared, fewer than 80% of studies passed when the relative AUC_{0-t} ratios were 88% or less. When G(2) versus R were compared, fewer than 80% of studies passed when the relative AUC_{0-t} ratios were 113% or greater. When Generic 2 and Generic 1 were compared, fewer than 80% of studies passed when the relative AUC_{0-t} ratios deviated from the reference by 7% or more. DISCUSSION: Despite limitations, this simulation indicates that two bioequivalent (vs. reference) generic warfarin products may not be bioequivalent to each other. Alternative methods of assessing bioequivalence are needed when more than one generic of narrow therapeutic index drug exists in the market.

Bioavailability of carbamazepine from four different products and the occurrence of side effects
Olling M, Mensinga TT, Barends DM, Groen C, Lake OA, Meulenbelt J. Biopharm Drug Dispos 1999 Jan; 20(1):19–28. The relative bioavailability of four different carbamazepine products, showing large differences in in vitro dissolution profiles, was studied in healthy volunteers to correlate the occurrence of side effects with a measure of the rate of absorption in vivo for bioequivalence testing. Two out of the three generic products investigated showed bioequivalence with respect to the extent of absorption with Tegretol. In vivo, the differences found in absorption rate were reflected in the occurrence of side effects, especially dizziness. As a measure for the rate of absorption, the partial AUC did not seem to be a good characteristic to test bioequivalence, as the variability is very high and dependent on the AUC taken. The C_{max}/AUC_{part} seems more promising, especially the partial AUC directly after completion of the absorption process. The variability is low in the case of carbamazepine after a single dose. However, as long as no consensus on the use of other metrics and the objective (clinical or quality control aspects) of bioequivalence testing is reached, and no other pharmacokinetic

characteristic is validated, C_{max} should be the characteristic of choice for the rate of absorption in single-dose studies with carbamazepine products.

Bioequivalence assessment of three different estradiol formulations in postmenopausal women in an open, randomized, single-dose, 3-way cross-over study

Timmer CJ, Geurts TB. Eur J Drug Metab Pharmacokinet 1999 Jan–Mar; 24(1):47–53. OBJECTIVE: The aim of the study was to assess the bioavailability of estradiol (E2) following oral, single-dose administration of equimolar doses of three HRT preparations in a three-way crossover study in postmenopausal women. METHODS: Eighteen healthy subjects were enrolled. Free E2 and estrone (E1) serum concentrations were determined using commercially available immunoassay kits. Bioequivalence testing was performed between the following oral formulations: (*i*) 1.5 mg E2 tablets versus 2 mg E2V tablets and (*ii*) 1.5 mg E2 plus 0.15 mg DSG tablets versus 1.5 mg E2 tablets. RESULTS: For both E2 and E1, the E2 tablet was bioequivalent with both the E2V and the E2/DSG tablet with respect to the rate and the extent of absorption (bioavailability). Although the mean t_{max} values of the three tablet formulations were similar, the variability was too large to prove formal bioequivalence. CONCLUSION: E2 tablets and E2/DSG tablets were bioequivalent and also bioequivalence of E2 tablets with commercially available E2V was found, which ensures a sequential HRT preparation without large variations in estrogen serum concentrations.

Bioequivalence of ivermectin formulations in pigs and cattle

Lifschitz A, Pis A, Alvarez L, Virkel G, Sanchez S, Sallovitz J, Kujanek R, Lanusse C. J Vet Pharmacol Ther 1999 Feb; 22(1):27–34. The vehicle in which endectocide compounds are formulated plays a relevant role in their absorption kinetics and resultant systemic availability. The pharmaceutical bioequivalence and comparative plasma disposition kinetics of ivermectin (IVM), following the subcutaneous administration of two injectable formulations to pigs and cattle were investigated using parallel experimental designs. Sixteen parasite-free male Duroc Jersey–Yorkshire crossbred pigs (90–110 kg) (Expt 1) and 16 parasite-free male Holstein calves (100–120 kg) (Expt 2) were divided into two groups and treated subcutaneously at either 300 (pigs) or 200 (calves) μg/kg with two different propylene glycol/glycerol formal (60:40) based IVM formulations; in both experiments pigs or calves in Group A received the test (IVM-TEST) formulation and those in Group B were treated with the reference formulation (IVM-CONTROL). Heparinized blood samples were taken from 0 hours up to either 20 (pigs) or 30 (calves) days post-treatment and plasma was extracted, derivatized and analyzed by high performance liquid chromatography (HPLC) using fluorescence detection. Early detection of IVM (12 hours) with a peak plasma concentration (C_{max}) between 33 and 39 ng/mL was observed in pigs. The drug was detected in plasma up to 20 days post-administration of either formulation, resulting in elimination half-lives between 3.47 and 3.80 days. There were no differences between the IVM-TEST and IVM-CONTROL formulations in the kinetic parameters (except t_{max}) obtained in pigs. IVM was detected in plasma between 12 hours and 30 days post-administration of both formulations under investigation in cattle. The plasma disposition kinetics of IVM in calves was similar following treatment with both formulations. C_{max} values (between 40.5 and 46.4 ng/mL) were achieved at two days post-administration of both formulations. None of the estimated kinetic parameters were statistically different between drug formulations. The injectable IVM formulations investigated were bioequivalent after their subcutaneous administration to both pigs and calves at recommended dose rates.

Bioequivalence review for drug interchangeability

Chow SC, Shao J. J Biopharm Stat 1999 Aug; 9(3):485–97. To monitor the performance of the approved generic copies of a brand-name drug, we propose some methods in assessing bioequivalence among generic copies and the brand-name drug, and among generic copies themselves, using data from several bioequivalence studies adopting the standard 2×2 crossover design without carryover effects. We propose a meta-analysis method that increases statistical power when the between-subject variability is not large. A non-meta-analysis is also considered. A numerical example of applying both methods is presented for illustration.

Bioequivalence studies: biometrical concepts of alternative designs and pooled analysis
Zintzaras E, Bouka P. Eur J Drug Metab Pharmacokinet 1999 Jul–Sep; 24(3):225–32. A bioequivalence study compares the bioavailability between a test and a reference drug product in terms of the rate and extent of drug absorption. Area under the plasma concentration–time curve (AUC) and maximum plasma concentration (C_{max}) are the pharmacokinetic parameters that serve as characteristics for the assessment of the extent and rate of absorption, respectively. The experimental design of a bioequivalence study is usually a crossover and rarely a parallel or a paired comparative. The statistical assessment of bioequivalence is based on the 90% confidence interval for the ratio of the test mean to the reference mean for AUC and C_{max} The aims of this paper are to: (*i*) investigate alternative designs to a crossover design for conducting bioequivalence studies, (*ii*) propose the statistical analysis of different designs for bioequivalence studies on the same products, and (*iii*) discuss their usefulness for the approval of new generic drug products. For this purpose, three case studies are illustrated and analyzed. The first case study concerns the investigation of the merits of a crossover design relative to a parallel group design for highly variable drugs using as an example a bioequivalence study of tamoxifen products. The second case study concerns the pooled statistical analysis of two bioequivalent studies of the same levodopa products. The analyses of the individual studies failed to meet the regulatory criteria for bioequivalence. The one study design was a paired comparative and the other one a crossover. Under some assumptions, the crossover design may be considered as a paired comparative and the data from the two studies may be analyzed together as a paired comparative design. The third case study concerns the statistical pooled analysis of two bioequivalent studies of the same clodronate products. The one study was a three-period crossover pilot study and it was used to identify the variability of the active substance. Then, this variability was used to determine the number of subjects for the main pivotal study which was a two-period crossover. The pilot study design was converted into a two-period crossover design and the data from the two studies were analyzed together as a two-period crossover design. The original data of the studies were modified accordingly.

Comparative bioequivalence study of rifampicin and isoniazid combinations in healthy volunteers
Padgaonkar KA, Revankar SN, Bhatt AD, Vaz JA, Desai ND, D'Sa S, Shah V, Gandewar K. Int J Tuberc Lung Dis 1999 Jul; 3(7):627–31. OBJECTIVE: To assess the bioavailability of rifampicin (RMP) in three brands of combination formulations of anti-tuberculosis drugs. DESIGN: A three-way double-blind, crossover bioavailability study of RMP and isoniazid (INH), consisting of a comparison of a two-drug combination of tablets of RMP and INH each separately (reference brand R) and a tablet of RMP+INH (brand N), and a capsule of RMP+INH (brand L) was carried out in 12 healthy male volunteers. Coded plasma samples were analyzed for levels of RMP as well as INH and acetylisoniazid (ACINH) by two high performance liquid chromatography (HPLC) methods. RESULTS: The mean values of RMP in brand N (C_{max} 6.49$\pm$0.52 μg/mL, T_{max} 2.33$\pm$0.18 h, AUC_{0-24h} 39.83$\pm$3.44 μg/mL h) were comparable with those obtained with brand R (C_{max} 5.22$\pm$0.59 μg/mL, T_{max} 2.50$\pm$0.12 h, AUC_{0-24h} 33.33$\pm$3.47 μg/mL h). The mean values of RMP in brand L (C_{max} 3.05$\pm$0.52 μg/mL, T_{max} 3.79$\pm$0.57 h and AUC_{0-24h} 21.78$\pm$3.67 μg/mL h) were significantly different from those in brand R. Nevertheless, all of the pharmacokinetic parameters obtained for INH and ACINH in all three brands were comparable. CONCLUSION: Using brand R as a comparison, brand N was bioequivalent and brand L was not bioequivalent.

The development of a standardized screening protocol for the in vivo assessment of rifampicin bioavailability
McIlleron H, Gabriels G, Smith PJ, Fourie PB, Ellard GA. Int J Tuberc Lung Dis 1999 Nov; 3(11 Suppl. 3):S329–35; discussion S351–2. SETTING: The prerequisite for in vivo bioavailability testing of rifampicin in fixed-dose combination (FDC) formulations is widely accepted. However, many smaller drug regulatory authorities and drug manufacturers have difficulty implementing costly and cumbersome testing procedures. OBJECTIVE: To test whether a

simplified blood sampling schedule can be used for the determination of drug bioequivalence in randomized, single dose, crossover studies of FDCs and appropriate reference formulations. METHOD: The results of three bioavailability and bioequivalence studies of different rifampicin-containing FDCs were analyzed. The relationship between the number of time points employed and precision of estimated relative bioavailability was explored. The relative bioavailabilities of the drug components in the test FDCs were calculated using maximal concentration and area under the curve estimates based on an extended blood sampling schedule of up to 15 time points over 48 hours, and a contracted sampling scheme with only six blood samples over eight hours. RESULTS: Estimates of relative bioavailability calculated using the contracted blood sampling protocol were closely similar to those derived using the extended sampling schedules. CONCLUSION: Considerable cost and convenience benefits can be gained by using the contracted sampling schedule with only a minor reduction in the precision of the estimation of relative rifampicin bioavailability.

In vitro/in vivo correlations of dissolution data of carbamazepine immediate release tablets with pharmacokinetic data obtained in healthy volunteers

Lake OA, Olling M, Barends DM. Eur J Pharm Biopharm 1999 Jul; 48(1):13–9. The aim of the study was to select a dissolution test method for carbamazepine (CBZ) immediate release tablets, giving the best in vitro/in vivo correlations (IVIVC) and to determine the potential of this method as an estimate for bioequivalence testing. Four 200 mg CBZ products which are sold on the Dutch market, covering the innovator and three generic products, were selected. They had been tested in a randomized, four-way crossover bioavailability study in healthy volunteers. Their dissolution rate behavior in vitro was investigated in two dissolution media: (*i*) 1% sodium lauryl sulphate in water (SLS), in accordance with the United States Pharmacopeia (USP) and (*ii*) 0.1 mol/L hydrochloric acid in water (HC). In the bioavailability study these products had shown no large differences in the extent of absorption ($AUC_{0-\infty}$); but large differences in absorption rate. The products now also showed large differences in dissolution rate in vitro in both dissolution media, the rank order being the same as for the absorption rate. It was concluded that the absorption rate in vivo depends on the dissolution rate in vivo. "Level C" IVIVC according to the USP were optimized by plotting percentages dissolved on selected time points (D values) or their reciprocals ($1/D$ values), against several pharmacokinetic parameters primarily related to the absorption phase and against $AUC_{0-\infty}$. In this way for each IVIVC the optimum D or $1/D$ value, was calculated. For both media, no meaningful IVIVC were obtained with $AUC_{0-\infty}$, but favorable IVIVC were obtained with the parameters primarily related to the absorption phase. In the bioavailability study indicated above, it was found that, among the pharmacokinetic characteristics primarily related to the absorption phase, C_{max} is the most promising in expressing rate of absorption in bioequivalence testing in single dose studies with CBZ immediate release tablets. Consequently, C_{max} was selected for expressing rate of absorption. The most favorable IVIVC were obtained with $D(20)$ in SLS versus C_{max}. From this IVIVC and the requirements for bioequivalence ($AUC_{0-\infty}$: 0.8–1.25 and C_{max}: 0.75–1.35; 90% confidence interval), a specification for dissolution testing in SLS was calculated as follows: "after 20 minutes, 34–99% dissolved". Owing to the fact that the rate of absorption in vivo depends on i.a. the dissolution rate in vivo, it can be concluded that with this specification bioequivalence with respect to both rate of absorption and extent of absorption is ensured. As this specification is comparable with the USP specification: "not less than 75% dissolved after one hour", it is concluded that the USP specification is suitable to ensure bioequivalence of CBZ immediate release tablets.

An individual bioequivalence approach to compare the intrasubject variability of two ciclosporin formulations, SangCya and Neoral

Canafax DM, Irish WD, Moran HB, Squiers E, Levy R, Pouletty P, First MR, Christians U. Pharmacology 1999 Aug; 59(2):78–88. A novel bioequivalence testing approach was used to determine intrasubject variability and switchability of two ciclosporin formulations, SangCya (test) and Neoral (reference). Twenty healthy volunteers were enrolled into a single-dose, randomized, open-label, four-period, two-sequence study with a crossover replicate design. Subject-by-formulation interaction variances were compared using a mixed effects linear

model. Intrasubject variability for ln $AUC_{0-\infty}$ and ln C_{max} of SangCya and Neoral were not significantly different. The 95% confidence intervals of the intrasubject variability of $AUC_{0-\infty}$ (0.94) and C_{max} (1.28) as determined using the bootstrap nonparametric percentile method ($n=2000$) were below the individual bioequivalence limit estimated at 2.25. We concluded equivalent intrasubject variability of ciclosporin pharmacokinetics and switchability between SangCya and Neoral.

Pharmacokinetics and bioequivalence testing of generic ondansetron preparations in healthy Thai male volunteers

Rojanasthien N, Manorot M, Kumsorn B. Int J Clin Pharmacol Ther 1999 Nov; 37(11):548–54. SUBJECTS, MATERIAL, AND METHODS: Pharmacokinetics and bioequivalence of oral preparations of generic ondansetron were investigated in healthy Thai males. The test preparations were Vomitron 8 and Vomitron 4, the reference was Zofran. The three products were administered as an 8 mg single oral dose, in a three-period four-sequence crossover design with one-week washout period. An intravenous 8 mg Zofran was administered on the forth visit. Plasma ondansetron concentrations were determined by HPLC and the pharmacokinetic parameters were analyzed by non-compartmental analysis. RESULTS: Following i.v. ondansetron, the mean values of its elimination half-life, its plasma clearance, and its volume of distribution were 4.5 hours, 398 ml/min, and 130 L, respectively. Its oral bioavailability averaged 67%, and the elimination half-life after oral administration was 5.6 hours. The time to reach the maximal concentration (T_{max}, hour) of Zofran (1.21 ± 0.26) was statistically faster than that of Vomitron 8 (1.33 ± 0.54) and Vomitron 4 (1.46 ± 0.50). The 90% confidence intervals of the $AUC_{0-\infty}$ and C_{max} ratios μ_T/μ_R for (Vomitron 8/Zofran) were 0.88 to 1.12 and 0. 85 to 1.08, respectively. Similarly, the 90% CI of the-$AUC_{0-\infty}$ and C_{max} ratios for (Vomitron 4/Zofran) were 0.96 to 1.17 and 1.01 to 1.19, respectively. CONCLUSION: These values were within the acceptable range of 0.80 to 1.25, thus our study demonstrated the bioequivalence of Vomitron and Zofran with respect to the rate (C_{max}) and extent of absorption ($AUC_{0-\infty}$).

A relative bioavailability study of 2 oral formulations of omeprazole after their administration in repeated doses to healthy volunteers

Richards JP, Gimeno M, Moreland TA, McEwen J. Gastroenterol Hepatol 1999 Apr; 22(4):171–5. To determine the relative bioavailability of Ulceral (study formula) with respect to Losec (reference standard formula) and establish their bioequivalence daily doses of 20 mg of omeprazole were given during five consecutive days to 24 healthy volunteers. No significant differences were observed in the area under the curve (AUC_{0-t}), a parameter directly related to the inhibition of acid secretion induced by omeprazole. The confidence interval of 90% for the difference between the two formulations for AUC_{0-t} was within the interval of acceptance (0.80–1.25). The confidence interval for the difference between the two formulations for C_{max} was also within the range of acceptance (0.70–1.43). In relation to the time for achieving (C_{max} [t_{max})], the difference between the two formulations and the confidence interval of 95% for the t_{max} was 0.75 (-0.5–1.75) hours, indicating that no significant differences were observed between the two treatments. This study confirms the bioequivalence of Ulceral with the standard reference formulation as well as the tolerability of the two formulae.

Some observations on current and possible future developments in bioequivalency testing

Rhodes CT. Drug Dev Ind Pharm 1999 Apr; 25(4):559–62. Present trends in the evolution of the design and interpretations of bioequivalency studies are reviewed. It is suggested that, although such tests are now being increasingly regarded as clinical mirrors rather than simply quality control tests for final product testing, there is still the possibility of simplifying such procedures. However, care must be exercised to ensure that changes in bioequivalency tests are introduced only after careful public discussions, which should involve both regulators and pharmaceutical scientists from academia and industry. Further, it is important that bioequivalency standards shall be internally consistent and applied in a politically neutral manner.

A study of the relative bioavailability of cysteamine hydrochloride, cysteamine bitartrate and phosphocysteamine in healthy adult male volunteers
Tenneze L, Daurat V, Tibi A, Chaumet-Riffaud P, Funck-Brentano C. Br J Clin Pharmacol 1999 Jan; 47(1):49–52. AIMS: Cysteamine, the only drug available for the treatment of cystinosis in paediatric patients, is available as the hydrochloride, the bitartrate and as sodium phosphocysteamine salts. It has been suggested that cysteamine bitartrate and phosphocysteamine are better tolerated and may have a better bioavailability than cysteamine hydrochloride. This has, however, never been demonstrated. METHODS: We compared the pharmacokinetics and tolerance of these three formulations of cysteamine in 18 healthy adult male volunteers in a double-blind, latin-square, three-period, single oral dose crossover relative bioavailability study. RESULTS: No statistical difference was found between relative bioavailabilities, $AUC_{0,\infty}$ (geometric mean and SD in μmol l(−1) hours: 169±51, 158±46, 173±49 with cysteamine hydrochloride, phosphocysteamine, and cysteamine bitartrate, respectively), C_{max} (geometric mean and SD in μmol l(−1); 66±25.5, 59±12, 63±20) and t_{max} [median and range in hours: 0.88 (0.25–2), 1.25 (0.25–2), 0.88 (0.25–2)] with each of the three forms of cysteamine tested. Bioequivalence statistics (90% confidence intervals) showed non equivalence of C_{max} of cysteamine base as the only non equivalence of pharmacokinetics between the three formulations: 90% CI for C_{max} relative ratios to cysteamine hydrochloride were [75.6–105.81] for phosphocysteamine and [74.2–124.2] for cysteamine bitartrate. The only significant adverse event was vomiting whose frequency was inversely correlated with body weight (Spearman's $r=-0.76$, $p<0.001$). The nature of the salt tested did not influence vomiting. CONCLUSIONS: While none of the three forms of cysteamine tested has a clear advantage over the others in terms of pharmacokinetics and tolerance profile, this should now however be addressed in patients treated for cystinosis during repeat administrations.

Variability of the model-independent AUC: the one sample per individual case
Jawien W. J Pharmacokinet Biopharm. 1999 Aug; 27(4):437–64. A theory is developed for estimation of a population value of AUC along with its standard deviation, in the case, when only one concentration–time (*C–t*) sample is available for each individual. This theory is based on model-independent pharmacokinetics. Integration methods are classified due to their applicability to the presented approach. The main goal of this work is to establish a statistical hypothesis-testing procedure which would make single *C–t* samples usable for bioequivalence studies. An application of the theory to a number of integration methods currently in use is analyzed in detail. A real data illustration is included.

1998

Assessment of metabolites in bioequivalence studies: should bioequivalence criteria be applied on the sum of parent compound and metabolite?
Mahmood I. Int J Clin Pharmacol Ther 1998 Oct; 36(10):540–4. OBJECTIVES: The objective of this study was to demonstrate the impact of the sum of parent compound and metabolite in bioequivalence assessment. METHODS: Four drugs with active metabolite were selected to assess bioequivalence. Bioequivalence criteria of 80–125% were applied to the parent compound, the metabolite, and the sum of parent compound and metabolite. RESULTS: The results indicated that the application of 80–125% bioequivalence criteria to the sum of parent compound and metabolite might be misleading. CONCLUSION: The 90% confidence interval should be applied separately to the parent compound and each metabolite.

Bioequivalence of a combination of levothyroxine and iodine in comparison with levothyroxine only. A controlled double-blind study of bioavailability
Forster G, Hansen C, Morsch F, al-Hakim K, Beyer J, Kahaly G. Med Klin (Munich) 1998 Jul 15;93(7):401–6. BACKGROUND: Iodine deficiency is the main cause of endemic goitre. Iodine supplementation and decrease of pituitary TSH are the therapeutical aims. In this study, bioavailability of levothyroxine combined with iodide and the same dose of levothyroxine alone were compared. PATIENTS AND METHODS: Forty-eight subjects aged 18 to 40 years were randomly assigned for six days either 150 μg levothyroxine and 150 μg iodide (group A,

$n=25$) or 150 μg levothyroxine (group B, $n=23$). Baseline TSH and thyroid hormones were measured two days before starting therapy as well as daily till day 6. TRH-test (delta TSH) and thyroid sonography were performed at days −2 and 6. RESULTS: During therapy, baseline TSH decreased markedly from 1.26 to 0.35 mU/mL (median) in group A and from 1.37 to 0.39 to 0.39 mU/mL in group B (both $p<0.001$), as well as delta TSH (A from 5.66 to 2.61 mU/mL; B from 6.3 to 2.95 mU/mL; $p<0.001$). The difference in delta TSH (day −2 vs. day 6) was negatively correlated to body surface ($r=-0.307$; $p<0.05$). TT4 levels increased in both groups (A from 7.1 to 9.1 μU/dL; B from 7.2 to 9.4 μU/dL; $p<0.005$). No significant differences were noted between both groups for thyroid-related parameters. In both groups, confidence intervals for baseline TSH and TT4 were in the expected range. CONCLUSION: In this study, similar bioavailability and bioequivalence for levothyroxine and the combination of levothyroxine with iodide were demonstrated.

Biopharmaceutical characterization of oral controlled/modified-release drug products. In vitro/in vivo correlation of roxatidine

Frick A, Moller H, Wirbitzki E. Eur J Pharm Biopharm 1998 Nov; 46(3):313–9. From the marketed drug product Roxane(R) 75 mg C/MR capsules (roxatidine controlled/modified-release capsules), an in vitro/in vivo comparison was performed to demonstrate a 1:1 correlation between in vitro and in vivo dissolution, and, furthermore, to ensure bioequivalence of the roxatidine controlled/modified-release (C/MR) capsules exhibiting dissolution profiles within the defined acceptance criteria. This 1:1 in vitro/in vivo comparison was calculated using a model independent numerical deconvolution method. The high degree of correlation is extremely rare; nevertheless, it allows to omit the testing of clinical side batches for the setting of acceptance criteria for the in vitro dissolution of roxatidine controlled/modified-release (C/MR) capsules. The 1:1 in vitro/in vivo correlation can be explained by the biopharmaceutical characteristics of the drug substance as well as the drug product, that is, pH-independent high solubility of the drug substance as well as dissolution which is independent of pH and agitation. These facts lead to a controlled/modified-release formulation. Therefore, it is important to keep in mind that in most cases in which a pH-dependent solubility/dissolution as well as permeability characteristics can be found, a 1:1 in vitro/in vivo correlation could not be expected. Copyright 1998 Elsevier Science B.V.

The comparative bioavailability of a generic and the innovator fluconazole preparations in healthy Thai volunteers

Teekachunhatean S, Rojanastein N, Manorot M, Sangdee C, Apisariyakul A, Ajayutphokin U. J Med Assoc Thai 1998 Oct; 81(10):772–8. We studied the pharmacokinetics and compared the oral bioavailability of the "generic" (Biozole, Biolab Company, Thailand) and the "innovator" (Diflucan, Pfizer Incorporation, U.S.A.) fluconazole preparations in 12 healthy Thai volunteers. A 200 mg single oral dose of each preparation was given to the subjects in a randomized double-blind two-period crossover design with two weeks washout period. Blood samples were collected just before and at 0.5, 1, 2, 2.5, 3, 4, 24, 48, 56, and 72 hours after drug administration. Serum fluconazole concentrations were determined by using high performance liquid chromatography. Individual concentration–time profiles and the pharmacokinetic parameters were analyzed by the noncompartmental pharmacokinetic method [TOPFIT, a pharmacokinetic data analysis program]. The pharmacokinetic parameters (T_{max}, C_{max}, V_d, C_l) of fluconazole in Thai healthy volunteers were comparable to those values observed in Caucasian subjects. The relative bioavailability of the generic Biozole was 102.38±9.79% of Diflucan. The means and 90% confidence intervals (90% CI) of the [Biozole/Diflucan] ratio of AUC_{0-72}, $AUC_{0-\infty}$ and C_{max} were 1.02 (0.98–1.06), 0.99 (0.95–1.03), and 1.13 (1.03–1.25), respectively. These values were well within the acceptable bioequivalence ranges of 0.8–1.25 proposed by the U.S. FDA. The means and 90% CI of T_{max} differences [Biozole–Diflucan] were −0.46 [(−1.03)–(0.12)]. This value was outside the stipulated bioequivalence range of ±0.41 h (±20% of the T_{max} of the reference formulation). Nevertheless, the T_{max} difference was not expected to be related to the differences in safety and efficacy of the drug. Hence, Biozole and Diflucan were bioequivalent with respect to the extent of absorption (AUC), and the C_{max}, and could be used interchangeably.

Comparative studies of quality and bioavailability of methotrexate in Thai patients with rheumatoid arthritis

Manorot M, Rojanasathien N, Louthrenoo W, Tonsuwannont W, Teekachunhatean S. J Med Assoc Thai 1998 Dec; 81(12):978–85. The bioavailability of the two generic methotrexate oral preparations (Emtrexate, Pharmachemie Company, Holland and Methotrexate Remedica, Remedica, Cyprus as the test preparations), were compared to the innovator (Methotrexate Lederle, Lederle, U.S.A. as the reference) in 10 patients with rheumatoid arthritis. A single 7.5 mg oral dose of each preparation was given to the subjects in a randomized, double-blind, three-period crossover design with a one week washout period. Serum methotrexate concentrations were determined by using Fluorescence Polarization Immunoassay (Abbott TDx). No significant differences in pharmacokinetic parameters (AUC, C_{max}, and T_{max}) were observed between the test and reference preparations. The mean and 90% CI of the ratio Emtrexate/Methotrexate Lederle and Methotrexate Remedica/Methotrexate Lederle of the C_{max}, AUC_{0-8}, and $AUC_{0-\alpha}$ were 0.93 (0.87–1.00), 0.9 (0.82–0.98), 0.88 (0.79–0.99) and 0.97 (0.93–1.02), 0.95 (0.90–0.99), 0.94 (0.86–1.02), respectively. These values were well within the acceptable bioequivalence range of 0.8–1.25. The mean and 90% CI of T_{max} difference between Emtrexate–Methotrexate Lederle and Methotrexate Remedica–Methotrexate Lederle also overlapped the stipulated bioequivalence range of the T_{max} differences of ± 0.25 hour. Thus, Emtrexate and Methotrexate Remedica were considered bioequivalent to the reference Methotrexate Lederle regarding the rate of absorption and the extent of absorption.

Dissolution testing as a prognostic tool for oral drug absorption: immediate release dosage forms

Dressman JB, Amidon GL, Reppas C, Shah VP. Pharm Res 1998 Jan; 15(1):11–22. Dissolution tests are used for many purposes in the pharmaceutical industry: in the development of new products, for quality control and, to assist with the determination of bioequivalence. Recent regulatory developments such as the Biopharmaceutics Classification Scheme have highlighted the importance of dissolution in the regulation of post-approval changes and introduced the possibility of substituting dissolution tests for clinical studies in some cases. Therefore, there is a need to develop dissolution tests that better predict the in vivo performance of drug products. This could be achieved if the conditions in the gastrointestinal tract were successfully reconstructed in vitro. The aims of this article are, first, to clarify under which circumstances dissolution testing can be prognostic for in vivo performance, and second, to present physiological data relevant to the design of dissolution tests, particularly with respect to the composition, volume, flow rates, and mixing patterns of the fluids in the gastrointestinal tract. Finally, brief comments are made in regard to the composition of in vitro dissolution media as well as the hydrodynamics and duration of the test.

A generic drug primer: regulatory aspects and scientific concepts

Henderson JD, White GL Jr. Mil Med 1998 Apr; 163(4):193–7. The regulatory aspects of generic drug substitution and the scientific concepts that serve as the basis for generic drug approval are discussed, with emphasis on the source of therapeutic equivalence information compiled by the Food and Drug Administration in Approved Drug Products with Therapeutic Equivalence Evaluations. The Food and Drug Administration's determination of bioequivalence for immediate-release and extended-release dosage forms is summarized, with a discussion of the underlying assumptions and current issues regarding bioequivalence testing. Medical practitioners must comply with the regulations stated in each state's Pharmacy Practice Act when allowing generic substitution and should ensure that the substituted product is therapeutically equivalent to the prescribed product.

The impact of formulation and process changes on in vitro dissolution and the bioequivalence of piroxicam capsules

Piscitelli DA, Bigora S, Propst C, Goskonda S, Schwartz P, Lesko LJ, Augsburger L, Young D. Pharm Dev Technol 1998 Nov; 3(4):443–52. The purpose of this research was to determine the effect of major compositional changes on the bioavailability of piroxicam from immediate-release formulations filled in hard gelatin capsules. The capsules were manufactured according to a $2(5-1)+$star point (resolution V) experimental design to investigate the effects of sodium

lauryl sulfate level, magnesium stearate level, lactose/microcrystalline cellulose ratio, piroxicam particle size, and lubricant blending time. Sodium lauryl sulfate level, lactose level, and piroxicam particle size were the most important main effects affecting dissolution. Lubricant level and lubricant blending time were either not significant (5% level) or were among the lowest ranking of factors affecting dissolution in standardized Pareto analysis. Three of these formulations exhibiting slow, medium, and fast dissolution were compared to a single lot of the Innovator (commercial) product in a small bioavailability study. The slow formulation did not meet the USP dissolution specification for piroxicam capsules. Compositionally, the experimental formulations represented major changes in piroxicam particle size, level of filler, and level of sodium lauryl sulfate. Sixteen healthy volunteers received each formulation (20 mg) in a four-way crossover design. The three Maryland manufactured formulations were bioequivalent with the commercial product and were also bioequivalent among themselves. The major changes incorporated into these formulations did not result in major differences in bioavailability. The dissolution profiles which discriminated between the formulations in vitro did not accurately represent the in vivo bioavailability results. The results of this study are part of the research database that supports SUPAC-IR, an FDA guidance that provides relaxed testing and filing requirements for scale-up and post-approval changes to immediate-release oral solid dosage forms.

Is one paracetamol suppository of 1000 mg bioequivalent with two suppositories of 500 mg

Narvanen T, Halsas M, Smal J, Marvola M. Eur J Drug Metab Pharmacokinet 1998 Apr–Jun; 23(2):203–6. A common belief is that one tablet or suppository containing, e.g. 100 mg of a drug can be substituted, without any changes in the therapeutic effect, with two units of the same brand containing 50 mg of the drug. In the present study, a single dose of paracetamol was administered to healthy volunteers as (*i*) two tablets of 500 mg, (*ii*) two suppositories of 500 mg, and (*iii*) one suppository of 1000 mg. There were statistically significant differences in all bioavailability parameters (t_{max}, C_{max}, and AUC) between the three treatments. The relative bioavailability of the 500 mg suppositories was 77% and that of the 1000 mg suppositories 66%. The absorption rate from suppositories was markedly lower than from the tablets. Especially low absorption rate was obtained with the suppository of 1000 mg. The two strengths, although having the same trade name, were not therefore bioequivalent.

Relative bioavailability of different oral sustained release oxprenolol tablets

Leucuta SE, Follidis M, Capalneanu R, Mocan A. Eur J Drug Metab Pharmacokinet 1998 Apr–Jun; 23(2):178–84. The bioequivalence of oral dosage forms of oxprenolol was assessed in a triple crossover study on two groups of 12 volunteers each. Single 160 mg doses of oxprenolol hydrochloride were given after an overnight fast of either oxprenolol sustained-release tablets in a megaloporous system, a hydrophil matrix and Slow-Trasicor (Ciba-Geigy) in the first group, or floating slow-release tablets administered with food or in absence of food, and rapid release Oxprenolol (Terapia, Cluj-Napoca) tablets, in the second group. Serum oxprenolol concentrations were measured by a gas chromatographic method. Pharmacokinetic parameters which describe bioavailability and general kinetic behavior of the drug were calculated from individual serum profiles. They were subjected to statistical analysis (paired Student's *t*-test, $p < 0.05$). The customary bioequivalence criterion was used: $0.8 <$ parameter ratio(tested/standard) < 1.2. Megaloporous tablets showed bioequivalence with the reference sustained release product Slow-Trasicor. Hydrophil tablets showed moderate sustained-release characteristics. Floating tablets showed significantly greater oxprenolol absorption when taken with food and were non-bioequivalent with floating tablets without food, as well as with the reference rapid release tablets, of oxprenolol. However, fasting tablets were bioequivalent to the Slow-Trasicor product, when taken with food.

Why rate of absorption inferences in single dose bioequivalence studies are often inappropriate

Basson RP, Ghosh A, Cerimele BJ, DeSante KA, Howey DC; Pharm Res. 1998 Feb; 15(2):276–9. PURPOSE: Peak drug concentration (C_{max}) measures the extremity of drug exposure and is a secondary indicator of the extent of absorption after area under the concentration time curve

(AUC). C_{max} serves as the indicator of absorption rate in bioequivalence (BE) studies in the United States (1). The use of C_{max}, not the time to C_{max} (T_{max}), as the metric to assess absorption rate causes erratic inferences in BE studies, and incorrect conclusions for some. We can improve BE efficiency (i.e., get the answer right the first time), by properly analyzing the time to C_{max} (T_{max}) instead of C_{max}. METHODS: We have previously redirected attention to T_{max} as the unconfounded absorption rate variable, instead of C_{max}, and have called for equally spaced sampling times during the suspected absorption phase to improve the performance of the rate metric (2). Equal spacing converts T_{max} easily into a count variable and we illustrated an appropriate statistical analysis for counts. This paper provides some measurement theory concepts to help judge which is the more appropriate analysis, and also provides parametric confidence limits for T_{max} treatment differences. Three separate BE studies are then analyzed by both methods. RESULTS: By focusing on the differences in conclusions, or inferences, this paper identifies three major issues with the current FDA "recommended" analysis of BE studies. First, C_{max}, a continuous variable peak-height or extent measure has usurped T_{max}'s function and performs erratically as a substitute measure for the rate of absorption. Second, T_{max}, should be analyzed as a discrete attribute, not as a continuous variable. Third, since several extent measures (AUC, C_{max}), not one, are actually being analyzed, an adjustment for multiple testing is mandatory if we are to maintain the size of the test at the desired alpha level (13), and not inadvertently use a narrower bioequivalence window than is intended. These actions all can have serious unintended consequences on inferences, including making inappropriate ones

1997

Absolute and comparative subcutaneous bioavailability of ardeparin sodium, a low molecular weight heparin

Troy S, Fruncillo R, Ozawa T, Mammen E, Holloway S, Chiang S. Thromb Haemost 1997 Aug; 78(2):871–5. Ardeparin sodium (Normiflo, Wyeth-Ayerst) is a low molecular weight heparin undergoing clinical evaluation as an antithrombotic agent. The objective of this study was to evaluate the absolute and comparative bioavailability of ardeparin following subcutaneous administration of three different formulations [two formulations of ardeparin at 10,000 anti-factor Xa (aXa) U/mL, but with different preservatives, and one at 20,000 aXa U/mL]. The study was conducted using a randomized four-period crossover design (three subcutaneous treatments and one intravenous treatment) in 24 healthy subjects, and the pharmacokinetics of ardeparin were characterized by plasma anti-factor IIa (aIIa) and aXa activities. The mean absolute bioavailability of ardeparin based on aIIa activity ranged from 62% to 64% and the mean absolute bioavailability based on aXa activity ranged from 88% to 97%. Based on bioequivalence testing criteria, the three ardeparin formulations were bioequivalent.

An adjusted two one-sided *t*-test for the assessment of bioequivalence with multiple doses

Wang W, Hsuan F, Chow SC. J Biopharm Stat 1997 Mar; 7(1):157–70. In medical practice, it has been realized that noncompliance may have an impact on the therapeutic effect of a drug therapy, regardless of race, gender, and education of patients. Therefore, it is of interest to study the impact of noncompliance on drug absorption through in vivo testing. Efron and Feldman examined dose–response relationship when noncompliance is an issue. In this paper, we study bias and variation induced by noncompliance for pharmacokinetic parameters such as the area under the curve. We use bioequivalence testing as an example to demonstrate that a false conclusion could be drawn if one ignores the effect of noncompliance. We propose a new test for the assessment of bioequivalence in multiple doses. The proposed test appears to have a substantial improvement over the usual two one-sided tests based on a simulation study.

Analysis of metabolites: a new approach to bioequivalence studies of spironolactone formulations

Vergin H, Mahr G, Metz R, Eichinger A, Nitsche V, Martens H. Int J Clin Pharmacol Ther 1997 Aug; 35(8):334–40. The aldosterone antagonist spironolactone undergoes extensive and complex biotransformation. For investigation of bioequivalence of two oral spironolactone

formulations, Spironolacton 50 Heumann and Aldactone 50, the pharmacokinetics and bioequivalence of the parent drug and two predominant active metabolites, canrenone and 7-α-thiomethylspirolactone, were determined in a two-way crossover study in 24 young healthy male volunteers after multiple oral dosing of 100 mg once daily. Plasma samples were measured by a newly developed HPLC assay and individual pharmacokinetic parameters of the three compounds were calculated by use of noncompartmental techniques. Statistical analysis was performed by ANOVA and nonparametric methods. Spironolactone was rapidly cleared from plasma. Therefore, only $C_{ss,max}$ and $t_{ss,max}$ were determined. Concerning $C_{ss,max}$ bioequivalence was found with 90% classical shortest confidence interval ranging from 80.7% to 112.4%. The intrasubject variability for $C_{ss,max}$ was determined to be 28.1%. Higher and persisting concentrations were observed for the metabolites. For canrenone, 90% classical shortest confidence intervals were calculated as 95.4 to 105.0% for $AUC_{ss,\tau}$, as 92.9 to 105.8% for $C_{ss,max}$, and as 89.1 to 106.3% for peak trough fluctuation (PTF). In the case of 7-α-thiomethylspirolactone, the values were 84.2% to 103.0% for $AUC_{ss,\tau}$, 77.0% to 98.6% for $C_{ss,max}$, and 85.0% to 100.4% for PTF. For $t_{ss,max}$ nonparametric, 90% confidence intervals were determined as 0.00 to 1.50 hours for spironolactone and canrenone and as −0.50 to 1.00 hour for 7-α-thiomethylspirolactone. The intraindividual variability was below 30% for all pharmacokinetic parameters in the case of the metabolites. Thus, bioequivalence of the test and the reference formulation can be concluded. The study suggests the inclusion of parent compound and metabolites for bioequivalence testing of spironolactone formulations. Intraindividual subject variability was clearly diminished by investigating bioequivalence under steady-state conditions.

Bioanalytic examination and pharmacokinetics of captopril. Bioequivalence studies of different captopril-containing Tensiomin preparations

Klebovich I, Benkone MS. Acta Pharm Hung 1997 Jul; 67(4):123–35. The authors of the present report reviewed the literature of various bioanalytical methods for the pharmacokinetics and metabolism of captopril and present their own results obtained in bioanalytical and pharmacokinetic studies. The authors performed a detailed comparative clinical, pharmacokinetic, and bioequivalence study, in Hungary, with three different captopril tablets, 50 mg each, namely with Tensiomin (EGIS Pharmaceuticals Ltd) as test preparation and Capoten (E.R. Squibb & Sons, Inc.) and Lopirin (Squibb Pharma GmbH) as reference preparations. Bioequivalence study of Tensiomin and Capoten preparations both containing 100 mg of captopril was carried out in the United States. Relative bioavailability and comparative pharmacokinetic parameters were determined in 24 and 25 healthy volunteers, respectively, in single-dose, randomized studies of three-way (50 mg) or two-way (100 mg) crossover design. The individual pharmacokinetic parameters determined for the test and reference preparations with different active principle content were t_{max}, C_{max}, AUC_{0-t}, $AUC_{0-\infty}$ $t_{½}$, $C_{max}/AUC_{0-\infty}$. Statistical evaluation of the results of bioequivalence studies was made using confidence interval calculation and the Test/Reference ratio (50 and 100 mg), and by Schuirmann's, Hauck-Anderson's, Westlake's, Wilcoxon's methods (50 mg), and power test (100 mg). All the above tests indicated statistical equivalence between the test and reference preparations. The test and reference preparations of different strengths had identical relative bioavailability. Accordingly, the clinical and biological equivalence of 50 and 100 mg Tensiomin tablets with 50 and 100 mg Capoten tablets and 50 mg Lopirin tablet have been demonstrated in two independent comparative bioequivalence studies.

A comment on so-called individual criteria of bioequivalence

Wellek S. J Biopharm Stat 1997 Mar; 7(1):17–21. The idea of defining bioequivalence in terms of the proportion of individuals exhibiting a drug formulation discrepancy that does not exceed some prespecified limit seems natural enough. Since the derivation of formal statistical procedures for testing the associated hypotheses is more or less straightforward, it is surprising that the approach was pursued in a systematic manner not earlier than in 1990 (by Anderson and Hauck and Wellek). In discussing the relative merits of the approach, we will stress the importance of a careful understanding of its conceptual basis. It will be argued that the most severe limitation on the usefulness of such probability-based criteria of individual

bioequivalence arises from the fact that large intraindividual formulation discrepancies provide evidence against equivalence only in definite absence of period effects.

Comparative study of bioavailability and clinical efficacy of carbamazepine in epileptic patients

Silpakit O, Amornpichetkoon M, Kaojarern S. Ann Pharmacother 1997 May; 31(5):548–52. OBJECTIVE: To compare the bioavailability of three generic brands of carbamazepine tablets with that of a proprietary brand in adult patients with epilepsy. DESIGN: A double-blind, randomized, three-phase crossover study. SETTING: A psychiatric facility. PARTICIPANTS: Eighteen patients with epilepsy who had taken carbamazepine at least five months before entering the study. MAIN OUTCOME MEASURES: Ten blood specimens from each patient were collected at steady state. Plasma concentration of carbamazepine was analyzed for pharmacokinetic parameters such as maximum plasma concentration (C_{max}), mean time to reach maximum concentration (t_{max}), and mean AUC. RESULTS: There were no statistically significant differences in these parameters among four brands of carbamazepine. However, when comparing the 90% CI of AUC of three generic brands with that of the proprietary brand, the AUC of two generic brands lay within a range of 80% to 120%. The effects of gender and each brand of carbamazepine on these pharmacokinetic parameters were also analyzed. Breakthrough seizures occurred even though the plasma concentration of carbamazepine was therapeutic. CONCLUSIONS: The bioavailability of two generic brands of carbamazepine tablets (Carmapine and Carzepine) and the proprietary brand (Tegretol) were equivalent in this sample of adult patients with epilepsy.

A comparison of the standard approach and the NONMEM approach in the estimation of bioavailability in man

Combrink M, McFadyen ML, Miller R. J Pharm Pharmacol 1997 Jul; 49(7):731–3. There has recently been concern about confidence intervals calculated using the standard error of parameter estimates from NONMEM, a computer program that uses a nonlinear mixed-effects model to calculate relative bioavailability (F), because of possible downward bias of these estimates. In this study, an alternate approach, the log-likelihood procedure, was used to calculate the confidence intervals for F from NONMEM. These were then compared with those calculated using the standard error of the parameter estimates, the traditional NONMEM approach, and the standard model-independent method, to determine whether bias exists. By use of data from a single-dose, open crossover study of ibuprofen using 14 healthy male volunteers, NONMEM was shown to give results consistent with those obtained using the standard model-independent method of analysis and could be a useful tool in the determination of F where conditions for using the standard method of analysis are not optimum. The width of the confidence interval for F using the log-likelihood procedure was narrower and nonsymmetrical when compared with that obtained using the traditional NONMEM approach. The width of the confidence interval obtained using the traditional NONMEM method was similar to that from the standard approach, however, the parameter estimate for F was higher than that obtained from the standard method. This could have been because of an outlier in the dataset to which the standard approach is more sensitive. No downward bias was found in the confidence intervals from NONMEM. The bioavailability dataset was of relatively low variability and more research with highly variable data is necessary before it can be concluded that the confidence intervals calculated from NONMEM can be used for hypothesis testing.

Evaluation of the proposed FDA pilot dose–response methodology for topical corticosteroid bioequivalence testing

Demana PH, Smith EW, Walker RB, Haigh JM, Kanfer I. Pharm Res 1997 Mar; 14(3):303–8. PURPOSE: The American FDA has recently released a Guidance document for topical corticosteroid bioequivalence testing. The purpose of this study was to evaluate the recommendations of this document for appropriateness. The new specifications require a dose–vasoconstriction response estimation by the use of a Minolta chromameter in a preliminary pilot study to determine the parameters for use in a pivotal bioequivalence study. METHODS: The visually assessed human skin balancing assay methodology routinely practiced in our

laboratories was modified to comply with the requirements of the pilot study so that visual and chromameter data could be compared. Two different cream formulations, each containing 0.12% betamethasone 17-valerate, were used for this comparison. RESULTS: Visual data showed the expected rank order of AUC values for most dose durations whereas the chromameter data did not show similar results. The expected rank order of AUC values for both chromameter and visual data was not observed at very short dose durations. In fitting the data to pharmacodynamic models, equivalent goodness of fit criteria were obtained when several different parameter estimates were used in the model definition, however, the visual data were best described by the sigmoid E_{max} model while the chromameter data were best described by the simple E_{max} model. CONCLUSIONS: The E_{max} values predicted by the models were close to the observed values for both datasets and in addition, excellent correlation between the AUC values and the maximum blanching response (R_{max}) ($r > 0.95$) was noted for both methods of assessment. The chromameter ED50 values determined in this study were approximately two hours for both preparations. At this dose duration, the instrument would not be sensitive enough to distinguish between weak blanching responses and normal skin for bioequivalence assessment purposes.

Meta-analysis for bioequivalence review
Chow SC, Liu J. J Biopharm Stat 1997 Mar; 7(1):97–111. The problem of drug interchangeability among a brand-name drug and its generic copies is considered. Under current Food and Drug Administration (FDA) regulation, a patient may switch from the brand-name drug to a generic drug if the generic drug is shown to be bioequivalent to the brand-name drug based on bioequivalence testing. After the patent of a brand-name drug is expired, usually there will be a number of generic copies available on the market. The FDA does not indicate that a patient may switch from a generic to another even though both of the generic drugs are bioequivalent to the brand-name drug. As a result, drug interchangeability among the brand name and its generic copies is a safety concern. In this paper, we propose to perform a meta-analysis for an overview of bioequivalence. The proposed meta-analysis provides an assessment of bioequivalence among generic copies of a brand name that can be used as a tool to monitor the performance of the approved generic copies of the brand-name drug. In addition, it provides more accurate estimates of inter- and intrasubject variabilities of the drug product.

A note on sample size determination for bioequivalence studies with high-order crossover designs
Chen KW, Chow SC, Li G. J Pharmacokinet Biopharm 1997 Dec; 25(6):753–65. Similar to Liu and Chow, approximate formulas for sample size determination are derived based on Schuirmann's two one-sided tests procedure for bioequivalence studies for the additive and the multiplicative models under various higher order crossover designs for comparing two formulations of a drug product. The higher order crossover designs under study include Balaam's design, the two-sequence dual design, and two four-period designs (with two and four sequences), which are commonly used for assessment of bioequivalence between formulations. The derived formulas are simple enough to be carried out with a pocket calculator. The number of subjects required for each of the four higher order designs is tabulated for selected powers and various parameter values.

Comment in: Biometrics. 1999 Dec; 55(4):1314–5. Optimum allocation of treatments for Welch's test in equivalence assessment
Dette H, Munk A. Biometrics 1997 Sep; 53(3):1143–50. As an extension of Welch's test to equivalence trials in a matched pair design, we determine the sample sizes n_1 and n_2 that maximize the power at given alternatives for a given total sample size $n = n_1 + n_2$. Although the optimal allocation is obtained asymptotically when the ratio of the standard deviations in both treatment groups equals the ratio of the sample sizes, numerical investigations show that this result does not hold for sample sizes where $n < \text{or} = 25$. For convenience, we provide tables containing the optimal combinations for the bioequivalence problem as well as for the problem of testing a clinically relevant difference. Results indicate that for small sample sizes, the optimum allocations of the treatments differ significantly in both testing problems, although they are asymptotically identical. In addition, we provide simple approximate equations that

can be used for the determination of the required sample sizes to control a preassigned probability of type II error.

Sample size determination for repeated measurements in bioequivalence test
Lui KJ. J Pharmacokinet Biopharm 1997 Aug; 25(4):507–13. When the measurement of outcome is unreliable or the cost of obtaining an additional subject is relatively high compared to the cost of obtaining an additional measurement from the same subject, it may be desirable to consider taking more than one measurement per subject to increase power or to minimize the cost in a clinical trial. When each subject in two comparison groups has a fixed number of repeated measurements, this paper develops an asymptotic procedure to calculate the number of subjects per group required to achieve a given power for an a-level bioequivalence test. Furthermore, Monte Carlo simulation is used to evaluate the accuracy of the approximate sample size calculation procedure and a brief discussion on how to determine the optimal number of repeated measurements is included.

Comment in: Ther Drug Monit 1998 Dec; 20(6):722–3. Simple bioequivalence criteria: are they relevant to critical dose drugs? Experience gained from cyclosporine
Johnston A, Keown PA, Holt DW. Ther Drug Monit 1997 Aug; 19(4):375–81. A critique of the current bioequivalence regulations is presented with reference to critical dose drugs. Using the development of a new cyclosporine formulation as an example, the deficiencies in current bioequivalence testing guidelines are examined and discussed. Based on the experience gained with cyclosporine, recommendations are made on how therapeutic equivalence, rather than just bioequivalence, should be established.

Statistical methods for two-sequence three-period crossover designs with incomplete data
Chow SC, Shao J. Stat Med 1997 May 15;16(9):1031–9. In clinical trials, and in bioavailability and bioequivalence studies, one often encounters replicate crossover designs such as a two-sequence three-period crossover design to assess treatment and carryover effects of two formulations of a drug product. Because of the potential dropout (or for some administrative reason), however, the observed dataset from a replicate crossover design is incomplete or unbalanced so that standard statistical methods for a crossover design may not apply directly. For inference on the treatment and carryover effects, we propose a method based on differences of the observations that eliminates the random subject effects and thus does not require any distributional condition on the random subject effects. When no datum is missing, this method provides the same results as the ordinary least squares method. When there are missing data, the proposed method still provides exact confidence intervals for the treatment and carryover effects, as long as the dropout is independent of the measurement errors. We provide an example for illustration.

Tolerance intervals for assessing individual bioequivalence
Brown EB, Iyer HK, Wang CM. Stat Med 1997 Apr 15;16(7):803–20. Evaluation of equivalence of two formulations of a drug typically entails the comparison of average bioavailabilities. Recently, however, authors have become aware that this may be insufficient to assess individual bioequivalence, that is, interchangeability of formulations on an individual basis. This paper outlines a tolerance interval procedure to assess individual bioequivalence based on a model that includes a subject by formulation interaction. We give methods for several higher-order crossover designs along with examples.

A two one-sided tests procedure for assessment of individual bioequivalence
Liu J, Chow SC. J Biopharm Stat 1997 Mar; 7(1):49–61. In this paper, we propose a two one-sided tests procedure for assessment of individual bioequivalence based on the concept of individual equivalence ratios proposed by Anderson and Hauck. The proposed procedure is derived under the normality assumption for the logarithmic transformation of pharmacokinetic responses obtained from a standard two-sequence, two-period crossover design. We show that the hypotheses for individual bioequivalence are equivalent to the hypotheses for testing whether the upper (or lower) pth quantile of the distribution of the differences between the test and reference formulations from the same subject is not greater (or not smaller) than some

prespecified equivalence limits. Under this setting, we examine the relationship between average and individual bioequivalence. There exists the uniformly most powerful invariant test for each of the two one-sided hypotheses. In addition, the proposed two one-sided tests procedure is a test of size alpha (i.e., $\leq \alpha$). We demonstrate that the determination of critical values, the enumeration of power, and the estimation of sample sizes require noncentral *t*-distributions but do not necessarily require the estimation of unknown population mean and variance for noncentrality parameters. We discuss possible extensions to other crossover and replicated crossover designs. A numerical example illustrates the proposed procedure.

Veterinary drug bioequivalence determination
Toutain PL, Koritz GD. J Vet Pharmacol Ther 1997 Apr; 20(2):79–90. A bioequivalence trial is a statistically based comparison of two formulations to demonstrate with a controlled consumer (patient) risk that two formulated drug products are interchangeable. The basic assumption underlying a bioequivalence trial is that essentially the same plasma time-course leads to essentially the same effect allowing two formulations to be interchanged. Bioequivalence is generally assessed using kinetic end points and in practice, two formulations in which bioavailability parameters (rate and extent) differ by 20% or less, with a 90% degree of confidence, are considered to be bioequivalent. In this review, the design and evaluation of bioequivalence studies are presented with special attention given to scientific issues.

1996

Absorption rate versus exposure: which is more useful for bioequivalence testing?
Tozer TN, Bois FY, Hauck WW, Chen ML, Williams RL. Pharm Res 1996 Mar; 13(3):453–6. PURPOSE: The goals were to evaluate the usefulness of C_{max}/AUC_{lqc}, ratio of the maximum plasma drug concentration to the area under the plasma concentration–time curve to the time of the last quantifiable concentration, in bioequivalence testing and to explore the use of exposure as a replacement for the concepts of rate and extent of drug absorption. METHODS: The bioequivalence of products differing in both rate (k_a) and extent (F) of absorption was assessed under conditions similar to those encountered in a typical trial. A one-compartment model drug with first-order absorption (rate constant$=k_a$) and eliminations was used. Variability was introduced in all model parameters using Monte Carlo techniques. The results were expressed in terms of the probability of declaring bioequivalence in a crossover trial with 24 subjects using C_{max}/AUC_{lqc}, AUC_{lqc}, and C_{max} as bioequivalence measures. RESULTS: The outcome of a bioequivalence trial was shown to depend on the measure. C_{max}/AUC_{lqc} reflected changes in k_a, but not in F. AUC_{lqc} showed dependence on F, but virtually no dependence on k_a. For C_{max}, a three to fourfold increase in k_a and a concomitant 20% decrease in F, as well as corresponding changes in the opposite directions, resulted in bioequivalent outcomes. CONCLUSIONS: It was concluded that use of C_{max}/AUC_{lqc} should be discouraged and that defining bioequivalence in terms of rate and extent of absorption has major problems. The goal of bioequivalence trials should be to assure that the shape of the concentration–time curve of the test product is sufficiently similar to that of the reference product. To this end, the use of "exposure" rather than "rate and extent of absorption" concepts is encouraged.

The assessment of individual and population bioequivalence
Chinchilli VM. J Biopharm Stat 1996 Mar; 6(1):1–14. We develop a statistical methodology for the assessment of individual bioequivalence when a crossover design is invoked. The location parameters for our model consist of population-averaged parameters for formulation, population-averaged parameters for nuisance effects (sequence, period, carryover, etc.), and subject-specific parameters for formulation. We do not impose any distributional assumptions other than the existence of first- and second-order moments. We derive unbiased estimators for all of the parameters in the model and construct subject-specific bioequivalence scores which exclude the effects of the nuisance parameters. We assess

individual bioequivalence by constructing distribution-free tolerance intervals based on the sample of bioequivalence scores.

Bioequivalence studies of celiprolol in healthy human volunteers
Iyer EK, Tipnis HP. Indian J Med Sci 1996 Jul; 50(7):234–8. Thus, bioequivalence between the two products was established by undertaking this study. From Table 1, it can be seen that the standard deviation at the various sampling points is high indicating varying absorption rates in individual volunteers, but this was observed in case of both the products. Also, since the study design was complete crossover, this high standard deviation was not due to any study design variable. As celiprolol shows nonlinear dose-related absorption kinetics, this high value of standard deviation may be due to the intersubject variation during the absorption process. However, all the pharmacokinetic parameters showed a comparable profile when statistically evaluated for any significant difference between the two products.

Comment in: Ann Pharmacother. 1997 Apr; 31(4):501–2. Comparative bioavailability and safety of two intramuscular ceftriaxone formulations
Suarez EC, Grippi JR. Ann Pharmacother 1996 Nov; 30(11):1223–6. OBJECTIVE: To determine if two ceftriaxone solutions of different concentrations are bioequivalent when administered intramuscularly. DESIGN: Double-blind, single-dose, two-period, randomized crossover study. SETTING: A clinical research center. SUBJECTS: Seventeen healthy volunteers. INTERVENTION: Ceftriaxone 500 mg administered in either 2 or 1.4 mL of lidocaine 1% solution, with final ceftriaxone concentrations of 250 and 350 mg/mL, respectively. MAIN OUTCOME MEASURES: Blood samples were assayed for ceftriaxone concentrations with HPLC and pharmacokinetic parameters were calculated from the resulting plasma-concentration time profiles: maximum plasma concentration (C_{max}) of ceftriaxone and areas under the concentration–time curve (AUC) from 0 to 36 hours and 0 to ∞ were the primary parameters considered in the determination of bioequivalence. RESULTS: The two solutions were generally well tolerated and had similar safety profiles. Administration of both solutions resulted in similar mean values for all pharmacokinetic parameters. Statistical analysis showed no significant differences between the two solutions in any pharmacokinetic parameter, indicating that the two solutions are statistically bioequivalent ($p \leq 0.05$). The 90% CI for the ratio of the means for $AUC_{0–36}$ (0.86–1.11), $AUC_{0.36}$ (0.89–1.14), and C_{max} (0.84–1.12) are within the Food and Drug Administration range of bioequivalence (0.80–1.25). CONCLUSIONS: These results demonstrate that the more concentrated solution of ceftriaxone (350 mg/mL) is bioequivalent to the currently marketed solution of 250 mg/mL.

Design and analysis of intrasubject variability in crossover experiments
Chinchilli VM, Esinhart JD. Stat Med 1996 Aug 15;15(15):1619–34. Recently, interest has grown in the development of inferential techniques to compare treatment variabilities in the setting of a crossover experiment. In particular, comparison of treatments with respect to intrasubject variability has greater interest than has intersubject variability. We begin with a presentation of a general approach for statistical inference within a crossover design. We discuss three different statistical models where model choice depends on the design and assumptions about carryover effects. Each model incorporates t-variate random subject effects, where t is the number of treatments. We develop maximum likelihood and restricted maximum likelihood approaches to derive parameter estimators and we consider a special case in which closed-form expressions for the variance component estimators are available. Finally, we illustrate the methodologies with the analysis of data from three examples.

Determination of bioequivalence of two furosemide preparations: the effect of high doses of furosemide on some pharmacokinetic parameters
Wolf-Coporda A, Lovric Z, Huic M, Francetic I, Vrhovac B, Plavsic F, Skreblin M. Int J Clin Pharmacol Res 1996;16(4–5):83–8. The bioequivalence of two oral preparations of the diuretic furosemide, namely (*i*) a Croatian pharmaceutical product (test preparation A) and (*ii*) a reference preparation B, both in a dose of 500 mg was assessed in an open, crossover, randomized trial in 15 healthy male volunteers, in whom the HPLC method with a fluorescent detector was used to determine its concentrations. The test preparation (A) was found to

achieve a considerably higher concentration (17.2 ± 9.304 mg/L) than the reference preparation (11.1 ± 6.484 mg/L); the time to peak concentrations was statistically significantly shorter for the test preparation (1.033 ± 0.743 hours) than for the reference preparation (1.656 ± 0.586 hours); and the areas under the concentration curves were statistically significantly greater for the examined preparation (65.9 mg hr/L) than for the reference preparation (46.845 mg hr/L). The relative bioavailability of the test preparation was 129%, i.e., it was not bioequivalent with the reference preparation. This finding was consistent with the previously performed laboratory quality testing in vitro, where the release of the reference preparation was found to be considerably slower and weaker than that of the test preparation. High doses of furosemide exemplified by 500 mg were found to affect only some of the pharmacokinetic parameters, i.e., they induce an accelerated absorption, an increase in serum concentration, and a prolongation of its half-life.

Multiple dose bioequivalence study with josamycin propionate, a drug with highly variable kinetics, in healthy volunteers

Van Hoogdalem EJ, Terpstra IJ, Krauwinkel WJ, Volkers-Kamermans NJ, Baven AL, Verschoor JS. Int J Clin Pharmacol Ther 1996 May; 34(5):202–7. Josamycin is a macrolide antibiotic with considerable intra- and interindividual variability in kinetics. In the present study, bioequivalence of an intact and dispersed josamycin Solutab tablet, containing 1000 mg of josamycin in the form of josamycin propionate ester, was tested versus a Josacine 1000 mg reference sachet. The design of this bioequivalence study was adapted to the drug's pharmacokinetic variability, comprising testing in steady state, testing the reference in replicate, and maintaining a widened bioequivalence margin. The study was performed in a group of 24 male and 12 female healthy subjects, according to a three-treatment four-period crossover design. Blood sampling for establishing josamycin propionate and josamycin base serum level profiles were collected during the 12 hours dosing interval on day 4. Steady-state serum levels were reached on day 4. With the reference sachet, mean peak levels of 1.02 μg/mL and 0.36 μg/mL were observed for parent drug and metabolite, respectively, reached at peak times of 1.5 and 1.8 hours. Comparable profiles were observed with the intact and dispersed Solutab tablets, both tending toward higher serum levels than the sachet. In terms of josamycin propionate levels as well as josamycin base levels, the intact and dispersed Solutab tablet was bioequivalent with the referent sachet within the preset 0.70–1.43 margins. Variability in josamycin kinetics proved to be substantial, maximum differences in peak levels and AUC values being about 10-fold between individuals and 3-fold within individuals. Retrospectively, the multiple dosing regimen appeared not to result in a clear reduction of intrasubject variability.

Randomization tests for assessing the equality of area under curves for studies using destructive sampling

Bailer AJ, Ruberg SJ. J Appl Toxicol 1996 Sep–Oct; 16(5):391–5. Testing the equality of the area under a curve (AUC) for different dose groups is frequently done in pharmacokinetic research. Equality of AUCs is one indicator of bioequivalence. When the experimental unit must be sacrificed to obtain a response, AUC can be simply estimated using a linear combination of response means at various time points. The distribution of this estimator is simply obtained using standard statistical theory, and statistical hypothesis tests are easily constructed. These tests assume a normal distribution of responses at each time point (or at least large enough samples to assure that the mean response is normally distributed). The applicability of this test to cases of non-normal response distributions when small numbers of observations are sampled at each time point is questionable. Randomization tests are suggested for this problem. These tests provide a valuable alternative to this normal-theory test. Discussion of the assessment of dose proportionality is also presented.

Sequential designs for equivalence studies

Whitehead J. Stat Med 1996 Dec 30;15(24):2703–15. Sequential designs are increasingly being used in major clinical trials concerning life-threatening diseases. So far most applications have concerned trials designed to establish whether an experimental treatment is superior to a control. However, many trials are conducted with the objective of showing that an experimental treatment is equivalent to a control. This paper concerns the application of sequential designs to

equivalence trials. Criteria for claiming equivalence are reviewed and compared, and methods first developed in the context of bioequivalence are described. Appropriate sequential procedures are identified. A simulated example, based on a clinical comparison of bronchodilators, is used to illustrate both the double triangular test and a comparable procedure constructed from alpha-spending functions.

A three-step procedure for assessing bioequivalence in the general mixed model framework

Vuorinen J, Turunen J. Stat Med 1996 Dec 30;15(24):2635–55. Bioavailability data arising from a standard two-period crossover study are routinely analyzed to establish bioequivalence between test and reference formulations. Current regulatory guidelines only require evidence of equivalence in average bioavailability for the assessment of bioequivalence. Under normality assumptions, this is achieved by demonstrating equivalence between the formulation means (step 1). However, the equivalence of formulation variances should also be assessed to get evidence of population bioequivalence (step 2), since a difference in variability of bioavailability may also pose significant problems in drug safety and efficacy. On the other hand, even population bioequivalence does not ensure that an individual subject could be expected to respond similarly to the two formulations. Therefore, whenever individual bioequivalence is the ultimate goal, the magnitude of intrasubject correlation should always be examined as the final stage (step 3). In this paper, these three successive concepts of bioequivalence are cast into the general mixed model framework and a stepwise testing procedure for the global assessment of bioequivalence is proposed. In addition to this, important issues addressed in the regulatory guidelines, such as verification of the model assumptions and application of the log-transformation, are discussed. Lastly, an example is presented to illustrate the proposed three-step procedure on the original and log-transformed scale of measurement.

The transitivity of bioequivalence testing: potential for drift

Anderson S, Hauck WW. Int J Clin Pharmacol Ther 1996 Sep; 34(9):369–74. During the drug development process bioequivalence studies are required as formulations are refined. At the end of this chain of innovator-conducted studies, generic manufacturer(s) conduct bioequivalence studies comparing their generic(s) to the current marketed formulation. The question we pose is: How transitive is bioequivalence? That is, if formulation B is bioequivalent to formulation A, and C to B, what can one say about the bioequivalence of C and A? We consider 1 (A–B), 2 (A–C), 3 (A–D), and 6 (A–G) bioequivalence steps and restrict attention to the current practice of average bioequivalence with an (80%, 125%) equivalence criterion. For the cases of no to small differences in bioavailability, our results suggest that one can be fairly confident of the bioequivalence of formulation C to formulation A, and of D to A, but not of G to A. The transitivity of bioequivalence decreases with additional steps and with increasing power of the individual bioequivalence studies.

1995

Absorption and bioavailability of pentaerithrityl-tetranitrate (PETN, Dilcoran 80)

Haustein KO, Winkler U, Loffler A, Huller G. Int J Clin Pharmacol Ther 1995 Feb; 33(2):95–102. The effects of 80 mg pentaerithrityl-tetranitrate (PETN) as suspension or formulated as tablets were compared to placebo in a single-blind, randomized, crossover study in 18 healthy subjects (study A), and the bioequivalence of two tablet formulations (marketed Dilcoran 80 vs. a new formulation) was studied in 24 healthy subjects after administration of single oral doses of 80 mg PETN according to a placebo controlled, randomized, double-blind, two-way crossover study design (study B). The perfusion of the right middle finger was measured by rheography (altitude A of the changes of resistance and of the incisure D) before and 24-hour postdose, and blood pressure and heart rate were measured in supine position at the same time. The values of area under curve (AUC) of the ratio A/D were calculated by the trapezoidal rule. In study A the mean A/D values were reduced

from about 2.0 to about 1.3 after intake of PETN (solution or tablet) with a minimum 60 to 90 minutes postdose (solution) and two-hour postdose (tablet). A significant reduction in this ratio was seen up to 8 (solution) or 12 hours (tablet) postdose. Changes in blood pressure were not observed while the heart rate decreased in the subjects of all three groups one- to two-hour postdose followed by an increase by 6 to 10 beats per minute. After subtraction of the AUC values of placebo from the PETN-derived AUC values, mean values of 6.61 (SD 1.52, solution) and 7.25 (SD 1.48, A/D×h, tablet) were calculated ($p > 0.1$, study A).(ABSTRACT TRUNCATED AT 250 WORDS)

Bioequivalence: an updated reappraisal addressed to applications of interchangeable multi-source pharmaceutical products

Marzo A, Balant LP. Arzneimittelforschung 1995 Feb; 45(2):109–15. This paper reviews study procedures for bioequivalence trials, mainly addressed to the New Drug Application (NDA) of generic drugs, strictly referring to EU and U.S.A. guidelines on this matter. Specific attention is devoted to the most appropriate experimental designs, the size of the volunteer sample, the ethical issues involved, statistics to assess bioequivalence, and the accepted standard format for final research reports. Some aspects which create serious problems in bioequivalence trials, most of which not fully covered by the EU and USA specific guidelines, are comprehensively discussed. These include (*i*) drugs with elevated variability, (*ii*) endogeneous substances and the management of baseline value, (*iii*) modified release formulations, (*iv*) prodrugs, (*v*) restrictions to be contained in forthcoming guidelines on chiral medicinal products, (*vi*) superbioavailability, (*vii*) drugs with elevated half-life, and (*viii*) cases in which bioequivalence trials should not be needed. As generic drugs cost less than the innovator product, agencies have facilitated their NDA procedures by requiring a dossier on chemistry and pharmacy and a pivotal bioequivalence study to demonstrate that the generic formulation is fully interchangeable with the innovator product. Bioequivalence is thus the key requirement for an NDA of a generic drug, and trials should be planned, conducted, and reported in the most appropriate way. With this in mind, this review is an up-to-date reappraisal that should stimulate the attention of scientists and regulatory authorities on some open questions on bioequivalence.

Comparison of the bioavailabilities of erythromycin estolate and erythromycin ethylsuccinate dry suspension preparations in steady state

Potthast H, Schug B, Elze M, Schwerdtle R, Blume H. Pharmazie 1995 Jan; 50(1):56–60. Relative bioavailability of erythromycin was determined after multiple-dose administration of erythromycin estolate in comparison to erythromycin ethylsuccinate both given as oral suspensions to 12 healthy volunteers. The daily erythromycin dose of erythromycin ethylsuccinate was 50% higher than the respective dose of erythromycin estolate; the dosage interval *tau* was 12 hours for erythromycin estolate and eight hours for erythromycin ethylsuccinate. This scheme was planned in accordance to advices of the respective manufactures. Results of the study confirm the differences in extent of bioavailability of both erythromycin derivatives known from single-dose investigations. Furthermore, the experimental data show that a twice daily administration of 1000 mg erythromycin as erythromycin estolate resulted in sufficiently high plasma concentration of the active compound.

Group sequential extensions of a standard bioequivalence testing procedure

Gould AL. J Pharmacokinet Biopharm 1995 Feb; 23(1):57–86. Bioequivalence trials compare the relative bioavailability of different formulations of a drug. Regulatory requirements for demonstrating average bioequivalence of two formulations generally include showing that a (say) 90% confidence interval for the ratio of expected pharmacologic end point values of the formulations lies between specified end points, e.g., 0.8–1.25. The likelihood of demonstrating bioequivalence when the formulations truly are equivalent depends on the sample size and on the variability of the pharmacologic end point. Group sequential bioequivalence testing provides a statistically valid way to accommodate mis-specification of the variability in designing the trial by allowing for additional observations if a clear decision to accept or reject bioequivalence cannot be reached with the initial set of observations. This paper describes group sequential bioequivalence designs applicable in

most practical situations that allow a decision to be reached with fewer observations than fixed-sample designs about 60% of the time at approximately the same average cost. The designs can be used in trials where the formulations are expected to have equal bioavailability and in trials where the formulations are expected to differ slightly. Data analyses are carried out exactly as for fixed-sample designs. Providing the capability of sequential decisions modestly affects the nominal significance levels, e.g., the required confidence level may be 93–94% instead of 90%.

Influence of human serum albumin content in formulations on the bioequivalency of interferon alfa-2a given by subcutaneous injection in healthy male volunteers
Zhi J, Teller SB, Satoh H, Koss-Twardy SG, Luke DR. J Clin Pharmacol 1995 Mar; 35(3):281–4. To determine the influence of human serum albumin (HSA) content in formulations on the bioequivalency of recombinant interferon alfa-2a, a double-blind, randomized, two-way crossover study was conducted in 24 healthy male volunteers. Subjects received a single subcutaneous injection of 18 million IU of Roferon-A reconstituted with either the diluent containing 10 mg of HSA or the HSA-free diluent; final HSA contents in the two formulations were 15 mg and 5 mg, respectively. Administration of the two formulations resulted in similar 48-hour Roferon-A serum concentration–time profiles and comparable frequency and intensity of adverse events. The statistical analysis using the two one-sided tests procedure showed that both formulations were bioequivalent for pharmacokinetic parameters such as C_{max}, t_{max}, AUC_{48}, and AUC. We conclude that a threefold change in HSA content in formulations does not alter the bioequivalency of Roferon-A.

A new statistical procedure of interval hypothesis testing for bioequivalence studies
Ohki T, Suzuki T, Goto S; Biol Pharm Bull 1995 Jan; 18(1):192–4. A simplified procedure of interval hypothesis testing was investigated and established for bioequivalence studies. The characteristics of this procedure were compared with those of other statistical procedures.

A pilot study on the determination of the relative bioavailability of levo-thyroxine
Trantow T, Herzog R, Fuder H, Ise J, Lucker PW. Methods Find Exp Clin Pharmacol 1995 Jun; 17(5):333–43. A promising new design aimed at testing bioequivalence of levo-thyroxine preparations in male euthyreotic volunteers was investigated in a pilot study. Healthy volunteers received a single oral dose of levo-thyroxine (200 μg of the same formulation, 2 tablets of 100 μg each) in four subsequent periods with washout times of one week between administrations. Consistent increases in serum levo-thyroxine concentrations were observed after intake of each dose. The number of volunteers included, in our case 12, was sufficient to state bioequivalence of the medication given in four subsequent periods as assessed by area under data after subtraction of area under basal levo-thyroxine concentrations determined on the day before drug administration, and allowed an estimate of the minimum number required for future studies. Furthermore, combination of individual values from different periods of identical treatment may lead to a reduction of minimum sample size. The design tested could also be used as a crossover design to obtain a reliable parameter related to relative levo-thyroxine bioavailability and is a promising alternative to another model where bioavailability can be tested in athyreotic patients. The medication was well tolerated and no adverse events related to medication were found. Safety parameters failed to reveal any marked change during the four study periods.

Relative bioavailability of the antiarrhythmic agent tiracizine and its metabolites
Berndt A, Oertel R, Richter K, Terhaag B, Gramatte T. Arzneimittelforschung 1995 Mar; 45(3):250–3. Relative bioavailability of a 100 mg tablet formulation of the antiarrhythmic agent tiracizine (CAS 78816-67-8) compared to a 50 mg formulation was assessed in a simple crossover study after single administration of a 100 mg dose to 12 healthy volunteers. Tiracizine and three of its metabolites (M1, M2, and M3) were measured in serum and urine by high pressure liquid chromatography. AUC (means after administration of the test preparation and 95% nonparametric confidence interval for the ratio test preparation/reference preparation) were 391.5 ng hr/mL and 0.87 to 1.11 for tiracizine, 5184.5 ng hr/mL and 0.94 to 1.26 for M1, and 1319.9 ng hr/mL and 0.88 to 1.16 for M2. Mean maximum serum concentrations after the test

preparation and corresponding 95% confidence interval were 111.2 ng/mL and 0.86 to 1.20 for tiracizine, 301.2 ng/mL and 0.98 to 1.22 for M1, 54.6 ng/mL and 0.86 to 1.17 for M2, and 35.2 ng/mL and 0.82 to 1.17 for M3. t_{max} did not differ after the two preparations for tiracizine, M2 and M3, but was significant lower for M1 after administration of the test preparation (2.2 ± 0.7 hours vs. 3.0 ± 1.2 hours). Total urinary recovery (sum of parent compound and metabolite recovery) up to 32 hours after intake of the test preparation was 31.2% of the administered dose. The corresponding 95% confidence interval was 0.84 to 1.08. Statistical evaluation of all parameters revealed bioequivalence between the two preparations if a single dose of 100 mg is administered.

Safety and bioequivalency of three formulations of respiratory syncytial virus-enriched immunoglobulin

Groothuis JR, Simoes EA, Lehr MV, Kramer AA, Hemming VG, Rodriguez WJ, Arrobio J, Welliver RC, Siber GR. Antimicrob Agents Chemother 1995 Mar; 39(3):668–71. Respiratory syncytial virus (RSV) causes serious illness (lower respiratory illness) in preterm infants. RSV antibody-enriched immunoglobulin (RSVIG) that was lyophilized (LYO) protected against RSV lower respiratory illness. The Food and Drug Administration now requires an additional viral inactivation step (VI). We compared LYO, LYO-VI, and a more convenient liquid RSVIG (LIQ-VI) in 30 preterm infants (median age, 7 months; median weight, 5.4 kg). Infants were randomized to receive LYO ($n=10$), LYO-VI ($n=10$), or LIQ-VI ($n=10$) in monthly infusions of 750 mg/kg of body weight per dose (December–March). Children were monitored closely for adverse reactions to RSVIG and for RSV illness.

The solubility and bioequivalence of silymarin preparations

Schulz HU, Schurer M, Krumbiegel G, Wachter W, Weyhenmeyer R, Seidel G. Arzneimittel-forschung 1995 Jan; 45(1):61–4. Seven silymarin products (pharmacies only), two of them with two batches each, were analyzed for their ingredients, in particular silibinin (CAS 22888-70-6) and tested in vitro for their liberation of active agents. Founded on the results of these tests three products were checked for bioequivalence. Therefore, a typical phase I 3-fold crossover study was performed showing one product (Legalon) to be qualified by approximately 2-fold higher silibinin availability compared to the two other preparations.

A spreadsheet program for simulation of bioequivalence and bioavailability studies

Abdallah HY, Ludden TM. Comput Biol Med 1995 May; 25(3):349–54. This paper presents a spreadsheet for Excel for Windows, which simulates bioequivalence (BE) trials. The program incorporates inter- and intrasubject variability in drug absorption and disposition as well as assay precision and the uniformity of the administered dose. The output provides confidence intervals and a pass/fail code for each study. This program is useful for simulating BE trials using widely available and simple-to-use spreadsheet programs. An example of the application of the program in assessing the influence of intrasubject variability on the outcome of BE testing of two identical formulations is also presented.

Use of the repeated crossover designs in assessing bioequivalence

Liu JP. Stat Med 1995 May 15–30;14(9–10):1067–78; discussion 1079–80. We consider applications of the repeated 2×2 crossover design to evaluating bioequivalence between the two formulations. The repeated 2×2 crossover design allows us not only to assess bioequivalence on average bioavailability and to examine the subject-by-formulation interaction but also to obtain independent unbiased estimates of intrasubject variability. One consequence of unequal intrasubject variabilities is that the sum of squares of intersubject residuals and the sum of squares of subject-by-formulation residuals are not independent. We also discuss the relative merits of this design as compared to the standard 2×2 crossover design without repeated measurements in terms of precision and sample size with respect to the ratio of the number of subjects to the repeated measurements per subject. We investigate other uses of the 2×2 crossover in examining the bioequivalence between the two different dosing regimens. Possible applications of other repeated crossover designs to bioequivalence for more than two formulations are explored. A numerical example illustrates the proposed procedure.

1994

An assessment of the 4-6-20 rule for acceptance of analytical runs in bioavailability, bioequivalence, and pharmacokinetic studies
Kringle RO. Pharm Res 1994 Apr; 11(4):556–60. A recent conference report described a decision rule, hereafter referred to as the 4-6-20 rule, for acceptance/rejection of analytical runs in bioavailability, bioequivalence, and pharmacokinetic studies. This procedure requires that quality control specimens at three concentrations (low, medium, and high) be assayed in duplicate in each run. For run acceptance, at least four of the six assay values must be within $\pm 20\%$ of their respective nominal concentrations, and at least one of the two values at each concentration must be within these limits. An inherent flaw in this decision rule is that the risk of rejecting runs, when the assay performance has in fact not deteriorated, varies for each assay and is neither known nor controlled. In this paper, simulation methods are used to evaluate the operating characteristics of the 4-6-20 rule in comparison to those of classical statistical quality control procedures.

Bioequivalence of a generic slow-release theophylline tablet in children
Kanthawatana S, Ahrens RC, McCubbin M, Bronsky E, Blake K, Hendeles L. J Pediatr 1994 Dec; 125(6 Pt 1):987–91. OBJECTIVE: To determine whether a generic slow-release theophylline tablet (manufactured by Sidmak Laboratories, Inc.) is therapeutically equivalent to a proprietary theophylline tablet, Theo-Dur, in children. DESIGN: Prospective, randomized, double-blind, crossover trial. SETTING: Multicenter clinics. PATIENTS: Thirty-eight children, 6–16 years of age, with asthma. INTERVENTIONS: Individualized doses of Theo-Dur or generic tablet every 12 hours for five days. MEASUREMENTS AND MAIN RESULTS: During the last 24 hours of each regimen, theophylline serum concentrations were measured serially and a standardized exercise stress test was performed at 24 hours (trough serum concentration). Neither formulation effectively blocked the response to exercise; the maximum decrease in forced expiratory volume in the first second was $26.1\% \pm 18.9\%$ with Theo-Dur and $24.8\% \pm 19.7\%$ with the generic product ($p = 0.68$; $\beta = 0.08$). The mean $\pm$ SD peak serum concentrations were 18.0 ± 3.0 μg/ml with Theo-Dur and 18.7 ± 3.7 μg/ml with the generic tablet; the trough serum concentration was <10 μg/ml in 15 subjects after administration of Theo-Dur and in 20 subjects after administration of the generic product. There were no significant differences in relative extent of absorption or the time to reach peak serum concentration. CONCLUSIONS: This generic formulation and Theo-Dur are bioequivalent in children. However, these results cannot be extrapolated to slow-release theophylline formulations that have not been approved by the U.S. Food and Drug Administration as equivalent to Theo-Dur.

Effect of meals and dosage-form modification on theophylline bioavailability from a 24-hour sustained-release delivery system
Gonzalez MA, Straughan AB. Clin Ther 1994 Sep–Oct; 16(5):804–14. The bioavailability of theophylline from an extended-release formulation (Uni-Dur) intended for once-daily administration was assessed in a randomized, single-dose, five-way crossover study to determine the effects of food and breaking the tablet, and the bioequivalence of two dosage strengths. The five treatments given at one-week interval were (*i*) immediate-release theophylline (Slo-Phyllin) 5×100 mg to fasting subjects as a reference treatment; (*ii*) sustained-release Uni-Dur 600-mg theophylline tablet to fasting subjects; (*iii*) Uni-Dur 600-mg tablet after a high-fat meal; (*iv*) Uni-Dur 600-mg dose administered as two half tablets to fasting subjects; and (*v*) Uni-Dur 400-mg tablet to fasting subjects. Serial blood samples were collected immediately before and for 57 hours after dosing. The mean relative extents of absorption for the four Uni-Dur treatments were not significantly different from Slo-Phyllin treatment or from each other ($84.30 \pm 23.6\%$, 600 mg, fasting; $88.73 \pm 18.63\%$, 600 mg, fed; $93.65 \pm 19.67\%$, half tablet; and $92.87 \pm 19.5\%$, 400 mg, fasting). The maximum theophylline serum concentrations with Uni-Dur were significantly lower and the times to reach peak concentrations were significantly longer than with Slo-Phyllin. Differences noted among the four Uni-Dur treatments were as follows: the time to peak theophylline concentration was significantly longer in the fed state (17.09 hours) as were the times to 50% (11.73 hours) and 80% (18.46 hours) absorption compared with fasting (13.57, 8.57, and 14.07 hours, respectively). The Uni-Dur 400-mg treatment resulted in a significantly higher maximum

theophylline serum concentration (6.64 μg/ml) compared with the Uni-Dur 600-mg fasting treatment (5.33 μg/ml); however, the correlation between in vivo and in vitro data supports the bioequivalence of the two strengths. This study shows that theophylline is slowly and consistently absorbed from the Uni-Dur 24-hour sustained-release form, and food or breaking the tablet does not alter the extent of absorption. Thus, Uni-Dur potentially provides greater ease of administration and convenience for patients while maintaining therapeutic theophylline serum levels over the 24-hour dosing interval.

Comment in: J Pharm Sci 1997 Mar; 86(3):401–2. Evaluation of different indirect measures of rate of drug absorption in comparative pharmacokinetic studies
Lacey LF, Keene ON, Duquesnoy C, Bye A. J Pharm Sci 1994 Feb; 83(2):212–5. As indirect measures of rate of drug absorption (metrics), maximum plasma concentration (C_{max}) is confounded by extent of drug absorption and the time to reach C_{max} (t_{max}) is a discrete variable, dependent on blood sampling frequency. Building on the work of Endrenyi et al., we have compared different metrics, including C_{max}/area under the curve of concentration versus time from time zero to infinity (AUC_∞), partial AUC from zero to t_{max} (AUC_p), and C_{max}, t_{max} with simulated experiments. Importantly, the performance of these metrics was assessed with the results of actual pharmacokinetic studies involving Glaxo drugs. The results of the simulated and real experiments were consistent and produced the following unambiguous findings: (*i*) C_{max}/AUC_∞ is a more powerful metric than C_{max} in establishing bioequivalence when the formulations are truly bioequivalent; (*ii*) C_{max}/AUC_∞ is more sensitive than C_{max} at detecting differences in rate of absorption when they exist; and (*iii*) the treatment ratios for AUC_p, AUC_p/AUC_∞, and C_{max}, t_{max} are very imprecisely estimated and are of no practical value as measures of rate of absorption. Of the metrics examined, C_{max}/AUC_∞ is the most sensitive and powerful indirect measure of rate of drug absorption in comparative pharmacokinetic studies involving immediate-release dosage forms and should be used instead of C_{max} in bioequivalence testing.

Fieller's confidence intervals for the ratio of two means in the assessment of average bioequivalence from crossover data
Vuorinen J, Tuominen J. Stat Med 1994 Dec; 15–30;13(23–24):2531–45. The two-period crossover design is the most commonly used study design for bioequivalence of one test formulation to be assessed in comparison to one reference formulation. Consequently, in this paper, all derivation is based on this particular design. It is assumed that for the underlying statistical model the usual assumptions of normality and additivity are satisfied on the original scale of measurement and that it is wanted to base the assessment of average bioavailability on the ratio of the unknown population means for the test and reference formulation. The purpose of this paper is to illustrate that it is reasonable to assume a uniform covariance structure for the two-period crossover design, because the demand of equal variability in bioavailabilities, in addition to equal average bioavailabilities, for the reference and test formulation makes the assumption of uniform covariance structure very realistic, and also because the properties of a decision rule based on a Fieller's confidence interval under a uniform covariance structure are competitive with those of the corresponding rule based on a general covariance structure.

Measuring switchability and prescribability: when is average bioequivalence sufficient?
Hauck WW, Anderson S. J Pharmacokinet Biopharm 1994 Dec; 22(6):551–64. Recent work, beginning with that of Anderson and Hauck in 1990, has led to a general acceptance of the need to ensure switchability in bioequivalence testing for approval of generic drugs. In other applications of bioequivalence testing, prescribability may be sufficient. However, there is less acceptance of the need to change statistical procedures and study designs from those currently used to assess the current criterion of average bioequivalence. We propose easily interpreted measures of switchability and prescribability. These measures provide bases for assessing conditions under which average bioequivalence is not sufficient to ensure switchability and prescribability, and hence for which a procedure for individual or population bioequivalence is required. The required conditions are sufficiently tight that they cannot be presumed to hold. Thus, there are reasonable conditions for which current practice is not sufficient. An outcome of this development is a connection between two current approaches for assessing individual bioequivalence.

Presentation of the intrasubject coefficient of variation for sample size planning in bioequivalence studies
Hauschke D, Steinijans WV, Diletti E, Schall R, Luus HG, Elze M, Blume H. Int J Clin Pharmacol Ther 1994 Jul; 32(7):376–8. Bioequivalence studies are generally performed as crossover studies and, therefore, information on the intrasubject coefficient of variation is needed for sample size planning. Unfortunately, this information is usually not presented in publications on bioequivalence studies, and only the pooled inter- and intrasubject coefficient of variation for either test or reference formulation is reported. Thus, the essential information for sample size planning of future studies is not made available to other researchers. In order to overcome such shortcomings, the presentation of results from bioequivalence studies should routinely include the intrasubject coefficient of variation. For the relevant coefficients of variation, theoretical background together with modes of calculation and presentation is given in this communication with particular emphasis on the multiplicative model.

Robust and bootstrap testing procedures for bioequivalence
Shen CF, Iglewicz B. J Biopharm Stat 1994 Mar; 4(1):65–90. A common problem encountered in bioequivalence studies is the presence of outliers. In this situation, the two one-sided *t*-tests proposed by Schuirmann fail to provide reasonable power for concluding bioequivalence. In contrast, our proposed 2 beta trimmed-*t* procedure has the following advantages: (*i*) it has higher efficiency for non-normal symmetric distributions, (*ii*) it is resistant to outliers, and (*iii*) it is relatively easy to compute. Two bootstrap procedures introduced here provide further justification for the proposed trimmed *t*-test procedure. Results from Monte Carlo studies illustrate the power of the proposed procedures under various distributional assumptions for a 2×2 crossover trial.

1993

The application of new bioavailability parameters in the bioequivalence testing of antimicrobial agents
Wessels JC, Koeleman HA, Steyn HS, Ellis SM. Int J Clin Pharmacol Ther Toxicol 1993 Nov; 31(11):542–6. Two new bioavailability parameters were recently suggested [Koeleman et al. 1991] to define (*i*) the time that the concentration in the blood stays above a defined minimum effective concentration, t_e and (*ii*) the onset of the effect, t_o. In addition to conventional bioequivalence parameters, the new bioavailability parameters (t_o and t_e) were calculated in this study and statistically compared for penicillin, chloroquine, oxytetracycline, amoxycillin, and flucloxacillin from available bioequivalence data. For oxytetracycline, flucloxacillin, and amoxycillin, the conventional bioavailability parameters indicated partial equivalence whereas using the t_e and t_o parameters, more realistic indications of the possible extent of the performance of a drug from dosage forms were obtained than with the conventional bioequivalence parameters. The new parameters gave additional information for a better evaluation of the performance of a drug from a dosage form.

Applying Bayesian ideas in drug development and clinical trials
Spiegelhalter DJ, Freedman LS, Parmar MK. Stat Med 1993 Aug; 12(15–16):1501–11; discussion 1513–7. The Bayesian paradigm emphasizes that studies are not performed in isolation, and that external evidence can be used formally in the design, monitoring and reporting of clinical trials. A variety of tools for assessing the current evidence for treatment efficacy are presented, making use of graphical display to provide insight into ethical and efficiency issues in starting and stopping trials—these are illustrated with a trial in osteosarcoma that is currently taking place. Finally, we recommend that an additional "interpretation" section is placed in clinical reports to provide a bridge between "results" and "discussion"—it is this section that would contain the Bayesian perspective.

Bioequivalence revisited: non-parametric analysis of two-period crossover studies
Wijnand HP. Comput Methods Programs Biomed 1993 Aug; 40(4):249–59. Hauschke et al.'s non-parametric bioequivalence procedure for treatment effects and some aspects of computer

implementation, among them Meineke and De Hey's algorithm and a recursive algorithm, are explored. For studies with up to 60 subjects, a table of indices of the ranked intersubject–intergroup mean ratios or differences is given, to establish non-parametric 90% confidence intervals. It is shown that non-parametric analysis is not limited to treatment effects: it can also be applied to period and sequence effects. This extended procedure can be seen as the non-parametric analog of analysis of variance on two-period crossover studies. A FORTRAN program (BIOEQNEW) incorporating Meineke and De Mey's algorithm is presented. This program provides non-parametric point estimates for treatment and period effects, 90% and 95% confidence intervals for test-versus-reference treatments, the 95% confidence interval for periods and a test on sequence effects, so that it can also be used for other than bioequivalence studies. BIOEQNEW can handle ratios ("multiplicative model") as well as differences ("additive model"). It optionally provides the complete non-parametric posterior probability distribution for treatment ratios or differences, so that Schuirmann's "two one-sided tests procedure" can also be performed in a non-parametric way.

Comparative bioequivalence study of different brands of acetyl salicylic acid in human volunteers

Valecha N, Gupta U, Mehta VL. Eur J Drug Metab Pharmacokinet 1993 Jul–Sep; 18(3):251–3. A double-blind crossover randomized study was conducted in seven normal healthy volunteers. Single dose (700 mg) of buffered aspirin or aspirin with calcium carbonate or aspirin with caffeine was administered orally, at least three days apart. Blood samples were drawn at different time intervals after administration of drug for estimation of salicylate levels. The values of different pharmacokinetic parameters ($AUC_{0-\infty}$, C_{max} and t_{max}) did not show any significant difference, suggesting that these three brands of aspirin are biologically equivalent.

Comparative studies on the in vitro drug dissolution profiles for hydroxyzine hydrochloride tablets

Loucas SP, Maager P, Mehl B. Ann Pharmacother 1993 Jan; 27(1):13–8. OBJECTIVE: A significant practical problem in the standardization of dissolution testing is addressed. In vitro releasing characteristics of hydroxyzine hydrochloride tablets are presented for further documentation of bioequivalency criteria. DESIGN: The assessment model compares the official United States Pharmacopeia disintegration approach for dissolution analysis with that of the Food and Drug Administration's recommended rotating paddle technique for inducing aqueous disruption of the solid oral dosage form. RESULTS: The rationale and significance of the study focus attention on the variation in release of the active ingredient observed relative to the four formulation strengths. With differences in the extent of dissolution noted and official standards in mind, emphasis is placed on the development of an alternate test protocol. CONCLUSIONS: Dissolution data derived via ultraviolet spectrophotometry revealed statistically significant differences in the amount of hydroxyzine hydrochloride being released from its coated structure, the extent of which was found to be dependent on the acid nature of the simulated gastric dissolution medium used and intensity of mixing action employed.

A log-normal model for individual bioequivalence

Phillips KF. J Biopharm Stat 1993 Sep; 3(2):185–201. A log-normal model is developed for testing *pi* 1, the probability that a subject's response will fall within given bioequivalence limits. The model is a parametric analog of Anderson and Hauck's TIER rule. Confidence intervals and hypothesis tests are derived. Statistical power is compared with that of the TIER rule. The probability of demonstrating mean bioequivalence is shown to greatly exceed that of showing individual bioequivalence.

Pharmacokinetic study of the relative bioavailability and bioequivalence after oral intensive or repeated short-term treatment with two polyamino acid formulations

Matera M, Castana R, Insirello L, Leonardi G. Int J Clin Pharmacol Res 1993;13(2):93–105. The authors studied the relative bioequivalence and bioavailability of two oral polyamino acid formulations (packet and flacon), based on four amino acids (l-glutamine, l-phosphoserine,

l-phosphothreonine and l-arginine) in association with vitamin B12 (Bio-logos, Sigma Tau Pharma S.A). Open-trial testing was carried out after intensive treatment and on the attainment of sustained levels. Fifty healthy volunteers (27 males and 23 females), ranging in age from 23 to 32 years, were included in the study. The pharmacokinetic behaviur of the various active ingredients was examined at a hematic level. Possible undesirable side effects, resulting from treatment, were also examined during the study. The mean pharmacokinetic constants considered (K_e 1, C_{max}, and $t_{1/2}$) generated an almost overlapping AUC (area under the curves) for all homologous components contained in both pharmaceutical forms. This indicates almost complete bioequivalence. The mean index for the rate of relative bioavailability was, in fact, estimated to be $106.3 \pm 12.4\%$. Repeated treatment did not appear to disturb the absorption mechanisms of the active ingredients contained in either of the two formulations examined, maintaining the relative bioavailability relationship within a negligible range, with a statistically non-significant difference (Student's *t*-test for coupled data). A few episodes, characterized by slight increases in excitability, were reported for both preparations in two patients (4%).

Pharmacodynamic bioequivalence: Evaluation of different brands of terfenadine hydrochloride

Tekur U, Gupta U, Mehta VL. Indian J Physiol Pharmacol 1993 Oct; 37(4):345–6. Terfenadine is a selective histamine H1 receptor antagonist which binds preferentially to peripheral receptors in vivo and is devoid of central nervous system depressant activity and thus has an improved adverse effect profile. Hence, terfenadine may be considered to be a first-line agent in the treatment of allergic rhinitis and chronic urticaria. In man, terfenadine is rapidly absorbed following a single oral dose and a peak terfenadine plasma concentration is reached within one to two hours after the drug administration. The present study was carried out to compare the bioequivalence of two terfenadine hydrochloride preparations marketed by Kopran Chem. Co. and Merrel Dow, U.K. (Triludan) by evaluating their ability to inhibit the skin reaction to intradermally injected histamine.

1992

Bioavailability and bioequivalence of veterinary drug dosage forms, with particular reference to horses: an overview

Baggot JD. J Vet Pharmacol Ther 1992 Jun; 15(2):160–73. The route of administration and formulation of the dosage form affect the bioavailability (rate and extent of absorption) of a drug and may thereby influence the intensity and duration of the pharmacologic effect. Location of injection site may affect the plasma concentration profile of drugs administered as aqueous suspensions or sustained release parenteral preparations (procaine penicillin G). When absorption influences the rate of elimination ("flip-flop" phenomenon), the apparent half-life of the drug will be increased (cefazolin sodium, i.m; meclofenamic acid, p.o.). Absorption generally approximates a first-order process and either the absorption half-life or the mean absorption time (statistical moment term) will provide an estimate of the rate of absorption. The method of corresponding areas is the usual technique employed in estimating the extent of absorption (systemic availability). Inherent in this technique is the assumption that clearance of the drug remains unchanged. In horses, the time of feeding relative to oral dosing has been shown to affect systemic availability (rifampin, trimethoprim) and pattern of absorption (phenylbutazone). Oral paste formulations (trimethoprim-sulfadiazine, ivermectin) are convenient to administer, allow precision in dosage compared with powders or granules added to feed, and could provide sustained release. Assessment of bioequivalence is based on relative bioavailability, using a reference dosage form, together with a measure of the uncertainty (variance) of the estimate. Bioequivalence relies on the concept that preparations of a drug which provide essentially equivalent plasma concentration profiles should produce the same therapeutic effect.

Bioequivalence of quinidine in two sustained-release preparations

Garty M, Rachmel A, Ilfeld D, Sinai Y, Paz R. Isr J Med Sci 1992 Jun; 28(6):357–61. The bioequivalence of two sustained-release preparations of quinidine bisulfate from Teva

(Israel) and from Astra (Sweden) was assessed in an acute, single-dose randomized crossover study in seven healthy subjects. There was no significant difference in time to peak, peak serum concentration, area under the concentration time curve from 0 to infinity, and the fraction absorbed between quinidine bisulfate 500 mg from Teva and from Astra. In addition, quinidine bisulfate 250 mg from Teva was compared with the short-acting quinidine sulfate 200 mg. The quinidine bisulfate from Teva had a significantly $p < 0.025$ decreased peak serum concentration and an increased time to peak compared with the short-acting quinidine sulfate, although these two drugs are similar for the area under the curve from 0 to infinity. Our pharmaceutical records show that 85% of outpatients receiving quinidine are given the sustained-release quinidine bisulfate. However, only 36% of the outpatients prescribed sustained-release quinidine bisulfate are appropriately prescribed for twice-daily treatment. Thus, the quinidine bisulfate from Teva is a sustained-release preparation with bioequivalence to the reference sustained-release preparation and can be administered twice daily.

The bioequivalence of two different batches of two nifedipine controlled-release preparations with deficient batch conformity in in vitro liberation studies
Theiss U, Gebhardt E, Muller J. Arzneimittelforschung 1992 May; 42(5):629–32. The pharmacokinetics of two batches of two different nifedipine (CAS 21829-25-4) controlled-release formulations (product 1 or 2, respectively) were investigated during two randomized double-blind crossover studies testing two treatments each in two separate treatment periods with a total of 40 (2×20) healthy male volunteers. Plasma concentrations of nifedipine were determined up to 48 hours after administration of 20 mg nifedipine. In spite of different in vitro release rates a positive bioequivalence was determined for product 1, while no bioequivalence could be proven with the investigated batches of product 2. This is compatible with the different in vivo release rates. Therefore, a different in vitro release of a drug does not necessarily lead to a negative bioequivalence decision. On the other hand, deficient conformity of batches in vitro can go along with missing bioequivalence in vivo. A harmless substitution of formulations containing equal substances requires their therapeutical equivalence. A constantly high pharmaceutical quality is required to avoid therapeutic risks.

Bioequivalence studies of topical preparations: statistical considerations
Hauck WW. Int J Dermatol 1992 Oct; 31 Suppl. 1:29–33. To be approved for marketing, a potential generic pharmaceutical product must demonstrate bioequivalence, that is, a rate and extent of absorption similar to those of the currently marketed ("innovator") product. For oral products, design, and statistical analysis for studies conducted to determine whether two products are bioequivalent have become reasonably standardized; the design is crossover, and analysis is based on the two one-sided tests principle. The purpose of this overview is to consider whether the practices for oral products apply to topical products, and where different procedures may be required. The principles behind the practices for oral products are seen, largely, to carry over to topical products.

Influence of higher rates of agitation on release patterns of immediate-release drug products
Shah VP, Gurbarg M, Noory A, Dighe S, Skelly JP. J Pharm Sci 1992 Jun; 81(6):500–3. The dissolution procedure serves as a quality control test to assure batch-to-batch uniformity and bioequivalence of a product once the bioavailability of the product has been established. It can also be used to detect manufacturing and/or process variations that could reduce product bioavailability. Dissolution testing must be conducted at an appropriate agitation rate. Tests conducted at high agitation rates may lose the ability to differentiate between good and bad products. Although the effect of high agitation rates has been known for some time, several immediate-release drug products still have United States Pharmacopeia (USP) monograph dissolution procedures that require very high agitation rates. A systematic survey was conducted on marketed tablets of chloroquine phosphate, griseofulvin, hydroxychloroquine sulfate, isocarboxazide, primaquine phosphate, and sulfadiazine. Each of these products has a USP monograph requiring a dissolution test at a paddle speed of 100 rpm. To study the influence of agitation rate on the dissolution rate of these products, dissolution studies were conducted at

paddle speeds of 50, 75, and 100 rpm with the USP apparatus 2 (paddle method). The dissolution rate increased with an increase in the agitation rate from 50 to 75 rpm. However, no significant increase in the dissolution rate was noted with an increase in the agitation rate from 75 to 100 rpm. The data support the position that the higher agitation rate of 100 rpm is not necessary for a quality control procedure or a compendial standard for the products tested.

On the assessment of bioequivalence in a two-period crossover design
Wijnand HP. Comput Methods Programs Biomed. 1992 Mar;37(2):151–7. The results obtained with Meineke and De Mey's algorithm for posterior probability distributions in the nonparametric evaluation of two-period cross-over bioequivalence studies are critically discussed. Suggestions for improvement of their program NEWPARM are given. It is shown that this program in its present form cannot handle the results from bioequivalence studies in which more than 15 subjects participated. For larger study sizes, conversion to a more powerful programming language capable of handling large three-dimensional arrays, is mandatory. An alternative algorithm, allowing large sample sizes to be analysed nonparametrically without program conversion, is offered. A general bioequivalence program written by the author, into which this alternative algorithm is implemented, has been in use by a number of pharmaceutical companies and drug regulatory agencies since February 1990; its most recent update is Version 3.7 (BIOEQV37.EXE) of October 1990.

Pharmacodynamics as a tool to assess the bioequivalence of non-systemically available drugs: size of the sample required
Du Souich P, Besner JG, Caille G. Biopharm Drug Dispos 1992 May; 13(4):233–42. Bioequivalence studies of drugs which are not systematically available must rely on the measure of the pharmacologic response. Detection of a difference between two such preparations is often hampered by the need to include an elevated number of subjects. The number of subjects can be reduced whenever: (*i*) the characteristics of the subjects are well defined, (*ii*) the selection of the baseline target effect is done rigorously, (*iii*) the target effect can be quantified reliably, (*iv*) the effect is measured when less variability is expected, e.g. at steady state, (*v*) the effect is measured repeatedly, and (*vi*) when possible, the predicted maximal effect (E_{max}) and the concentration to elicit 50% of E_{max} are estimated. A simple equation has been derived to estimate the number of subjects needed in these bioequivalence studies.

Presentation of results from bioequivalence studies
Sauter R, Steinijans VW, Diletti E, Bohm A, Schulz HU. Int J Clin Pharmacol Ther Toxicol 1992 Jul; 30(7):233–56. Based on general guidelines and requirements for the design and analysis of bioequivalence studies, specific recommendations are made for the presentation of results, both in tabular and graphical form. This is done by means of two examples, one of a single-dose study and one of a multiple-dose study. The recommendations in this paper are twofold. Firstly, a complete and rather detailed presentation of results is given, which practically corresponds to the standard of research reports. Secondly, a subset of this is suggested for publication. It gives the essential results for bioequivalence assessment in a standardized form. From an editorial point of view, it would be highly appreciated if the papers submitted for publication were always accompanied by a complete presentation including the individual concentration/time data and the various steps of calculation. This would speed up peer review and ultimately improve and harmonize the standard of bioequivalence publications.

A retrospective assessment of the 75/75 rule in bioequivalence
Dobbins TW, Thiyagarajan B. Stat Med 1992 Jul; 11(10):1333–42. The 75/75 rule was originally proposed by the U.S. Food and Drug Administration (FDA) as an alternative means of testing the bioequivalence of two formulations of a pharmaceutical agent. The rule specified that the ratio of test-to-reference formulation of a bioavailability measure arising in a bioequivalence study must be between 75% and 125% of unity in at least 75% of subjects to declare two formulations bioequivalent. The rule has garnered criticism in the literature and the FDA no longer uses the rule formally in assessing bioequivalence.

The basis, however, for all criticism of the rule has been simulation arguments. In this paper, we derive the sampling model implied by the rule and place the rule in the framework of a statistical hypothesis test. We show how the significance level of the test depends upon variability of the formulations, and thus why the rule performs in the way that has received criticism.

Statistical aspects of bioequivalence: a review
Pidgen AW. Xenobiotica 1992 Jul; 22(7):881–93. 1. Over the past 20 years a number of statistical methods have been proposed for use in bioequivalence testing. This review examines these methods and reflects current thinking of regulatory authorities. 2. The standard bioequivalence study is conducted as a controlled, single-dose crossover design in a small number of healthy male adults. Blood and/or urine samples are taken at predetermined times for drug/metabolite assay from which pharmacokinetic parameters are derived and compared statistically. Sample size calculations should be determined by the error variance associated with the primary characteristic to be studied, the significance level, the power of the test, and the deviation from the reference product compatible with safety and efficacy. 3. In general, bioequivalence is assessed using three parameters namely, C_{max}, t_{max} and AUC. Urinary excretion data may also be used if the amount excreted unchanged is significant. These parameters are best obtained using a simple model-independent approach. 4. The parameters of C_{max} and AUC should be logarithmically transformed prior to analysis. For t_{max}, parametric statistical procedures are not appropriate. 5. Classical hypothesis testing using the power approach is not applicable to the practical problem under consideration in bioequivalence trials. 6. Classical 90% confidence limits and the two one-sided *t*-test approach are operationally identical and are the methods of choice for assessing bioequivalence (C_{max} and AUC). When t_{max} is an important parameter from the clinical point of view then the use of non-parametric confidence intervals is recommended.

Therapeutic bioequivalency study of brand name versus generic carbamazepine
Oles KS, Penry JK, Smith LD, Anderson RL, Dean JC, Riela AR. Neurology 1992 Jun; 42(6): 1147–53. We performed a randomized double-blind crossover therapeutic bioequivalency study of a generic (Epitol) versus a brand name (Tegretol) carbamazepine product under steady-state conditions in 40 epileptic patients. Each patient received 90-day supplies of Epitol or Tegretol and placebo, which replaced the usual dosage of the alternate product. Group A consisted of 20 seizure-free (from five months to two years) patients and group B of 20 patients with seizures refractory to drug therapy. In group A, four patients had seizures, two on both Epitol and Tegretol and two on Tegretol. In group B, the average seizure frequencies were 0.25 seizures per day on Epitol and 0.22 seizures per day on Tegretol. Average seizure frequencies were statistically the same (at a 20% difference, $p < 0.05$). Areas under the curve were statistically the same (at a 20% difference, $p = 0.05$). Average peak heights were statistically the same (at a 20% difference, $p < 0.05$). Average time to peak was earlier with Epitol. Epitol and Tegretol performed equally well in clinical efficacy and bioequivalency.

The vasoconstrictor assay in bioequivalence testing: practical concerns and recent developments
Stoughton RB. Int J Dermatol 1992 Oct; 31 Suppl. 1:26–8. The vasoconstrictor assay, when properly performed, is a highly reliable method to determine bioequivalence of generic formulations. Recent research has resolved some of the remaining questions concerning the practical application of the assay. Significant vehicle-related differences have been observed between the potency of different, supposedly equivalent formulations now on the market. Large differences in concentrations of the active agent in similar vehicles usually have not resulted in corresponding differences in vasoconstrictor assay results. Finally, the time course of drug effects may differ among highly potent and less potent corticosteroids. In general, the higher the potency of the topical corticosteroid, the earlier the maximal effect is observed. This finding suggests that short application of highly potent agents might minimize systemic absorption without sacrificing efficacy.

Appendix III: Dissolution Testing Methods of Approved Drugs

Drug name	Dosage form	USP apparatus	Speed (RPMs)	Medium	Volume (mL)	Recommended sampling times	Date updated
Abacavir	Tablet	II (paddle)	75	0.1 N HCl	900	5, 10, 15, and 30 min	03/22/2006
Abacavir sulfate/Lamivudine	Tablet	II (paddle)	75	0.1 N HCl	900	10, 20, 30, and 45 min	01/03/2007
Abacavir sulfate/Lamivudine/ Zidovudine	Tablet	II (paddle)	75	0.1 N HCl	900	5, 10, 15, 30, and 45 min	01/03/2007
Acamprosate calcium	Tablet (delayed release)	I (basket)	180	Acid stage: 0.1 N HCl buffer stage: "citrate-sodium hydroxide" buffer pH 6.8 (150 mL of 2 N NaOH, 21.014 gm of citric acid and ultra-pure water to 1000 mL) (method B)	1000	120 (acid) 30, 60, 90, 120, and 180 min (buffer)	12/20/2005
Acarbose	Tablet	II (paddle)	75	Water (deaerated)	900	10, 15, 20, 30 and 45 min	03/22/2006
Acetaminophen	Suppository	II (paddle)	50	Phosphate buffer, pH 5	900	15, 30, 45, 60 and 90 min	08/17/2006
Acetaminophen/ Butalbital	Tablet	II (paddle)	50	Water (deaerated)	900	15, 30, 45, 60 and 90 min	01/03/2007
Acetaminophen/ Butalbital/Caffeine/ Codeine phosphate	Capsule	II (paddle)	50	Water (deaerated)	900	10, 20, 30, 45 and 60 min	03/04/2006
Acetaminophen/ Caffeine/ Dihydrocodeine bitartrate	Capsules	I (basket)	100	Water	900	10, 20, 30, 45 and 60 min	01/03/2007
Acetaminophen/ Pentazocine HCl	Tablet	I (basket)	100	Water (deaerated)	900	10, 20, 30, 45 and 60 min	01/12/2004
Acetaminophen/ Tramadol HCl	Tablet	II (paddle)	50	0.1 N HCl	900	5, 10, 15, 20 and 30 min	03/04/2006
Acitretin	Capsule	I (basket)	100	3% SLS in water, pH 9.6	900	10, 20, 30 and 45 min	01/12/2004
Acrivastine/ Pseudoephedrine HCl	Capsule	II (paddle)	50	0.01 N HCl	900	5, 10, 15 and 30 min	01/12/2004
Acyclovir	Suspension	II (paddle)	50	0.1 N HCl	900	10, 20, 30, 45 and 60 min	02/20/2004
Almotriptan malate	Tablet	II (paddle)	50	0.1 N HCl	900	5, 10, 15, and 30 min	01/20/2006
Alosetron HCl	Tablet	II (paddle)	50 (for 1 mg) and 75 (for 0.5 mg)	Water (deaerated)	500	10, 20, 30 and 45 min	01/26/2006
Amantadine HCl	Tablet	II (paddle)	50	Water (deaerated)	500	10, 20, 30, 45 and 60 min	01/12/2004
Amiodarone HCl (test 1)	Tablet	II (paddle)	100	1% SLS in water	1000	10, 20, 30, 45, 60 and 90 min	01/12/2004

Amiodarone HCl (test 2)	Tablet	I (basket)	50	Acetate buffer, pH 4.0, with 1% Tween 80	900	10, 20, 30, 45, 60 and 90 min	01/12/2004
Amlodipine besylate	Tablet	II (paddle)	75	0.01 N HCl	500	10, 20, 30, 45 and 60 min	01/14/2004
Amlodipine besylate/ Benazepril HCl	Capsule	I (basket)	100	0.01 N HCl	500	10, 20, 30, 45 and 60 min	01/14/2004
Amoxicillin/Clavulanate potassium	Suspension	II (paddle)	75	Water (deaerated)	900	5, 10, 15 and 30 min	01/14/2004
Amphetamine ER	Capsule	II (paddle)	50	750 mL of dilute HCl, pH 1.1 for the first 2 hr, then add 200 mL of 200 mM phosphate buffer, and adjust to pH 6 (w/HCl or NaOH) for the remainder	750 mL of dilute HCl, 200 mL of phosphate buffer	1, 2, 3, 4, and 6 hr	08/17/2006
Ampicillin/Ampicillin trihydrate	For oral suspension	II (paddle)	25	Water (deaerated)	900	5, 10, 15, 20 min	01/03/2007
Anagrelide HCl	Capsule	I (basket)	100	0.1 N HCl	900	5, 10, 15, 30 and 45 min	01/14/2004
Anastrozole	Tablet	II (paddle)	50	Water	900	5, 10, 15, and 30 and 45 min	01/03/2007
Aprepitant	Capsule	II (paddle)	100	2.2% SDS in distilled water	900	10, 15, 20, 30 and 45 min	01/20/2006
Aripiprazole	Tablet	II (paddle)	60	pH 1.2 USP buffer (hydrochloric acid)	900	10, 20, 30 and 45 min	12/20/2005
Aspirin/Caffeine/ Orphenadrine citrate	Tablet	I (basket)	75	Water (deaerated)	900	10, 20, 30, 45 and 60 min	01/15/2004
Aspirin/Hydrocodone bitartrate	Tablet	II (paddle)	75	Acetate buffer, pH 4.5	900	10, 20, 30, 45, 60 and 90 min	01/15/2004
Aspirin/Meprobamate	Tablet	I (basket)	100	Water (deaerated)	900	10, 20, 30, 45, 60 and 90 min	01/15/2004
Aspirin/Methocarbamol	Tablet	II (paddle)	50	Water (deaerated)	900	10, 20, 30, 45, 60 and 90 min	01/15/2004
Atazanavir	Capsule	II (paddle)	50	0.025 N HCl	1000	10, 20, 30 and 45 min	01/20/2006
Atomoxetine HCl	Capsule	II (paddle)	50	0.1 N HCl	1000	10, 20, 30 and 45 min	12/20/2005
Atorvastatin calcium	Tablet	II (paddle)	75	0.05 M phosphate buffer, pH 6.8	900	5, 10, 15 and 30 min	01/15/2004
Atovaquone/ Proguanil HCl	Tablet	II (paddle) with PEAK vessels	50	40% isopropranol buffered to pH 8.0 with potassium dihydrogen phosphate	900	15, 30, 45 and 60 min	08/17/2006
Auranofin	Capsule	II (paddle)	50	Water (deaerated)	900	10, 20, 30, and 45 min	01/15/2004
Azithromycin	Oral suspension	II (paddle)	50	Phosphate buffer, pH 6.0	900	10, 20, 30, and 45 min	08/17/2006
Azithromycin dihydrate	Film-coated tablet	II (paddle)	75	Phosphate Buffer, pH 6.0	900	10, 20, 30, and 45 min	01/03/2007
Balsalazide disodium	Capsule	II (paddle) with sinker	50	pH 6.8 buffer	900	10, 20, 30, and 45 min	01/26/2006
Benazepril HCl	Tablet	II (paddle)	50	Water (deaerated)	500	10, 20, 30 and 45 min	01/16/2004
Benazepril HCl/ Hydro-chlorothiazide	Tablet	I (basket)	100	0.1 N HCl	500	10, 20, 30 and 45 min	01/16/2004
Benzonatate	Capsule	II (paddle)	50	Water (deaerated)	900	10, 20, 30, 45 and 60 min	03/04/2006
Benzphetamine HCl	Tablet	II (paddle)	50	Water (deaerated)	900	10, 20, 30 and 45 min	01/16/2004
Bepridil HCl	Tablet	I (basket)	100	0.1 N HCl	900	10, 20, 30, 45 and 60 min	01/16/2004

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Drug name	Dosage form	USP apparatus	Speed (RPMs)	Medium	Volume (mL)	Recommended sampling times	Date updated
Bexarotene	Capsule	II (paddle)	50	Tier 1 Medium: 0.5% HDTMA in 0.05 M phosphate buffer, pH 7.5 Tier 2 medium: 0.5% HDTMA in 0.05 M phosphate buffer, pH 7.5 with 0.05 g/L pancreatin enzyme	900	15, 30, 45 and 60 min	08/17/2006
Bicalutamide	Tablet	II (paddle)	50	1% SLS in water	1000	10, 20, 30, 45 and 60 min	12/15/2005
Bisoprolol fumarate/ Hydro-chlorothiazide	Tablet	II (paddle)	75	0.1 N HCl	900	5, 10, 20, 30 and 45 min	01/20/2004
Cabergoline	Tablet	II (paddle)	50	0.1 N HCl	500	5, 10, 15 and 30 min	01/20/2004
Candesartan cilexetil (16 mg)/ Hydrochlorothiazide (12.5)	Tablet	II (paddle)	50	0.35% polysorbate 20 in phosphate buffer pH 6.5	900	10, 20, 30, 45, and 60 min	03/04/2006
Candesartan Cilexetil (32 mg)/ Hydrochloro-thiazide (12.5)	Tablet	II (paddle)	50	0.70% polysorbate 20 in phosphate buffer pH 6.5	900	15, 20, 30, 45 and 60 min	03/04/2006
Candesartan Cilexetil (all strengths)	Tablet	II (paddle)	50	0.35% polysorbate 20 in 0.05 M phosphate buffer, pH 6.5	900	10, 20, 30, 45 and 60 min	03/04/2006
Capecitabine	Tablet	II (paddle)	50	Water (deaerated)	900	10, 20, 30 and 45 min	01/23/2004
Carbamazepine	Suspension	II (paddle)	50	Water (deaerated)	900	10, 20, 30, 45 and 60 min	01/20/2004
Carbidopa/Entacapone/ Levodopa	Tablet	I (basket)	Carbidopa and Levodopa: 50; Entacapone: 125	For both carbidopa and levodopa: 0.1 N HCl, For entacapone: phosphate buffer pH 5.5	Carbidopa and Levodopa: 750 mL. Entacapone: 900 mL	10, 20, 30, 45 and 60 min	01/03/2007
Carvedilol	Tablet	II (paddle)	50	SGF without enzyme	900	10, 20, 30 and 45 min	01/21/2004
Cefditoren piivoxil	Tablet	II (paddle)	75	Simulated gastric fluid without enzyme	900	5, 10, 15, 20 and 30 min	02/09/2006
Cefpodoxime proxetil	Suspension	II (paddle)	50	Glycine buffer (0.04 M) pH 3.0	900	10, 20, 30 and 45 min	12/20/2005
Cefprozil monohydrate	Suspension	II (paddle)	25	Water (deaerated)	900	5, 10, 15 and 30 min	01/21/2004
Ceftibuten dihydrate	Suspension	II (paddle)	50	0.05 M phosphate buffer, pH 7.0	1000	10, 20, 30 and 45 min	01/21/2004
Celecoxib	Capsule	II (Paddle)	100 mg and 200 mg: 50 rpm; 400 mg: 75 rpm	Tier 1 medium: 0.04 M tribasic sodium phosphate (pH 12) with 1% SLS Tier 2 initial medium: 750 mL of SGF, USP (includes pepsin) At 20 min, 180 mL of 5% SLS solution and 70 mL of 1.2 N NaOH are added to initial medium.Tier 2 final medium: 1% SLS, pH 12	Tier 1: 1000 mL Tier 2: 750 mL (initial) 1000 mL (final)	15, 30, 45 and 60 min	08/17/2006

Cetirizine HCl	Tablet (regular & chewable)	II (paddle)	50	Water (deaerated)	900	10, 20, 30 and 45 min	03/04/2006
Cevimeline HCl	Capsule	II (paddle) with option to use a sinker	50	0.1 N HCl	900	5, 10, 15, and 30 min	01/26/2006
Chlorambucil	Tablet	II (paddle)	75	0.1 N HCl	900	10, 20, 30, and 45 min	08/17/2006
Chlorpheniramine maleate/Ibuprofen/ Pseudoephedrine HCl	Tablet	II (paddle)	50	0.05 M phosphate buffer, pH 6.5	900	10, 20, 30 and 45 min	02/20/2004
Cilostazol	Tablet	II (paddle)	75	0.3% SLS in water	900	15, 30, 45, 60 and 90 min	08/17/2006
Cinacalcet HCl	Tablet	II (paddle)	75	0.05 N HCl	900	10, 20, 30 and 45 min	01/26/2006
Citalopram HBr	Tablet	I (basket)	100	Buffer (1 N HCl/1 N NaOH), pH 1.5	800	10, 20, 30 and 45 min	07/13/2004
Clarithromycin	Suspension	II (paddle)	50	0.05 M phosphate buffer, pH 6.8	900	10, 20, 30, 45 and 60 min	01/23/2004
Clopidogrel bisulfate	Tablet	II (paddle)	50	KCl/HCl buffer, pH 2.0	1000	10, 20, 30 and 45 min	01/23/2004
Clotrimazole (vag. tablets)	Tablet (vaginal)	II (paddle)	50	0.1 N HCl	900	10, 20, 30 and 45 min	01/24/2004
Cyclophosphamide	Tablet	I (basket)	100	Water (deaerated)	900	10, 20, 30, 45 and 60 min	01/24/2004
Cyclosporine (100 mg) (RLD: Neoral⊃®)	Capsule (liquid filled)	II (paddle)	75	0.1 N HCl containing 4 mg of *N,N*-dimethydodecylamine-*N*-oxide per mL	1000	10, 20, 30, 45, 60 and 90 min	02/20/2004
Cyclosporine (25 mg) (RLD: Neoral)	Capsule (liquid filled)	II (paddle)	75	0.1 N HCl containing 4 mg of *N,N*-dimethydodecylamine-*N*-oxide per mL	500	10, 20, 30, 45, 60 and 90 min	02/20/2004
Cysteamine bitartrate	Capsule	I (basket)	75	0.1 N HCl	900	10, 20, 30 and 45 min	01/24/2004
Dantrolene sodium	Capsule	I (basket)	100	0.5% Hyamine 10× in water, adjust to pH 6.8 with 0.1 N KOH or 0.1 N HCl	900	10, 20, 30, 40 and 60 min	01/27/2004
Darifenacin hydrobromide	Tablet (extended release)	I (basket)	100	0.01 M HCl comparative dissolution data should also be provided in 900 mL pH 4.5 buffer, pH 6.8 buffer, and water using apparatus I (basket) at 100 RPM.	900	1, 4, 8, 12, 16, 20 and 24 hr	01/20/2006
Darunavir ethanolate	Tablet	II (paddle)	75	2% Tween-20 in 0.05 M sodium phosphate buffer, pH 3.0	900	10, 20, 30, and 45 min	01/03/2007
Deferasirox	Tablet (for oral suspension)	II (paddle)	50	Phosphate buffer pH 6.8 with 0.5% Tween 20	900	10, 20, 30 and 45 min	06/21/2006
Desloratadine	Tablet	II (paddle)	50	0.1 N HCl	500	15, 20, 30 and 45 min	03/04/2006
Desmopressin acetate	Tablet	II (paddle)	75	Water (deaerated)	500	10, 20, 30 and 45 min	12/15/2005

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Drug name	Dosage form	USP apparatus	Speed (RPMs)	Medium	Volume (mL)	Recommended sampling times	Date updated
Desogestrel/Ethinyl estradiol	Tablet	II (paddle)	50	0.05% SLS in water	500	10, 20, 30 and 45 min	01/28/2004
Dextromethorphan polistirex	Suspension	II (paddle)	50	0.1 N HCl	500	30, 60, 90 and 180 min	03/04/2006
Diclofenac potassium	Tablet	II (paddle)	50	SIF without enzyme	900	10, 20, 30, 45, 60 and 90 min	01/27/2004
Diclofenac/ Misoprostol enteric coated (arthrotec)	Tablet	II (paddle) (diclo) II (paddle) (miso)	100 (diclo) 50 (miso)	Diclofenac: acid stage: 0.1 N HCl buffer stage: 750 mL 0.1 N HCl + 250 mL 0.2 M phos.buffer, pH 6.8 (method A) misoprostol: water (deaerated)	Diclo: acid: 750 buffer:1000 miso: 500	Diclo.: 120 (acid) 15, 30, 45 and 60 min (buffer). miso:10, 20 and 30 min	12/15/2005
Didanosine (chewable)	Tablet (chewable)	II (paddle)	75	Water (deaerated)	900	10, 20, 30 and 45 min	01/26/2004
Didanosine (delayed release)	Capsule (delayed release pellets)	I (basket)	100	Acid stage: 0.1 N HCl; buffer stage: 0.1 N HCl:0.2 M tribasic sodium phosphate (3:1), pH 6.8	1000	Acid stage: 60, 90 and 120 min; buffer stage: 10, 20, 30, 45 and 60 min	01/26/2004
Divalproex sodium	Capsule (sprinkle)	II (paddle)	50	0.05 M phosphate buffer, pH 7.5	500	2, 4, 6, 8 and 10 hr	03/04/2006
Dofetilide	Capsule	I (basket)	100	0.001 M HCl	900	10, 15, 30 and 45 min	01/20/2006
Donepezil	Tablet [orally disintegrating (ODT)]	II (paddle)	50	0.1 N HCl	900	10, 20, 30 and 45 min	03/04/2006
Donepezil HCl	Tablet	II (paddle)	50	0.1 N HCl	900	10, 20, 30 and 40 min	01/27/2004
Doxazosin mesylate	Tablet	II (paddle)	50	0.01 N HCl	900	10, 20, 30, 45 and 60 min	01/27/2004
Doxazosin mesylate	Tablet	II (paddle)	75	SGF without enzyme	900	4, 8, 16 hr	01/03/2007
Drospirenone/Estradiol	Tablet	II (paddle)	50	Water	900	10, 20, 30, and 45	01/03/2007
Duloxetine	Capsule (delayed release pellets)	I (basket)	100	[A] Gastric challenge: 0.1 N HCl [B] buffer medium: pH 6.8 phosphate buffer (USP)	1000	120 min (for A) 15, 30, 45, 60 and 90 min (for B)	03/22/2006
Dutasteride	Capsule (soft gelatin)	II (paddle)	50	Tier I: dissolution medium: 0.1 N HCl with 2% (w/v) SDS (900 mL) Tier II: dissolution medium: 0.1 N HCl with pepsin (1.6 g/L, label activity 1:3,000) (450 mL) for the first 25 min, followed by addition of 0.1 N HCl with SDS (4% w/v) (450 mL) for the remainder of the dissolution test.	900	15, 30, 45 and 60 min	01/26/2006
Efavirenz	Capsule	II (paddle) a sinker may be used with justification if necessary.	50	1% SLS in water	900	15, 30, 45 and 60 min	03/22/2006

Efavirenz 600 mg; Emtricitabine 200 mg; Tenofovir disoproxil fumarate 300 mg	Tablet	II (paddle)	100	2% SLS in water	1000	10, 20, 30, and 45 min	01/03/2007
Emtricitabine	Capsule	II (paddle)	50	Tier 1: 0.1 N HCl Tier 2: 0.1 N HCl containing Pepsin 750,000 USP units/L. Tier 2 is used after failure of Tier 1 testing	900	10, 20, 30 and 45 min	12/16/2005
Emtricitabine/ Tenofovir disoproxil fumarate	Tablet	II (paddle)	50	0.01 N HCl	900	5, 10, 15, 30 and 45 min	01/03/2007
Entacapone	Tablet	II (paddle)	50	Phosphate buffer, pH 5.5	900	10, 20, 30 and 45 min	01/29/2004
Entecavir	Tablet	II (paddle)	50	Phosphate buffer pH 6.8 (50 mM)	1000	10, 20, 30, and 45 min	06/21/2006
Eplerenone	Tablet	II (paddle)	50	0.1 N HCl	1000	10, 20, 30 and 45 min	12/19/2005
Erlotinib	Tablet	II (paddle)	75	0.1 N HCl containing 1% SDS	1000	15, 30, 45 and 60 min	03/22/2006
Erythromycin ethylsuccinate	Suspension	II (paddle)	75	Monobasic sodium phosphate, pH 6.8 buffer with 1% SLS buffer w/1% SLS	900	10, 20, 30, 45 and 60 min	01/27/2004
Escitalopram oxalate	Tablet	II (paddle)	75	0.1 N HCl	900	10, 20, 30 and 45 min	02/20/2004
Esomeprazole magnesium	Capsule (delayed release pellets)	II (paddle)	100	Acid stage: 0.1 N HCl; buffer stage: sodium phosphate buffer, pH 6.8	Acid stage: 300; buffer stage: 1000	Acid stage: 60, 90 and 120 min; buffer stage: 10, 20, 30, 45 and 60 min	02/26/2004
Estazolam	Tablet	II (paddle)	50	Water (deaerated)	900	10, 20, 30 and 45 min	01/27/2004
Estradiol	Vaginal ring	Incubator shaker	130	0.9% saline	250	1, 9, 16, 17, 18, 19, 45 days	01/03/2007
Estradiol/Norgestimate (1 mg/0.09 mg)	Tablet	II (paddle)	50	0.3% SLS in water	500	10, 20, 30 and 45 min	07/09/2004
Ethinyl estradiol/ Norgestimate (RLD: ortho cyclen-28)	Tablet	II (paddle)	75	0.05% Tween 20 in water	600	10, 20, 30 and 45 min	03/02/2004
Ethinyl estradiol/ Norgestimate (RLD: ortho tri-cyclen Lo)	Tablet	II (paddle)	75	0.05% Tween 20 in water	600	5, 10, 20 and 30 min	03/02/2004
Ethinyl estradiol/ Norgestimate (RLD: ortho tri-cyclen)	Tablet	II (paddle)	75	0.05% Tween 20 in water	600	5, 10, 20, 30 and 45 min	01/28/2004
Ethinyl estradiol/ Norgestrel	Tablet	II (paddle)	75	Water with 5 ppm of Tween 80	500	10, 20, 30, 45, 60 and 90 min	01/28/2004
Exemestane	Tablet	I (basket)	100	0.5% (w/v) SLS solution	900	10, 20, 30, and 45 min	08/17/2006
Ezetimibe/Simvastatin	Tablet	II (paddle)	50	0.01 M sodium phosphate, pH 7.0/0.5% SDS	900	5, 10, 20, and 30 min	01/03/2007

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Drug name	Dosage form	USP apparatus	Speed (RPMs)	Medium	Volume (mL)	Recommended sampling times	Date updated
Famotidine	Tablet (orally disintegrating)	II (paddle)	50	Water (deaerated)	900	2, 4, 6, 8 and 10 hr	01/29/2004
Famotidine	Tablet (chewable)	II (paddle)	50	0.1 M phosphate buffer, pH 4.5	900	10, 20, 30, 45 and 60 min	01/29/2004
Famotidine/Antacid combination berry and mint flavors	Tablet (chewable)	III (20 mesh top screen, 40 mesh bottom screen)	30 DPM	0.1 M acetate buffer, pH 4.5	900	10, 20, 39 and 45 min	03/04/2006
Felbamate	Suspension	II (paddle)	50	Water (deaerated)	900	5, 10, 15 and 30 min	01/28/2004
Felbamate	Tablet	II (paddle)	50	Water (deaerated)	900	10, 20, 30, 45, 60 and 90 min	01/28/2004
Fenofibrate	Tablet	II (paddle)	50	0.05 M SLS in water	1000	10, 20, 30 and 45 min	01/29/2004
Fenofibrate	Capsule (micronized)	II (paddle)	75	0.05 M SLS in water	1000	10, 20, 30, 40 and 60 min	01/29/2004
Fexofenadine HCl	Tablet	II (paddle)	50	0.001 N HCl	900	5, 10, 20, 30 and 45 min	02/19/2004
Fexofenadine HCl	Capsule	II (paddle)	50	Water (deaerated)	900	10, 20, 30, 45 and 60 min	01/29/2004
Flavoxate HCl	Tablet	I (basket)	100	0.1 N HCl	900	5, 10, 20 and 30 min	01/29/2004
Fluconazole	Tablet	II (paddle)	50	Water (deaerated)	900 (for 150, 200, 300 and 400 mg tabs) 500 (for 50 and 100 mg tabs)	10, 20, 30, 45 and 60 min	03/04/2006
Fluconazole (200 mg/5 mL)	Suspension	II (paddle)	50	Water (deaerated)	900	10, 20, 30 and 45 min	01/30/2004
Fluconazole (50 mg/5 mL)	Suspension	II (paddle)	50	Water (deaerated)	500	10, 20, 30 and 45 min	01/30/2004
Fluoxetine HCl	Tablet	I (basket)	100	0.1 N HCl	1000	5, 10, 15 and 30 min	01/03/2007
Fluoxetine/ Olanzapine	Capsule	II (paddle)	50	0.1 N HCl	900	10, 20, 30 and 45 min	08/17/2006
Fluvoxamine maleate	Tablet	II (paddle)	50	Water (deaerated)	900	10, 20, 30, and 45 min	01/03/2007
Fosamprenavir calcium	Tablet	II (paddle)	75	250 mM sodium Acetate/acetic acid buffer pH 3.5	900	10, 20, 30 and 45 min	12/16/2005
Fosinopril sodium	Tablet	II (paddle)	50	Water (deaerated)	900	10, 20, 30 and 45 min	01/30/2004
Fosinopril sodium/ Hydro-chloro thiazide	Tablet	II (paddle)	50	Water (deaerated)	900	10, 20, 30, 45 and 60 min	01/30/2004
Gabapentin	Tablet	II (paddle)	50	0.06 N HCl	900	10, 20, 30 and 45 min	01/30/2004
Gabapentin	Capsule	II (paddle)	50	0.06 N HCl	900	5, 10, 20 and 30 min	01/30/2004

Galantamine	Capsule (extended release)	II (paddle)	50	50 mM potassium dihydrogen phosphate buffer pH 6.5 comparative dissolution data should also be provided in 900 mL pH 0.1 HCl, pH 4.5 buffer, and water using apparatus II (paddle) at 50 RPM.	900	1, 4, 10 and 12 hr	01/20/2006
Galantamine HBr	Tablet	II (paddle)	50	Water (deaerated)	500	5, 10, 20 and 30 min	03/04/2006
Ganciclovir	Capsule	II (paddle)	60	Water (deaerated)	900	10, 20, 30, 45 and 60 min	02/02/2004
Gemifloxacin mesylate	Tablet	II (paddle)	50	0.01 N HCl	900	10, 20, 30, and 45 min	01/03/2007
Glimepiride	Tablet	II (paddle)	75	Phosphate buffer, pH 7.8	900	5, 10, 15 and 30 min	07/23/2004
Glimepiride/ Rosiglitazone maleate	Tablet	II (paddle)	75	0.01 M HCl with 0.5% SDS	900	5, 10, 15, 30, 45 and 60 min	01/03/2007
Glipizide/Metformin HCl	Tablet	II (paddle)	50	Phosphate buffer, pH 6.8	1000	10, 20,30, 45 and 60 min	03/04/2006
Glyburide (micronized)	Tablet	II (paddle)	50	0.05 M phosphate buffer, pH 7.5	900	10, 20, 30, 45 and 60 min	02/02/2004
Glyburide (non-micronized)	Tablet	II (paddle)	75	0.05 M borate buffer, pH 9.5	500	10, 20, 30, 45 and 60 min	02/02/2004
Glyburide and Metformin HCl	Tablet	II (paddle)	Glyburide: 75 Metformin: 50	Glyburide: borate buffer, pH 9.5 metformin: phosphate buffer, pH 6.8	Glyburide: 500 Metformin: 1000	5, 10, 15, and 30 min	01/03/2007
Granisetron HCl	Tablet	II (paddle)	50	Phosphate buffer, pH 6.5	500	10, 20, 30, 45 and 60 min	06/05/2006
Guaifenesin	Tablet (extended release)	I (basket)	75	0.1 N HCl	900	1, 2, 4, 6 and 12 hr	01/03/2007
Homatropine methylbromide/ Hydrocodone bitartrate	Tablet	II (paddle)	50	Water (deaerated)	900	10, 20, 30 and 45 min	02/03/2004
Hydrochlorothiazide	Capsule	I (basket)	100	0.1 N HCl	900	10, 20, 30 and 45 min	02/03/2004
Hydrochlorothiazide/ Lisinopril	Tablet	II (paddle)	50	0.1 N HCl	900	10, 20, 30, 45 and 60 min	02/03/2004
Hydrochlorothiazide/ Losartan potassium	Tablet	I (basket)	100	Water (deaerated)	900	10, 20, 30, 45 and 60 min	02/03/2004
Hydrochlorothiazide/ Moexipril HCl	Tablet	II (paddle)	50	0.1 N HCl	900	5, 10, 15 and 30 min	02/10/2004
Hydrochlorothiazide/ Quinapril HCl	Tablet	I (basket)	100	Water (deaerated)	900	5, 10, 20 and 30 min	02/03/2004
Hydrochlorothiazide/ Valsartan	Tablet	II (paddle)	50	Phosphate buffer pH 6.8	1000	10, 20, 30 and 45 min	02/03/2004
Hydrocodone bitartrate/Ibuprofen	Tablet	II (paddle)	50	Phosphate buffer, pH 7.2	900	5, 10, 15 and 30 min	02/04/2004

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Drug name	Dosage form	USP apparatus	Speed (RPMs)	Medium	Volume (mL)	Recommended sampling times	Date updated
Ibandronate sodium	Tablet	II (paddle)	50	Water	500	5, 10, 15, 30, and 45 min	01/03/2007
Ibuprofen (chewable tab)	Tablet (chewable)	II (paddle)	50	0.05 M phosphate buffer, pH 7.2	900	10, 20, 30 and 45 min	02/04/2004
Ibuprofen potassium (soft gelatin cap gel—liquid filled)	Capsule (soft gelatin/liquid fill)	I (basket)	150	phosphate buffer, pH 7.2	900	5, 10, 20 and 30 min	02/04/2004
Ibuprofen/ Diphenhydramine	Capsule	I (basket)	100	Phosphate buffer (200 mM), pH7.2	900	10, 20, 30, and 45 min	01/03/2007
Ibuprofen/ Pseudoephedrine HCl	Capsule	I (basket)	150	Tier 1: 0.05 M phosphate buffer, pH 7.2 Tier 2: 0.05 M phosphate buffer, pH 7.2 with NMT 1750 USP protease units/L of 1×USP pancreatin	900	10, 20, 30 and 45 min	03/04/2006
Ibuprofen/ Pseudoephedrine HCl	Suspension	II (paddle)	50	0.05 M phosphate buffer, pH 7.2	900	5, 10, 15 and 30 min	02/04/2004
Indinavir sulfate	Capsule	II (paddle)	50	0.1 M citrate buffer, pH 3.8	900	10, 15, 20 and 30 min	02/04/2004
Irbesartan	Tablet	II (paddle)	50	0.1 N HCl	1000	10, 20, 30 and 45 min	12/14/2004
Irbesartan/HCTZ	Tablet	II (paddle)	50	0.1 N HCl	1000	10, 20, 30, 45 and 60 min	01/03/2007
Isocarboxazid	Tablet	II (paddle)	50	0.1 N HCl	900	10, 20, 30, 45 and 60 min	02/04/2004
Isosorbide mononitrate	Tablet	II (paddle)	50	Water (deaerated)	900	5, 10, 15 and 30 min	02/04/2004
Isosorbide mononitrate	Tablet (extended release)	II (paddle)	50	0.1 N HCl containing 0.2% NaCl	500	1, 2, 6, 10, 12 hr	01/03/2007
Isradipine	Capsule	II (paddle)	50	0.1% lauryl dimethylamine oxide (LDAO) in water	500	10, 20, 30, 45 and 60 min	02/25/2004
Isradipine (10 mg)	Tablet (extended release)	II (paddle)	50	0.2% lauryl dimethylamine oxide (LDAO) in water	1000	2, 4, 8, 12, 16 and 24 hr	02/25/2004
Isradipine (5 mg)	Tablet (extended release)	II (paddle)	50	0.2% lauryl dimethylamine oxide (LDAO) in water	500	2, 4, 8, 12, 16 and 24 hr	02/25/2004
Itraconazole	Capsule	II (paddle)	100	SGF without enzyme	900	10, 20, 30, 45, 60 and 90 min	02/04/2004
Ivermectin	Tablet	II (paddle)	50	0.5% SDS in 0.01 M monobasic sodium phosphate, pH 7.0	900	10, 20, 30, 45 and 60 min	02/04/2004
Ketoconazole	Tablet	I (basket)	100	SGF w/o pepsin	800	15, 30, 45, 60 and 90 min	01/03/2007
Ketoprofen	Tablet	II (paddle)	50	SIF buffer without enzyme, pH 7.4	900	10, 20, 30, 45 and 60 min	02/05/2004
Ketoprofen	Capsule (IR)	II (paddle)	50	0.05 M phosphate buffer pH 7.4	1000	10, 20, 30 and 45 min	02/25/2004
Lamivudine (for 100 mg and 150 mg)	Tablet	II (paddle)	50	Water (deaerated)	900	10, 20, 30 and 45 min	03/22/2006
Lamivudine (for 300 mg only)	Tablet	II (paddle)	75	0.1 N HCl	900	5, 10, 15 and 30 min	03/22/2006

Lamivudine 150 mg/ Zidovudine 300 mg tablets and Abacavir sulfate 300 mg tablets-co-packaged	Tablet	II (paddle)	75	0.1 N HCl	900	5, 10, 15, 20, 30, 40 min	01/03/2007
Lamivudine/ Stavudine/ Nevirapine	Tablet	II (paddle)	75	0.1 N HCl	900	10, 20, 30, 45 and 60 min	01/03/2007
Lamivudine/ Zidovudine	Tablet	II (paddle)	75	0.1 N HCl	900	10, 20, 30 and 45 min	02/20/2004
Lamivudine/ Zidovudine + Efavirenz	Tablet (copackage)	II (paddle)	Lamivudine and Zidovudine: 75 Efavirenz: 50	Lamivudine and Zidovudine: 0.1 N HCl Efavirenz: 2% SLS in water	Lamivudine and Zidovudine: 1000 Efavirenz: 900	10, 20, 30, and 45 min	01/03/2007
Lamivudine/ Zidovudine + Nevirapine	Tablet (copackage)	II (paddle)	50	Lamivudine and Zidovudine: water Nevirapine: 0.06 M HCl (pH 1.2)	900	10, 15, 30, 45, 60 min	01/03/2007
Lamivudine/ Zidovudine/ Nevirapine	Tablet	II (paddle)	50	0.01 N HCl	900	10, 15, 30, 45, 60 min	01/03/2007
Lamotrigine	Tablet (regular)	II (paddle)	50	0.1 N HCl	900	5, 10, 15, 20 and 30 min	03/04/2006
Lamotrigine (25 mg)	Tablet (chewable dispersible)	II (paddle)	50	0.1 N HCl	900	5, 10, 15, 20 and 30 min	03/04/2006
Lamotrigine (5 mg)	Tablet (chewable dispersible)	II (paddle)	50	0.1 N HCl	500	5, 10, 15, 20 and 30 min	03/04/2006
Lanthanum Carbonate	Chewable tablet	Reciprocating cylinder (apparatus 3 modified)	10 dpm (dip RPM)	0.25 N HCl	900 (modified from the standard apparatus 3 vessel to achieve sink condition)	10, 20, 30 and 45 min	01/03/2007
Leflunomide	Tablet	II (paddle)	100	Water (deaerated)	1000	10, 20, 30 and 45 min	02/05/2004
Levetiracetam	Tablet	II (paddle)	50	Water (deaerated)	900	5, 10, 15 and 30 min	02/05/2004
Levonorgestrel	Tablet	II (paddle)	75	0.1 N HCl with 0.1% SLS	1000	10, 20, 30, 45, 60 and 90 min	02/05/2004
Lidocaine	Topical patch	Paddle over disk (apparatus 5)	50	Acetic acid/sodium acetate buffer, pH 4.0 at 32°C	500	10, 20, 30, 60, 120 and 180 min	01/03/2007
Lomefloxacin HCl	Tablet	II (paddle)	50	0.01 N HCl	900	10, 20, 30 and 45 min	02/05/2004
Loratadine (orally disintegrating tab)	Tablet (orally disintegrating)	I (basket)	50	SGF without enzyme	900	2, 4, 6 and 10 hr	02/05/2004

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Drug name	Dosage form	USP apparatus	Speed (RPMs)	Medium	Volume (mL)	Recommended sampling times	Date updated
Losartan potassium	Tablet	II (paddle)	50	Water (deaerated)	900	10, 20, 30 and 45 min	02/06/2004
Mefloquine HCl	Tablet	I (basket)	100	SGF without enzyme	900	10, 20, 30, 45 and 60 min	02/06/2004
Meloxicam	Suspension	II (paddle)	25	Phosphate buffer at pH 7.5	900	5, 10, 15 and 30 min	01/26/2006
Meloxicam	Tablet	II (paddle)	75	Phosphate Buffer, pH 7.5	900	10, 20, 30, 45 and 60 min	02/20/2004
Memantine HCIL	Tablet	I (basket)	100	0.1 N HCl with NaCl (12 g NaCl in 6 L water adjust pH to 1.2 with HCl)	900	10, 20, 30 and 45 min	12/16/2005
Mercaptopurine	Tablet	II (paddle)	50	0.1 N HCl	900	20, 30, 45, 60, 90 and 120 min	02/06/2004
Mesalamine	Suppository	II (paddle) with option to use a sinker	75 (for 500 mg) and 125 (for 1000 mg)	For 500 mg strength: 0.2 M phosphate buffer, pH 7.5 at 37°C For 1000 mg strength: 0.2 M phosphate buffer, pH 7.5 at 40°C	900	30, 60, 90, 120 and 150 min	01/30/2006
Mesna	Tablet	II (paddle)	50	0.06 N HCl	500	5, 10, 15, 20 and 30 min	02/09/2004
Metaxalone	Tablet	II (paddle)	100	0.5% SLS in water	900	30, 60, 90 and 120 min	02/06/2004
Metformin HCl	Tablet (extended release)	500 mg: paddle, 750 mg: basket	100	Phosphate buffer, pH 6.8	1000	1, 3, 6 and 10 hr	01/03/2007
Metformin/ Pioglitazone HCl	Tablet	II (paddle)	50	pH 2.5 mcilvaine buffer (0.1 M citric acid adjusted to pH 2.5 with 0.2 M Na_2HPO_4)	900	10, 20, 30, and 45 min	01/03/2007
Metolazone	Tablet	II (paddle)	75	2% SLS in 0.05 M sodium phosphate buffer, pH 7.5	900	30, 60, 90, 120 and 150 min	02/10/2004
Metronidazole	Capsule	I (basket)	100	0.1 N HCl	900	10, 20, 30 and 45 min	02/09/2004
Midodrine HCl	Tablet	II (paddle)	50	0.1 N HCl	900	5, 10, 15 and 30 min	02/06/2004
Miglustat	Capsule	I (basket)	100	0.1 N HCl	1000	10, 20, 30 and 45 min	01/03/2007
Mirtazapine	Tablet	II (paddle)	50	0.1 N HCl	900	5, 10, 15 and 30 min	02/10/2004
Mirtazapine	Tablet [orally disintegrating (ODT)]	II (paddle)	50	0.1 N HCl	900	5, 10, 15, 20 and 30 min	03/04/2006
Misoprostol	Tablet	II (paddle)	50	Water (deaerated)	500	5, 10, 20 and 30 min	02/10/2004
Modafinil	Tablet	II (paddle)	50	0.1 N HCl	900	10, 20, 30, 45 and 60 min	02/10/2004
Moexipril HCl	Tablet	II (paddle)	50	Water (deaerated)	900	5, 10, 15 and 30 min	02/10/2004
Montelukast	Tablet (chewable)	II (paddle)	50	0.5% SDS in water	900	5, 10, 20 and 30 min	03/04/2006
Morphine sulfate	Capsule (extended release)	II (paddle)	50	Phosphate buffer, pH 6.8	900	1, 3, 6, 12, 24 hr	01/03/2007
Morphine sulfate	Capsule (sustained release)	I (basket)	100	Acid stage: 0.1 N HCl buffer stage: phosphate buffer, pH 7.5	Acid: 600 Buffer: 500	1, 4, 6, 9, and 12 hr	01/03/2007
Mycophenolate mofetil	Suspension	II (paddle)	40	0.1 N HCl	900	5, 10, 20 and 30 min	02/10/2004
Mycophenolate mofetil	Capsule	II (paddle)	40	0.1 N HCl	900	5, 10, 20 and 30 min	02/10/2004

Mycophenolate mofetil	Tablet	II (paddle)	50	0.1 N HCl	900	5, 10, 15 and 30 min	02/10/2004
Naratriptan HCl	Tablet	I (basket)	100	0.1 M HCl	500	5, 10, 15 and 30 min	01/03/2007
Nateglinide	Tablet	II (paddle)	50	0.01 N HCl with 0.5% (w/v) SLS	1000	10, 20, 30, and 45 min	01/03/2007
Nefazodone HCl	Tablet	II (paddle)	50	0.1 N HCl	900	10, 20, 30, and 45 min	01/03/2007
Nefazodone HCl	Tablet	II (paddle)	50	0.1 N HCl	900	10, 20, 30, 45 and 60 min	02/11/2004
Nelfinavir mesylate	Tablet	II (paddle)	50	0.1 N HCl	900	5, 10, 15, 20, 30, 45, 60 and 90 min	01/03/2007
Nevirapine	Suspension	II (paddle)	25	0.1 N HCl	900	10, 20, 30, 45 and 60 min	02/11/2004
Nevirapine	Tablet	II (paddle)	50	0.1 M phosphate buffer, pH 2 (3.9 mL concentrated phosphoric acid and 5.73 g sodium phosphate monobasic monohydrate. QS to a 1 L with water. Adjust pH to 2.0 with phosphoric acid)	900	10, 20, 30, 45, 60 and 90 min	12/15/2005
Nicardipine HCl	Capsule	II (paddle)	50	0.033 M citric acid buffer, pH 4.5	900	10, 20, 30 and 45 min	02/11/2004
Nitazoxanide	Tablet	II (paddle)	75	Phosphate buffer at pH 7.5 with 6% hexadecyltrimethyl ammonium bromide, bath temperature at 25°C	900	10, 20, 30, 45, 60 min	01/03/2007
Norethindrone (nor-QD)	Tablet	II (paddle)	75	0.1 N HCl, 0.02% SLS	900	15, 30, 45, 60, and 75 min	01/03/2007
Norethindrone (ortho-micronor)	Tablet	II (paddle)	75	0.09% SLS in 0.1 N HCl (same as Noreth./EE USP method)	500	15, 30, 45, and 60 min	01/03/2007
Nystatin	Tablet	II (paddle)	75	Water with 0.1% SLS	900	15, 30, 45, 60 and 90 min	01/03/2007
Ofloxacin	Tablet	I (basket)	100	0.1 N HCl	900	10, 20, 30 and 45 min	02/12/2004
Olanzapine	Tablet	II (paddle)	50	0.1 N HCl	900	5, 10, 20 and 30 min	02/12/2004
Olanzapine (orally disintegrating)	Tablet (orally disintegrating)	II (paddle)	50	0.1 N HCl	900	5, 10, 15 and 30 min	02/12/2004
Olmesartan	Tablet	II (paddle)	50	JP fluid 2, pH 6.8 37°C	900	10, 20, 30 and 45 min	06/21/2006
Olmesartan medoxomil	Tablet	II (paddle)	50	250 mL 0.2 N KH_2PO_4 + 118 mL NaOH + q.s. water to 1000 mL	1000	10, 20, 30, and 45 min	01/03/2007
Olmesartan medoxomil/HCTZ	Tablet	II (paddle)	50	JP fluid 2 (pH 6.8)	900	5, 10, 15, 20, 30, 45 and 60 min	01/03/2007
Olsalazine sodium	Capsule	I (basket)	100	Phosphate buffer, pH 7.5	900	10, 20, 30 and 45 min	02/12/2004
Omeprazole (powder for suspension)	Powder (suspension)	II (paddle)	50	0.25 mM Na phosphate buffer, pH 7.4	900	5, 10, 15 and 30 min	12/13/2004
Omeprazole magnesium	OTC Tablet (delayed release)	II (paddle)	100	Tablets are preexposed to 300 mL of 0.1 M HCl for 2 hr and then 700 mL of 0.086 M Na_2HPO_4 is added to the medium containing the capsule to give 1000 mL with pH 6.8	300 mL for the acid stage; 1000 mL for the buffer stage	Sampling started at the buffer stage 10, 20, 30, 45 and 60 min	01/03/2007

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Drug name	Dosage form	USP apparatus	Speed (RPMs)	Medium	Volume (mL)	Recommended sampling times	Date updated
Ondansetron	Tablet (orally disintegrating)	II (paddle)	50	0.1 N HCl, pH 1.0	500	5, 10, 15 and 30 min	02/12/2004
Ondansetron HCl	Tablet	II (paddle)	50	Water (deaerated)	500	5, 10, 15 and 30 min	02/12/2004
Orlistat	Capsule	II (paddle)	75	3% SLS in 0.5% sodium chloride, pH 6.0	900	10, 20, 30, 45 and 60 min	02/12/2004
Oseltamivir phosphate	Capsule	II (paddle)	50	0.1 N HCl	900	5, 10, 20, and 30 min	01/03/2007
Oxaprozin	Tablet	II (paddle)	75	0.05 M phosphate buffer, pH 7.4	1000	10, 20, 30, 45 and 60 min	02/12/2004
Oxcarbazepine	Suspension	II (paddle)	75	1% SDS in water	900	10, 20, 30 and 45 min	02/12/2004
Oxcarbazepine (150 mg)	Tablet	II (paddle)	60	0.3% SDS in water	900	10, 20, 30, 45, 60 and 90 min	02/12/2004
Oxcarbazepine (300 mg)	Tablet	II (paddle)	60	0.6% SDS in water	900	10, 20, 30, 45, 60 and 90 min	02/12/2004
Oxcarbazepine (600 mg)	Tablet	II (paddle)	60	1% SDS in water	900	10, 20, 30, 45, 60 and 90 min	02/12/2004
Oxybutinin	Trans-dermal	Paddle over disk (apparatus 5)	50	Phosphate buffer, pH 4.5 @ 32°C	900	1, 4, 24 hr	01/03/2007
Pantoprazole sodium	Tablet (delayed release)	II (paddle)	100	Acid stage: 0.1 N HCl for 2 hr buffer stage: phosphate buffer, pH 6.8	1000	1, 2 hr (acid stage) 10, 20, 30, 45 and 60 min (buffer stage)	03/04/2006
Paromomycin sulfate	Capsule	I (basket)	50	0.05 M phosphate buffer, pH 6.8	900	5, 10, 15, 20, 30 and 45 min	02/13/2004
Paroxetine HCl	Suspension	II (paddle)	100	SGF without enzyme	900	10, 20, 30 and 45 min	02/13/2004
Pemoline	Tablet	II (paddle)	75	Water (deaerated)	900	10, 20, 30, 45, 60 and 90 min	02/13/2004
Pergolide mesylate	Tablet	II (paddle)	50	SGF TS with cysteine without enzymes	500	10, 20, 30 and 45 min	03/04/2006
Perindopril erbumine	Tablet	II (paddle)	50	0.1 N HCl	900	10, 20, 30 and 45 min	08/02/2004
Pilocarpine HCl	Tablet	II (paddle)	50	0.1 N HCl	500	10, 20, 30, 45 and 60 min	01/20/2004
Pioglitazone HCl	Tablet	II (paddle)	75	HCl-0.3 M KCl buffer, pH 2.0	900	5, 10, 15 and 30 min	02/13/2004
Pravastatin sodium	Tablet	II (paddle)	50	Water (deaerated)	900	5, 10, 20 and 30 min	02/13/2004
Pregabalin	Capsule	II (paddle)	50	0.06 N HCl	900	10, 20, 30 and 45 min	03/22/2006
Prochlorperazine	Rectal suppository	1 (suppository, dissolution baskets, palmieri type)	100	0.1 N HCl at 38°C	900	10, 20, 30 and 45 min	08/17/2006
Propafenone HCl	Tablet	II (paddle)	75	0.1 N HCl	900	10, 20, 30 and 45 min	02/13/2004
Quetiapine fumarate	Tablet	II (paddle)	50	Water (deaerated)	900	10, 20, 30 and 45 min	02/18/2004
Quinapril HCl	Tablet	I (basket)	100	Water (deaerated)	900	10, 20, 30 and 45 min	02/18/2004
Quinapril/ Hydro-chlorothiazide	Tablet	I (basket)	100	Water	900	10, 20, 30 and 45 min	08/17/2006
Rabeprazole sodium	Tablet (delayed release)	II (paddle)	100	700 mL 0.1 N HCl (acid stage), after two hours add 300 mL of 0.6 M Tris, pH 8.0 (buffer stage)	Acid: 700 Buffer: 1000	10, 20, 30, and 45 min	01/03/2007

Raloxifene HCl	Tablet	II (paddle)	50	0.1% polysorbate 80 in water	1000	10, 20, 30 and 45 min	02/18/2004
Ramipril	Capsule	II (paddle)	50	0.1 N HCl	500	10, 20, 30 and 45 min	02/18/2004
Ranitidine HCl	Capsule	II (paddle)	50	Water (deaerated)	900	10, 20, 30 and 45 min	02/18/2004
Repaglinide	Tablet	II (paddle)	75	McIlvaine's buffer	900	15, 30, 45 and 60 min	08/17/2006
Ribavirin	Tablet	II (paddle)	50	Water (deaerated)	900	10, 20, 30 and 45 min	02/18/2004
Ribavirin	Capsule	I (basket)	100	Water (deaerated)	900	10, 20, 30 and 45 min	02/18/2004
Rifapentine	Tablet	II (paddle)	50	0.8% SLS in phosphate buffer, pH 7.0	900	10, 20, 30, 45, 60 and 90 min	02/25/2004
Riluzole	Tablet	II (paddle)	50	0.1 N HCl	900	10, 20, 30, 45 and 60 min	02/18/2004
Rimantidine HCl	Tablet	II (paddle)	50	Water	900	10, 20, 30, and 45 min	01/03/2007
Risedronate sodium	Tablet	II (paddle)	50	Water (deaerated)	500	10, 20, 30 and 45 min	02/20/2004
Risedronate sodium/Calcium carbonate	Tablet (copackaged)	For risedronate tablets:paddle	For Risedronate tablets: 50	For risedronate tablets:water; for calcium carbonate tablets: using USP method.	For risedronate tablets: 500 mL	10, 20, 30 and 45 min	01/03/2007
Risperidone	Tablet	II (paddle)	50	0.1 N HCl	500	10, 20, 30, 45 and 60 min	03/04/2006
Risperidone (orally disintegrating)	Tablet (orally disintegrating)	II (paddle)	50	0.1 N HCl	500	5, 10, 15 min	07/23/2004
Ritonavir	Capsule	II (paddle)	50	0.1 N HCl with 25 mM polyoxyethylene 10 laurylether (POE10LE)	900	10, 20, 30 and 45 min	02/18/2004
Rivastigmine tartrate	Capsule	II (paddle)	50	Water (deaerated)	500	10, 20, 30 and 45 min	01/03/2007
Rizatriptan benzoate (orally disintegrating)	Tablet (orally disintegrating)	II (paddle)	50	Water (deaerated)	900	5, 10 and 15 min	02/18/2004
Rizatriptan benzoate (regular)	Tablet	II (paddle)	50	Water (deaerated)	900	5, 10, 15 and 30 min	02/18/2004
Ropinirole HCl	Tablet	I (basket)	50	Citrate buffer, pH 4.0	500	5, 10, 15 and 30 min	01/03/2007
Rosiglitazone maleate	Tablet	II (paddle)	50	0.01 M acetate buffer, pH 4.0	900	10, 20, 30 and 45 min	02/24/2004
Rosuvastatin calcium	Tablet	II (paddle)	50	0.05 M citrate buffer pH 6.6	900	10, 20, 30 and 45 min	12/20/2005
Sertraline HCl	Tablet	II (paddle)	75	0.05 M sodium acetate buffer, pH 4.5	900	10, 20, 30 and 45 min	02/20/2004
Sibutramine HCl	Capsule	II (paddle)	50	0.05 M acetate buffer, pH 4.0	500	10, 20, 30, 45 and 60 min	02/25/2004
Sildenafil	Tablet	I (basket)	100	0.01 N HCl	900	5, 10, 15 and 30 min	03/04/2006
Sirolimus 1 mg	Tablet	Basket (40 mesh)	100	0.4% SLS in water	500	10, 20, 30, 45, 60 and 120 min	11/26/2004
Sirolimus 2 mg	Tablet	Basket (40 mesh)	100	0.4% SLS in water	500	10, 20, 30, 45, 60 and 120 min	11/26/2004
Sirolimus 5 mg	Tablet	Basket (20 mesh)	120	0.4% SLS in water	500	10, 20, 30, 45, 60 and 120 min	11/26/2004
Stavudine	Capsule	II (paddle)	100	Water	900	10, 20, 30, and 45 min	01/03/2007
Succimer	Capsule	II (paddle)	50	0.01 N phosphoric acid	900	10, 20, 30, 45, 60 and 90 min	02/20/2004
Sucralfate	Suspension	II (paddle)	75	0.1 N HCl/0.067 M KCl, pH 1.0	900	10, 20, 30 and 45 min	03/04/2006
Sulfamethoxazole/ Trimethoprim	Suspension	II (paddle)	50	1 mL of 0.2 N HCl in water	900	10, 20, 30, 45, 60 and 90 min	02/25/2004
Sulfisoxazle acetyl	Oral suspension (pediatric)	II (paddle)	30	1% SLS in 0.1 N HCl	900	15, 30, 45, 60 and 90 min	08/17/2006
Sumatriptan succinate	Tablet	II (paddle)	30	0.01 M HCl	900	5, 10, 15 and 30 min	03/04/2006

(Continued)

(*Continued*)

Drug name	Dosage form	USP apparatus	Speed (RPMs)	Medium	Volume (mL)	Recommended sampling times	Date updated
Tacrolimus	Capsule	II (paddle)	50	Hydroxypropyl cellulose solution (1 in 20,000). Adjust to pH 4.5 by phosphoric acid	900	30, 60, 90 and 120 min	02/20/2004
Tadalafil	Tablet	II (paddle)	50	0.5% SLS	1000	10, 20, 30 and 45 min	01/26/2006
Tamsulosin HCl	Capsule	II (paddle)	50	0–2 hr: 0.003% polysorbate 80, pH 1.2 2–8 hr: phosphate buffer, pH 7.2	500	1, 2, 3, 6, 8, and 10 hr	01/03/2007
Telithromycin	Tablet	II (paddle)	50	0.1 N HCl	900	10, 20, 30 and 45 min	01/03/2007
Telmisartani	Tablet	II (paddle)	75	Phosphate buffer, pH 7.5	900	10, 20, 30 and 45 min	03/04/2006
Temozolomide	Capsule	I (basket)	100	Distilled water	500	10, 20, 30, and 45 min	01/03/2007
Tenofovir disoproxil fumarate	Tablet	II (paddle)	50	0.1 N HCl	900	10, 20, 30, and 45 min	01/03/2007
Terazosin HCl	Tablet	II (paddle)	50	Water (deaerated)	900	10, 20, 30, 45 and 60 min	02/20/2004
Terazosin HCl	Capsule	II (paddle)	50	Water (deaerated)	900	10, 20, 30, 45, 60 and 90 min	02/20/2004
Terbinafine HCl	Tablet	II (paddle)	50	Citrate buffer, pH 3.0 adjusted with HCl	500	10, 20, 30 and 45 min	02/20/2004
Testosterone	Buccal tablet (extended release)	II (paddle, may use sinker)	60	1% SDS in double distilled water	1000	1, 2, 4, 6, 10, 12 and 24 hr	01/03/2007
Thalidomide	Capsule	II (paddle)	100	1.5% (w/v) SLS (pH 3.0, adj w/HCl)	900	10, 20, 30, 60 and 90 min	03/04/2006
Theophylline (100 mg and 200 mg)	Tablet (extended release)	II (paddle)	50	SGF, pH 1.2 during 1st hour. SIF, pH 7.5 from end of hour 1 through 12th hour	900	1, 4, 8, 12 hr	01/03/2007
Theophylline (450 mg)	Tablet (extended release)	II (paddle)	50	SGF, pH 1.2 during 1st hour. SIF, pH 7.5 from end of hour 1 through 12th hour	900	1, 4, 8, 12 hr	01/03/2007
Tiagabine HCl	Tablet	II (paddle)	50	Water	900	5, 10, 15, 20, and 30 min	01/03/2007
Ticlopidine HCl	Tablet	II (paddle)	50	Water (deaerated)	900	10, 20, 30, 45 and 60 min	02/19/2004
Tinidazole	Tablet	I (basket)	100	Water (deaerated)	900	10, 20, 30 and 45 min	01/03/2007
Tizanidine HCl	Capsule	II (paddle)	50	0.01 N HCl	500	5, 10, 15 and 30 min	02/20/2004
Tizanidine HCl	Tablet	I (basket)	100	0.1 N HCl	500	5, 10, 15 and 30 min	02/20/2004
Tolcapone	Tablet	II (paddle)	75	Borate buffer, pH 6.8 with 1% SLS	900	10, 20, 30 and 45 min	02/20/2004
Tolterodine tartrate	Tablet	II (paddle)	50	SGF without enzymes, pH 1.2	900	5, 10, 15 and 30 min	02/20/2004
Topiramate	Capsule (sprinkle)	II (paddle)	50	Water (deaerated)	900	10, 20, 30, 45 and 60 min	02/19/2004
Topiramate	Tablet	II (paddle)	50	Water (deaerated)	900	5, 10, 20 and 30 min	02/19/2004
Toremifene citrate	Tablet	II (paddle)	50	0.02 N HCl	1000	10, 20, 30 and 45 min	02/20/2004
Torsemide	Tablet	II (paddle)	50	0.1 N HCl	900	5, 10, 15 and 30 min	02/20/2004
Tramadol	Tablet (extended release)	I (basket)	75	0.1 N HCl	900	2, 4, 8, 10, 16 hr	01/03/2007
Tramadol HCl	Tablet	I (basket)	100	0.1 N HCl	900	10, 20, 30 and 45 min	02/19/2004
Trandolapril	Tablet	II (paddle)	50	Water (deaerated)	500	10, 20, 30, 45 and 60 min	02/20/2004

Trimipramine maleate	Capsule	I (basket)	100	Water (deaerated)	1000	10, 20, 30, 45, 60 and 90 min	03/04/2006
Valacyclovir HCl	Tablet	II (paddle)	50	0.1 N HCl	900	10, 20, 30, 45 and 60 min	02/19/2004
Valsartan (tab and cap)	Tablet	II (paddle)	50	0.067 M phosphate buffer, pH 6.8	1000	10, 20, 30 and 45 min	12/13/2004
Vardenafil HCl	Tablet	II (paddle)	50	0.1 N HCl	900	5, 10, 15 and 30 min	12/20/2005
Venlafaxine HCl	Tablet	II (paddle)	50	Water (deaerated)	900	5, 10, 15 and 30 min	02/19/2004
Venlafaxine HCl	Capsule (extended release)	I (basket)	100	Water	900	2, 4, 8, 12, and 20 hr	01/03/2007
Verapamil HCl (100, 200, 300 mg)	Capsule (extended release)	I (basket)	75	Water, pH 3.0 (adjusted with 0.1 N or 2 N HCl)	1000	1, 4, 8, 11, and 24 hr	01/03/2007
Verapamil HCl (120, 180, 240, 360 mg)	Capsule (sustained release)	I (basket)	75	Water, pH 3.0 (adjusted with 0.1 N or 2 N HCl)	1000	1, 4, 8, 11, and 24 hr	01/03/2007
Voriconazole	For suspension	II (paddle)	50	0.1 N HCl	900	10, 20, 30 and 45 min	01/03/2007
Zaleplon	Capsule	II (paddle)	75	Deionized water	900	5, 10, 20, and 30 min	01/03/2007
Zidovudine	Tablet	II (paddle)	50	Water	900	10, 20, 30, and 45 min	01/03/2007
Zileuton	Tablet	II (paddle)	50	0.05 M SLS in water	900	10, 20, 30, 45 and 60 min	02/19/2004
Zinc acetate	Capsule	II (paddle)	50	0.1 N HCl	900	10, 20, 30 and 45 min	02/19/2004
Ziprasidone HCl	Capsule	II (paddle)	75	Tier I: 0.05 M Na phosphate buffer, pH 7.5 + 2% SDS (w/w) Tier II: 0.05 M Na phosphate buffer, pH 7.5 (700 mL) + 1% pancreatin. After 15 min. incubation, add 200 mL of phosphate buffer containing 9% SDS	900	10, 20, 30, 45 and 60 min	03/04/2006
Zolpidem tartrate	Tablet	II (paddle)	50	0.01 N HCl, pH 2.0	900	5, 10, 15 and 30 min	02/19/2004
Zonisamide	Capsule	II (paddle)	50	Water (deaerated)	900	10, 20, 30, and 45 min	01/03/2007

Abbreviations: HDTMA, hexadecyltrimethylammonium; RPM, rate per minute; SDS, sodium dodecyl sulfate; SGF, simulated gastric fluid; SIF, simulated intestinal fluid; SLS, sodium lauryl sulfate; USP, U.S. Pharmacopeia.

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about the book...

As the generic pharmaceutical industry continues to grow and thrive, so does the need to conduct efficient and successful bioequivalence studies. In recent years, there have been significant changes to the statistical models for evaluating bioequivalence, and advances in the analytical technology used to detect drug and metabolite levels have made bioequivalence testing more difficult to conduct and summarize. The ***Handbook of Bioequivalence Testing*** offers a complete and timely description of every aspect of bioequivalence testing, including worldwide regulatory requirements for filing for approval of generic drugs, applying for a waiver, securing regulatory approval of reports, and obtaining regulatory certification of facilities conducting bioequivalence studies.

A practical, "how-to" manual on bioequivalence testing, this guide describes the current analytical methods used in bioequivalence testing, as well as their respective strengths and limitations… covers GLP, GCP, and 21 CFR compliance requirements for qualifying studies for regulatory submission and facility certification…includes actual examples of reports approved by regulatory authorities to illustrate various scientific, regulatory, and formatting aspects…and provides a list of vendors for the software used to analyze bioequivalence studies and recommendations.

about the author...

SARFARAZ K. NIAZI is Chief Executive Officer, Pharmaceutical Scientist Inc., Deerfield, Illinois. Dr. Niazi possesses over 35 years of worldwide experience conducting multidisciplinary research and teaching in the fields of biopharmaceutics and clinical pharmacokinetics. He has published over 100 refereed research articles and abstracts and numerous technical and literary books, including several textbooks. The recipient of several research recognition awards, Dr. Niazi was a Volwiler Fellow at Abbott Laboratories, Abbott Park, Illinois, and is currently an Adjunct Professor of Pharmacy at the University of Houston, Texas. He previously served in a tenured position at the faculty of the College of Pharmacy, University of Illinois, Chicago. Dr. Niazi is a licensed practitioner of patent law before the U.S. Patent and Trademark Office, serves as a consultant to the global pharmaceutical and biotechnology industry, and holds several major U.S. and worldwide patents for his inventions. Dr. Niazi received the M.S. degree in biopharmaceutics from Washington State University, Pullman, Washington and the Ph.D. degree in pharmacokinetics from the University of Illinois, Chicago.

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healthcare
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52 Vanderbilt Avenue
New York, NY 10017

Telephone House
69-77 Paul Street
London EC2A 4LQ, UK