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Bioavailability and Bioequivalence: Introduction
A *multisource drug product* is a drug product that contains the same active drug substance in the same dosage form and is marketed by more than one pharmaceutical manufacturer. *Single-source drug products* are drug products for which the patent has not yet expired or has certain exclusivities so that only one manufacturer can make it. Single-source drug products are usually brand-name (innovator) drug products. After the patent and other exclusivities for the brand-name drug expires, a pharmaceutical firm may manufacture a generic drug product that can be substituted for the branded drug product. Since the formulation and method of manufacture of the drug product can affect the bioavailability and stability of the drug, the generic drug manufacturer must demonstrate that the generic drug product is bioequivalent and therapeutically equivalent to the brand-name drug product.
Drug product selection and generic drug product substitution are major responsibilities for physicians, pharmacists, and others who prescribe, dispense, or purchase drugs. To facilitate such decisions, the U.S. Food and Drug Administration (FDA) publishes annually, in print and on the Internet, *Approved Drug Products with Therapeutic Equivalence Evaluations*, also known as the *Orange Book* (www.fda.gov/cder/orange/default.htm). The *Orange Book* identifies drug products approved on the basis of safety and effectiveness by the FDA and contains therapeutic equivalence evaluations for approved multisource prescription drug products. These evaluations serve as public information and advice to state health agencies, prescribers, and pharmacists to promote public education in the area of drug product selection and to foster containment of health care costs. The following definitions are from the *2003 Orange Book, Code of Federal Regulations*, 21 CFR 320, and other sources.
Definitions

Bioavailability. Bioavailability means 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. For drug products that are not intended to be absorbed into the bloodstream, bioavailability 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.

Bioequivalence requirement. A requirement imposed by the FDA for *in-vitro* and/or *in-vivo* testing of specified drug products, which must be satisfied as a condition for marketing.

Bioequivalent drug products. This term describes pharmaceutical equivalent or pharmaceutical alternative products that display comparable bioavailability when studied under similar experimental conditions. For systemically absorbed drugs, the test (generic) and reference listed drug (brand-name) shall be considered bioequivalent if: (1) 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 (2) 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 attainment of effective body drug concentrations on chronic use, and is considered medically insignificant for the drug.
When the above methods are not applicable (eg, for drug products that are not intended to be absorbed into the bloodstream), other *in-vivo* or *in-vitro* test methods to demonstrate bioequivalence may be appropriate.
Bioequivalence may sometimes be demonstrated using an *in-vitro* bioequivalence standard, especially when such an *in-vitro* test has been correlated with human *in-vivo* bioavailability data. In other situations, bioequivalence may sometimes be demonstrated through comparative clinical trials or pharmacodynamic studies.
Bioequivalent drug products may contain different inactive ingredients, provided the manufacturer identifies the differences and provides information that the differences do not affect the safety or efficacy of the product.

Brand name. The trade name of the drug. This name is privately owned by the manufacturer or distributor and is used to distinguish the specific drug product from competitor's products (eg, Tylenol, McNeil Laboratories).

Chemical name. The name used by organic chemists to indicate the chemical structure of the drug (eg, N-acetyl-*p*-aminophenol).

Abbreviated New Drug Application (ANDA). Drug manufacturers must file an ANDA for approval to market a generic drug product. The generic manufacturer is not required to perform clinical efficacy studies or nonclinical toxicology studies for the ANDA.

Drug product. The finished dosage form (eg, tablet, capsule, or solution) that contains the active drug ingredient, generally, but not necessarily, in association with inactive ingredients.

Drug product selection. The process of choosing or selecting the drug product in a specified dosage form.

Drug substance. A drug substance is the active pharmaceutical ingredient (API) or component in the drug product that furnishes the pharmacodynamic activity.

Equivalence. Relationship in terms of bioavailability, therapeutic response, or a set of established standards of one drug product to another.

Generic name. The established, nonproprietary, or common name of the active drug in a drug product (eg, acetaminophen).

Generic substitution. The process of dispensing a different brand or an unbranded drug product in place of the prescribed drug product. The substituted drug product contains the same active ingredient or therapeutic moiety as the same salt or ester in the same dosage form but is made by a different manufacturer. For example, a prescription for Motrin brand of ibuprofen might be dispensed by the pharmacist as Advil brand of ibuprofen or as a nonbranded generic ibuprofen if generic substitution is permitted and desired by the physician.

Pharmaceutical alternatives. Drug products that contain the same therapeutic moiety but as different salts, esters, or complexes. For example, tetracycline phosphate or tetracycline hydrochloride equivalent to 250 mg tetracycline base are considered pharmaceutical alternatives. Different dosage forms and strengths within a product line by a single manufacturer are pharmaceutical alternatives (eg, an extended-release dosage form and a standard immediate-release dosage form of the same active ingredient). The FDA currently considers a tablet and capsule containing the same active ingredient in the same dosage strength as pharmaceutical alternatives.

Pharmaceutical equivalents. Drug products in identical dosage forms that contain the same active ingredient(s), ie, the same salt or ester, are of the same dosage form, use the same route of administration, and are identical in strength or concentration (eg, chlordiazepoxide hydrochloride, 5-mg capsules). Pharmaceutically equivalent drug products are formulated to contain the same amount of active ingredient in the same dosage form and to meet the same or compendial or other applicable standards (ie, strength, quality, purity, and identity), but they may differ in characteristics such as shape, scoring configuration, release mechanisms, packaging, excipients (including colors, flavors, preservatives), expiration time, and, within certain limits, labeling. When applicable, pharmaceutical equivalents must meet the same content uniformity, disintegration times, and/or dissolution rates. Modified-release dosage forms that require a reservoir or overage or certain dosage forms such as prefilled syringes in which residual volume may vary must deliver identical amounts of active drug ingredient over an identical dosing period.

Pharmaceutical substitution. The process of dispensing a pharmaceutical alternative for the prescribed drug product. For example, ampicillin suspension is dispensed in place of ampicillin capsules, or tetracycline hydrochloride is dispensed in place of tetracycline phosphate. Pharmaceutical substitution generally requires the physician's approval.

Reference listed drug. The reference listed drug (RLD) is identified by the FDA as the drug product on which an applicant relies when seeking approval of an Abbreviated New Drug Application (ANDA). The RLD is generally the brand-name drug that has a full New Drug Application (NDA). The FDA designates a single reference listed drug as the standard to which all generic versions must be shown to be bioequivalent. The FDA hopes to avoid possible significant variations among generic drugs and their brand-name counterparts. Such variations could result if generic drugs were compared to different reference listed drugs.

Therapeutic alternatives. Drug products containing different active ingredients that are indicated for the same therapeutic or clinical objectives. Active ingredients in therapeutic alternatives are from the same pharmacologic class and are expected to have the same therapeutic effect when administered to patients for such condition of use. For example, ibuprofen is given instead of aspirin; cimetidine may be given instead of ranitidine.

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 (a) contain identical amounts of the same active drug ingredient in the same dosage form and route of administration, and (b) meet compendial or other applicable standards of strength, quality, purity, and identity; (3) they are bioequivalent in that (a) they do not present a known or potential bioequivalence problem, and they meet an acceptable *in-vitro* standard, or (b) if they do present such a known or potential problem, they are shown to meet an appropriate bioequivalence standard; (4) they are adequately labeled; and (5) they are manufactured in compliance with Current Good Manufacturing Practice regulations. The 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.

Therapeutic substitution. The process of dispensing a therapeutic alternative in place of the prescribed drug product. For example, amoxicillin is dispensed instead of ampicillin or ibuprofen is dispensed instead of naproxen. Therapeutic substitution can also occur when one NDA-approved drug is substituted for the same drug which has been approved by a different NDA, eg, the substitution of Nicoderm (nicotine transdermal system) for Nicotrol (nicotine transdermal system).

Purpose of Bioavailability Studies

Bioavailability studies are performed for both approved active drug ingredients and therapeutic moieties not yet approved for marketing by the FDA. New formulations of active drug ingredients must be approved by the FDA before marketing. In approving a drug product for marketing, the FDA ensures that the drug product is safe and effective for its labeled indications for use. Moreover, the drug product must meet all applicable standards of identity, strength, quality, and purity. To ensure that these standards are met, the FDA requires bioavailability/pharmacokinetic studies and, where necessary, bioequivalence studies for all drug products (*FDA Guidance for Industry,* 2003). Bioavailability may be considered as one aspect of drug product quality that links *in-vivo* performance of the drug product used in clinical trials to studies demonstrating evidence of safety and efficacy. For unmarketed drugs that do not have full NDA approval by the FDA, *in-vitro* and/or *in-vivo* bioequivalence studies must be performed on the drug formulation proposed for marketing as a generic drug product. Furthermore, the essential pharmacokinetics of the active drug ingredient or therapeutic moiety must be characterized. Essential pharmacokinetic parameters, including the rate and extent of systemic absorption, elimination half-life, and rates of excretion and metabolism, should be established after single- and multiple-dose administration. Data from these *in-vivo* bioavailability studies are important to establish recommended dosage regimens and to support drug labeling. *In-vivo* bioavailability studies are also performed for new formulations of active drug ingredients or therapeutic moieties that have full NDA approval and are approved for marketing. The purpose of these studies is to determine the bioavailability and to characterize the pharmacokinetics of the new formulation, new dosage form, or new salt or ester relative to a reference formulation.

In summary, clinical studies are useful in determining the safety and efficacy of drug products. *Bioavailability* studies are used to define the effect of changes in the physicochemical properties of the drug substance and the effect of the drug product (dosage form) on the pharmacokinetics of the drug. *Bioequivalence* studies are used to compare the bioavailability of the same drug (same salt or ester) from various drug products. Bioavailability and bioequivalence can also be considered as performance measures of the drug product *in-vivo*. If the drug products are bioequivalent and therapeutically equivalent (as defined above), then the clinical efficacy and the safety profile of these drug products are assumed to be similar and may be substituted for each other.

Relative and Absolute Availability

The area under the drug concentration–time curve (AUC) is used as a measure of the total amount of unaltered drug that reaches the systemic circulation. The AUC is dependent on the total quantity of available drug, FD_0, divided by the elimination rate constant, k, and the apparent volume of distribution, V_D. F is the fraction of the dose absorbed. After IV administration, F is equal to unity, because the entire dose enters the systemic circulation. Therefore, the drug is considered to be completely available after IV administration. After oral administration of a drug, F may vary from a value of 0 (no drug absorption) to 1 (complete drug absorption).

Relative Availability

Relative (apparent) availability is the availability of the drug from a drug product as compared to a recognized

standard. The fraction of dose systemically available from an oral drug product is difficult to ascertain. The availability of drug in the formulation is compared to the availability of drug in a standard dosage formulation, usually a solution of the pure drug evaluated in a crossover study. The relative availability of two drug products given at the same dosage level and by the same route of administration can be obtained using the following equation:

where drug product B is the recognized reference standard. This fraction may be multiplied by 100 to give percent relative availability.
When different doses are administered, a correction for the size of the dose is made, as in the following equation:

Urinary drug excretion data may also be used to measure relative availability, as long as the total amount of intact drug excreted in the urine is collected. The percent relative availability using urinary excretion data can be determined as follows:

where $[D_u]^{\infty}$ is the total amount of drug excreted in the urine.
Absolute Availability
The absolute availability of drug is the systemic availability of a drug after extravascular administration (eg, oral, rectal, transdermal, subcutaneous) compared to IV dosing. The absolute availability of a drug is generally measured by comparing the respective AUCs after extravascular and IV administration. This measurement may be performed as long as V_D and k are independent of the route of administration. Absolute availability after oral drug administration using plasma data can be determined as follows:

Absolute availability, F, may be expressed as a fraction or as a percent by multiplying F x 100. Absolute availability using urinary drug excretion data can be determined by the following:

The absolute bioavailability is also equal to F, the fraction of the dose that is bioavailable. Absolute availability is sometimes expressed as a percent, ie, $F = 1$, or 100%. For drugs given intravascularly, such as by IV bolus injection, $F = 1$ because all of the drug is completely absorbed. For all extravascular routes of administration, such as the oral route (PO), the absolute bioavailability F may not exceed 100% ($F > 1$). F is usually determined by Equation 15.4 or 15.5, where PO is the oral route or any other extravascular route of drug administration.
Practice Problem
The bioavailability of a new investigational drug was studied in 12 volunteers. Each volunteer received either a single oral tablet containing 200 mg of the drug, 5 mL of a pure aqueous solution containing 200 mg of the drug, or a single IV bolus injection containing 50 mg of the drug. Plasma samples were obtained periodically up to 48 hours after the dose and assayed for drug concentration. The average AUC values (0–48 hours) are given in the table below. From these data, calculate (a) the relative bioavailability of the drug from the tablet compared to the oral solution and (b) the absolute bioavailability of the drug from the tablet.

Drug Product	**Dose (mg)**	**AUC (g hr/mL)**	**Standard Deviation**
Oral tablet	200	89.5	19.7
Oral solution	200	86.1	18.1
IV bolus injection	50	37.8	5.7

Solution
The relative bioavailability of the drug from the tablet is estimated using Equation 15.1. No adjustment for dose is necessary.

The relative bioavailability of the drug from the tablet is 1.04, or 104%, compared to the solution. In this study, the difference in drug bioavailability between tablet and solution was not statistically significant. It is possible for the relative bioavailability to be greater than 100%.
The absolute drug bioavailability from the tablet is calculated using Equation 15.4 and adjusting for the dose.

Because F, the fraction of dose absorbed from the tablet, is less than 1, the drug is not completely absorbed

systemically, as a result of either poor absorption or metabolism by first-pass effect. The relative bioavailability of the drug from the tablet is approximately 100% when compared to the oral solution.
Results from bioequivalence studies may show that the relative bioavailability of the test oral product is greater than, equal to, or less than 100% compared to the reference oral drug product. However, the results from these bioequivalence studies should not be misinterpreted to imply that the absolute bioavailability of the drug from the oral drug products is also 100% unless the oral formulation was compared to an intravenous injection of the drug.
Methods for Assessing Bioavailability
Direct and indirect methods may be used to assess drug bioavailability. The *in-vivo* bioavailability of a drug product is demonstrated by the rate and extent of drug absorption, as determined by comparison of measured parameters, eg, concentration of the active drug ingredient in the blood, cumulative urinary excretion rates, or pharmacological effects. For drug products that are not intended to be absorbed into the bloodstream, bioavailability 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. The design of the bioavailability study depends on the objectives of the study, the ability to analyze the drug (and metabolites) in biological fluids, the pharmacodynamics of the drug substance, the route of drug administration, and the nature of the drug product. Pharmacokinetic and/or pharmacodynamic parameters as well as clinical observations and *in-vitro* studies may be used to determine drug bioavailability from a drug product ().

Table 15.1 Methods for Assessing Bioavailability and Bioequivalence
Plasma drug concentration
Time for peak plasma (blood) concentration (t_{max})
Peak plasma drug concentration (C_{max})
Area under the plasma drug concentration–time curve (AUC)
Urinary drug excretion
Cumulative amount of drug excreted in the urine (D_u)
Rate of drug excretion in the urine (dD_u/dt)
Time for maximum urinary excretion (t)
Acute pharmacodynamic effect
Maximum pharmacodynamic effect (E_{max})
Time for maximum pharmacodynamic effect
Area under the pharmacodynamic effect–time curve
Onset time for pharmacodynamic effect
Clinical observations
Well-controlled clinical trials
***In-vitro* studies**
Drug dissolution

Plasma Drug Concentration
Measurement of drug concentrations in blood, plasma, or serum after drug administration is the most direct and objective way to determine systemic drug bioavailability. By appropriate blood sampling, an accurate description of the plasma drug concentration–time profile of the therapeutically active drug substance(s) can be obtained using a validated drug assay.
t $_{max}$**.** The *time of peak plasma concentration*, t_{max}, corresponds to the time required to reach maximum drug concentration after drug administration. At t_{max}, peak drug absorption occurs and the rate of drug absorption exactly equals the rate of drug elimination (). Drug absorption still continues after t_{max} is reached, but at a slower rate. When comparing drug products, t_{max} can be used as an approximate indication of drug absorption rate. The value for t_{max} will become smaller (indicating less time required to reach peak plasma concentration) as the absorption rate for the drug becomes more rapid. Units for t_{max} are units of time (eg, hours, minutes).

C $_{max}$**.** The *peak plasma drug concentration*, C_{max}, represents the maximum plasma drug concentration obtained after oral administration of drug. For many drugs, a relationship is found between the pharmacodynamic drug effect and the plasma drug concentration. C_{max} provides indications that the drug is sufficiently systemically absorbed to provide a therapeutic response. In addition, C_{max} provides warning of possibly toxic levels of drug. The units of C_{max} are concentration units (eg, mg/mL, ng/mL). Although not a unit for rate, C_{max} is often used in bioequivalence studies as a surrogate measure for the rate of drug bioavailability.
AUC. The *area under the plasma level–time curve*, AUC, is a measurement of the *extent* of drug bioavailability (). The AUC reflects the total amount of active drug that reaches the systemic circulation. The AUC is the area under the drug plasma level–time curve from $t = 0$ to $t = \infty$, and is equal to the amount of unchanged drug reaching the general circulation divided by the clearance.

where F = fraction of dose absorbed, D_0 = dose, k = elimination rate constant, and V_D = volume of distribution. The AUC is independent of the route of administration and processes of drug elimination as long as the elimination processes do not change. The AUC can be determined by a numerical integration procedure, such as the trapezoidal

rule method. The units for AUC are concentration time (eg, g hr/mL).
For many drugs, the AUC is directly proportional to dose. For example, if a single dose of a drug is increased from 250 to 1000 mg, the AUC will also show a fourfold increase (and).
In some cases, the AUC is not directly proportional to the administered dose for all dosage levels. For example, as the dosage of drug is increased, one of the pathways for drug elimination may become saturated (). Drug elimination includes the processes of metabolism and excretion. Drug metabolism is an enzyme-dependent process. For drugs such as salicylate and phenytoin, continued increase of the dose causes saturation of one of the enzyme pathways for drug metabolism and consequent prolongation of the elimination half-life. The AUC thus increases disproportionally to the increase in dose, because a smaller amount of drug is being eliminated (ie, more drug is retained). When the AUC is not directly proportional to the dose, bioavailability of the drug is difficult to evaluate because drug kinetics may be dose dependent.

Figure 15-1.

Plasma drug concentration–time curve.

Figure 15-2.

Plasma level–time curve following administration of single doses of **(A)** 250 mg, **(B)** 500 mg, and **(C)** 1000 mg of drug.

Figure 15-3.

Linear relationship between AUC and dose (data from).

Figure 15-4.

Relationship between AUC and dose when metabolism is saturable.

Urinary Drug Excretion Data
Urinary drug excretion data is an indirect method for estimating bioavailability. The drug must be excreted in significant quantities as unchanged drug in the urine. In addition, timely urine samples must be collected and the total amount of urinary drug excretion must be obtained (see).
D $^{\infty}$ **u.** The *cumulative amount of drug excreted in the urine*, D^{∞}_u, is related directly to the total amount of drug absorbed. Experimentally, urine samples are collected periodically after administration of a drug product. Each urine

specimen is analyzed for free drug using a specific assay. A graph is constructed that relates the cumulative drug excreted to the collection-time interval ().
The relationship between the cumulative amount of drug excreted in the urine and the plasma level–time curve is shown in . When the drug is almost completely eliminated (point *C*), the plasma concentration approaches zero and the maximum amount of drug excreted in the urine, D_u^{∞}, is obtained.
dD $_u$***/dt.*** The *rate of drug excretion*. Because most drugs are eliminated by a first-order rate process, the rate of drug excretion is dependent on the first-order elimination rate constant *k* and the concentration of drug in the plasma C_p. In , the *maximum rate of drug excretion*, $(dD_u/dt)_{max}$, is at point *B*, whereas the minimum rate of drug excretion is at points *A* and *C*. Thus, a graph comparing the rate of drug excretion with respect to time should be similar in shape as the plasma level–time curve for that drug ().
t $^{\infty}$**.** The *total time for the drug to be excreted*. In and , the slope of the curve segment *A–B* is related to the rate of drug absorption, whereas point *C* is related to the total time required after drug administration for the drug to be absorbed and completely excreted $t = \infty$. The t^{∞} is a useful parameter in bioequivalence studies that compare several drug products, as will be described later in this chapter.

Figure 15-5.
Corresponding plots relating the plasma level–time curve and the cumulative urinary drug excretion.

Figure 15-6.
Corresponding plots relating the plasma level–time curve and the cumulative urinary drug excretion.

Figure 15-7.
Corresponding plots relating the plasma level–time curve and the rate of urinary drug excretion.

Acute Pharmacodynamic Effect
In some cases, the quantitative measurement of a drug in plasma or urine lacks an assay with sufficient accuracy and/or reproducibility. For locally acting, nonsystemically absorbed drug products, such as topical corticosteroids, plasma drug concentrations may not reflect the bioavailability of the drug at the site of action. An acute pharmacodynamic effect, such as an effect on forced expiratory volume, FEV_1 (inhaled bronchodilators) or skin blanching (topical corticosteroids) can be used as an index of drug bioavailability. In this case, the acute pharmacodynamic effect is measured over a period of time after administration of the drug product. Measurements of the pharmacodynamic effect should be made with sufficient frequency to permit a reasonable estimate for a time period at least three times the half-life of the drug (). This approach may be particularly applicable to dosage forms that are not intended to deliver the active moiety to the bloodstream for systemic distribution.
The use of an acute pharmacodynamic effect to determine bioavailability generally requires demonstration of a dose–response curve (see). Bioavailability is determined by characterization of the dose–response curve. For bioequivalence determination, pharmacodynamic parameters including the total area under the acute pharmacodynamic effect–time curve, peak pharmacodynamic effect, and time for peak pharmacodynamic effect are obtained from the pharmacodynamic effect–time curve. The onset time and duration of the pharmacokinetic effect may also be included in the analysis of the data. The use of pharmacodynamic endpoints for the determination of bioavailability and bioequivalence is much more variable than the measurement of plasma or urine drug concentrations.
Clinical Observations
Well-controlled clinical trials in humans establish the safety and effectiveness of drug products and may be used to determine bioavailability. However, the clinical trials approach is the least accurate, least sensitive, and least reproducible of the general approaches for determining *in-vivo* bioavailability. The FDA considers this approach only when analytical methods and pharmacodynamic methods are not available to permit use of one of the approaches described above. Comparative clinical studies have been used to establish bioequivalence for topical antifungal drug products (eg, ketoconazole) and for topical acne preparations. 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 other approaches

In-Vitro Studies

Drug dissolution studies may under certain conditions give an indication of drug bioavailability. Ideally, the *in-vitro* drug dissolution rate should correlate with *in-vivo* drug bioavailability (see and on *in-vivo–in-vitro* correlation, IVIVC). Dissolution studies are often performed on several test formulations of the same drug. The test formulation that demonstrates the most rapid rate of drug dissolution *in vitro* will generally have the most rapid rate of drug bioavailability *in vivo*.

The FDA may also use other *in-vitro* approaches for establishing bioequivalence. For example, cholestyramine resin is a basic quaternary ammonium anion-exchange resin that is hydrophilic, insoluble in water, and not absorbed in the gastrointestinal tract. The bioequivalence of cholestyramine resin is performed by equilibrium and kinetic binding studies of the resin to bile acid salts (www.fda.gov/cder/guidance/cholesty.pdf).

Bioequivalence Studies

Differences in the predicted clinical response or an adverse event may be due to differences in the pharmacokinetic and/or pharmacodynamic behavior of the drug among individuals or to differences in the bioavailability of the drug from the drug product. Bioequivalent drug products that have the same systemic drug bioavailability will have the same predictable drug response. However, variable clinical responses among individuals that are unrelated to bioavailability may be due to differences in the pharmacodynamics of the drug. Differences in pharmacodynamics, ie, the relationship between the drug and the receptor site, may be due to differences in receptor sensitivity to the drug. Various factors affecting pharmacodynamic drug behavior may include age, drug tolerance, drug interactions, and unknown pathophysiologic factors.

The bioavailability of a drug may be more reproducible among fasted individuals in controlled studies who take the drug on an empty stomach. When the drug is used on a daily basis, however, the nature of an individual's diet and lifestyle may affect the plasma drug levels because of variable absorption in the presence of food or even a change in the metabolic clearance of the drug. reported that patients on a high-carbohydrate diet have a much longer elimination half-life of theophylline, due to the reduced metabolic clearance of the drug ($t_{1/2}$, 18.1 hours), compared to patients on normal diets ($t_{1/2}$ = 6.76 hours). Previous studies demonstrated that the theophylline drug product was completely bioavailable. The higher plasma drug concentration resulting from a carbohydrate diet may subject the patient to a higher risk of drug intoxication with theophylline. The effect of food on the availability of theophylline has been reported by the FDA concerning the risk of higher theophylline plasma concentrations from a 24-hour sustained-release drug product taken with food. Although most bioavailability drug studies use fasted volunteers, the diet of patients actually using the drug product may increase, decrease, or have no affect on the bioavailability of the drug ().

Bases for Determining Bioequivalence

Bioequivalence is established if the *in-vivo* bioavailability of a test drug product (usually the generic product) does not differ significantly (ie, statistically insignificant) in the product's rate and extent of drug absorption, as determined by comparison of measured parameters (eg, concentration of the active drug ingredient in the blood, urinary excretion rates, or pharmacodynamic effects), from that of the *reference listed drug* (usually the brand-name product) when administered at the same molar dose of the active moiety under similar experimental conditions, either single dose or multiple dose.

In a few cases, a drug product that differs from the reference listed drug in its rate of absorption, but not in its extent of absorption, may be considered bioequivalent if the difference in the rate of absorption is intentional and appropriately reflected in the labeling and/or the rate of absorption is not detrimental to the safety and effectiveness of the drug product.

Drug Products with Possible Bioavailability and Bioequivalence Problems

Lack of bioavailability or bioequivalence may be suspected when evidence from well-controlled clinical trials or controlled observations in patients of various marketed drug products do not give comparable therapeutic effects. These drug products need to be evaluated either *in vitro* (eg, drug dissolution/release test) or *in vivo* (eg, bioequivalence study) to determine if the drug product has a bioavailability problem (see also U.S. Code of Federal Regulations, 21 CFR 320.33).

In addition, during the development of a drug product, certain biopharmaceutical properties of the active drug substance or the formulation of the drug product may indicate that the drug may have variable bioavailability and/or a bioequivalence problem. Some of these biopharmaceutic properties include:

The active drug ingredient has low solubility in water (eg, less than 5 mg/mL).

The dissolution rate of one or more such products is slow (eg, less than 50% in 30 minutes when tested with a general method specified by the FDA).

The particle size and/or surface area of the active drug ingredient is critical in determining its bioavailability.

Certain structural forms of the active drug ingredient (eg, polymorphic forms, solvates, complexes, and crystal modifications) dissolve poorly, thus affecting absorption.

Drug products that have a high ratio of excipients to active ingredients (eg, greater than 5:1).

Specific inactive ingredients (eg, hydrophilic or hydrophobic excipients and lubricants) either may be required for absorption of the active drug ingredient or therapeutic moiety or may interfere with such absorption.

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.

The degree of absorption of the active drug ingredient, therapeutic moiety, or its precursor is poor (eg, less than 50%, ordinarily in comparison to an intravenous dose), even when it is administered in pure form (eg, in solution).

There is rapid metabolism of the therapeutic moiety in the intestinal wall or liver during the absorption process (first-order metabolism), so that the rate of absorption is unusually important in the therapeutic effect and/or toxicity of the drug product.

The therapeutic moiety is rapidly metabolized or excreted, so that rapid dissolution and absorption are required for effectiveness.

The active drug ingredient or therapeutic moiety is unstable in specific portions of the GI tract and requires special coatings or formulations (eg, buffers, enteric coatings, and film coatings) to ensure adequate absorption.

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 bioequivalence.

Design and Evaluation of Bioequivalence Studies

Bioequivalence studies are performed to compare the bioavailability of the generic drug product to the brand-name product. Statistical techniques should be of sufficient sensitivity to detect differences in rate and extent of absorption that are not attributable to subject variability. Once bioequivalence is established, it is likely that both the generic and brand-name dosage forms will produce the same therapeutic effect. The FDA publishes guidances for bioequivalence studies (www.fda.gov/cder/guidance; see also 21 CFR 320.25). Sponsors may also request a meeting with the FDA to review the study design for a specific drug product.

Design

The design and evaluation of well-controlled bioequivalence studies require cooperative input from pharmacokineticists, statisticians, clinicians, bioanalytical chemists, and others. The basic design for a bioequivalence study is determined by (1) the scientific questions to be answered, (2) the nature of the reference material and the dosage form to be tested, (3) the availability of analytical methods, and (4) benefit–risk and ethical considerations with regard to testing in humans. For some generic drugs, the FDA offers general guidelines for conducting these studies. For example, *Statistical Procedures for Bioequivalence Studies Using a Standard Two-Treatment Crossover Design* is available from the FDA; the publication addresses three specific aspects, including (1) logarithmic transformation of pharmacokinetic data, (2) sequence effect, and (3) outlier consideration. However, even with the availability of such guidelines, the principal investigator should prepare a detailed protocol for the study. Some of the elements of a protocol for an *in-vivo* bioavailability study are listed in . Bioavailability studies for controlled-release dosage forms are discussed in .

Table 15.2 Elements of a Bioavailability Study Protocol
I. Title
A. Principal investigator (study director)
B. Project/protocol number and date
II. Study objective
III. Study design
A. Design
B. Drug products

1. Test product(s)
2. Reference product
C. Dosage regimen
D. Sample collection schedule
E. Housing/confinement
F. Fasting/meals schedule
G. Analytical methods
IV. Study population
A. Subjects
B. Subject selection
1. Medical history
2. Physical examination
3. Laboratory tests
C. Inclusion/exclusion criteria
1. Inclusion criteria
2. Exclusion criteria
D. Restrictions/prohibitions
V. Clinical procedures
A. Dosage and drug administration
B. Biological sampling schedule and handling procedures
C. Activity of subjects
VI. Ethical considerations
A. Basic principles
B. Institutional review board
C. Informed consent
D. Indications for subject withdrawal
E. Adverse reactions and emergency procedures
VII. Facilities
VIII. Data analysis
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For bioequivalence studies, the test and reference drug formulations must contain the pharmaceutical equivalent drug in the same dose strength, in similar dosage forms (eg, immediate release or controlled release), and be given by the same route of administration. Both a single-dose and/or a multiple-dose (steady-state) study may be required. Before beginning the study, the *Institutional Review Board* (IRB) of the clinical facility in which the study is to be performed must approve the study. The IRB is composed of both professional and lay persons with diverse backgrounds, who have clinical experience and expertise as well as sensitivity to ethical issues and community attitudes. The IRB is responsible for safeguarding the rights and welfare of human subjects.

The basic guiding principle in performing studies is *do not do unnecessary human research*. Generally, the study is performed in normal, healthy male and female volunteers who have given informed consent to be in the study. Critically ill patients are not included in an *in-vivo* bioavailability study unless the attending physician determines that there is a potential benefit to the patient. The number of subjects in the study will depend on the expected intersubject and intrasubject variability. Patient selection is made according to certain established criteria for

inclusion into, or exclusion from, the study. For example, the study might exclude any volunteers who have known allergies to the drug, are overweight, or have taken any medication within a specified period (often 1 week) prior to the study. Smokers are often included in these studies. The subjects are generally fasted for 10 to 12 hours (overnight) prior to drug administration and may continue to fast for a 2- to 4-hour period after dosing.

Analytical Methods

The analytical method used in an *in-vivo* bioavailability or bioequivalence 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, must 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. For bioavailability studies, both the parent drug and its major active metabolites are generally measured. For bioequivalence studies, the parent drug is measured. The active metabolite might be measured for some very high hepatic clearance (first-pass metabolism) drugs when the parent drug concentrations are too low to be reliable.

Reference Standard

For bioequivalence studies, one formulation of the drug is chosen as a reference standard against which all other formulations of the drug are compared. The reference drug product should be administered by the same route as the comparison formulations unless an alternative route or additional route is needed to answer specific pharmacokinetic questions. For example, if an active drug is poorly bioavailable after oral administration, the drug may be compared to an oral solution or an intravenous injection. For bioequivalence studies on a proposed generic drug product the reference standard is the *reference listed drug* (RLD), which is listed in *Approved Drug Products with Therapeutic Equivalence Evaluations*—the *Orange Book* (www.fda.gov/cder/orange/default.htm), and the proposed generic drug product is often referred to as the "Test" drug product. The RLD is generally a formulation currently marketed with a fully approved NDA for which there are valid scientific safety and efficacy data. The RLD is usually the innovator's or original manufacturer's brand-name product and is administered according to the dosage recommendations in the labeling.

Before beginning an *in-vivo* bioequivalence study, the total content of the active drug substance in the test product (generally the generic product) must be within 5% of that of the reference product. Moreover, *in-vitro* comparative dissolution or drug-release studies under various specified conditions are usually performed for both test and reference products before performing the *in-vivo* bioequivalence study.

Extended-Release Formulations

The purpose of an *in-vivo* bioavailability study involving an extended-release drug product is to determine if (1) the drug product meets the controlled-release claims made for it, (2) the bioavailability profile established for the drug product rules out the occurrence of any *dose dumping*, (3) the drug product's steady-state performance is equivalent to that of a currently marketed non-extended-release formulation, and (4) the drug product's formulation provides consistent pharmacokinetic performance between individual dosage units. A comparison bioavailability study is used for the development of a new extended release drug product in which the reference drug product may be either a solution or suspension of the active ingredient or a currently marketed non-controlled release drug product such as a tablet or capsule. For example, the bioavailability of a non-controlled-release (immediate-release) drug product given at a dose of 25 mg every 8 hours is compared to an extended-release product containing 75 mg of the same drug given once daily. For a bioequivalence study of a new generic extended release drug product, the reference drug product is the currently marketed extended release drug product listed as the RLD in the Orange Book and is administered according to the dosage recommendations in the approved labeling.

Combination Drug Products

Generally, the purpose of an *in-vivo* bioavailability study involving a combination drug product containing more than one active drug substance is to determine if the rate and extent of absorption of each active drug ingredient or therapeutic moiety in the combination drug product is equivalent to the rate and extent of absorption of each active drug ingredient or therapeutic moiety administered concurrently in separate single-ingredient preparations. The reference material in such a bioavailability study should be two or more currently marketed, single-ingredient drug products, each of which contains one of the active drug ingredients in the combination drug product. The FDA may, for valid scientific reasons, specify that the reference material be a combination drug product that is the subject of an approved NDA.

Study Designs

For many drug products, the FDA, Division of Bioequivalence, Office of Generic Drugs, provides guidance for the performance of *in-vitro* dissolution and *in-vivo* bioequivalence studies. Similar guidelines appear in the United States Pharmacopeia NF. Currently, three different studies may be required for solid oral dosage forms, including (1) a fasting study, (2) a food intervention study, and/or (3) a multiple-dose (steady-state) study. Other study designs have been proposed by the FDA. For example, the FDA published two draft guidelines in October and December 1997 to consider the performance of individual bioequivalence studies using a replicate design and a two-way crossover food intervention study. Proper study design and statistical evolution are important considerations for the determination of

bioequivalence. Some of the designs listed above are summarized here.

Fasting Study

Bioequivalence studies are usually evaluated by a single-dose, two-period, two-treatment, two-sequence, open-label, randomized crossover design comparing equal doses of the test and reference products in fasted, adult, healthy subjects. This study is required for all immediate-release and modified-release oral dosage forms. Both male and female subjects may be used in the study. Blood sampling is performed just before (zero time) the dose and at appropriate intervals after the dose to obtain an adequate description of the plasma drug concentration–time profile. The subjects should be in the fasting state (overnight fast of at least 10 hours) before drug administration and should continue to fast for up to 4 hours after dosing. No other medication is normally given to the subject for at least 1 week prior to the study. In some cases, a parallel design may be more appropriate for certain drug products, containing a drug with a very long elimination half-life. A replicate design may be used for a drug product containing a drug that has high intrasubject variability.

Food Intervention Study

Co-administration of food with an oral drug product may affect the bioavailability of the drug. Food intervention or food effect studies are generally conducted using meal conditions that are expected to provide the greatest effects on GI physiology so that systemic drug availability is maximally affected. The test meal is a high-fat (approximately 50% of total caloric content of the meal) and high-calorie (approximately 800–1000 calories) meal. A typical test meal is two eggs fried in butter, two strips of bacon, two slices of toast with butter, 4 ounces of brown potatoes, and 8 ounces of milk. This test meal derives approximately 150, 250, and 500–600 calories from protein, carbohydrate, and fat, respectively (www.fda.gov/cder/guidance/4613dft.pdf).

For bioequivalence studies, drug bioavailability from both the test and reference products should be affected similarly by food. The study design uses a single-dose, randomized, two-treatment, two-period, crossover study comparing equal doses of the test and reference products. Following an overnight fast of at least 10 hours, subjects are given the recommended meal 30 minutes before dosing. The meal is consumed over 30 minutes, with administration of the drug product immediately after the meal. The drug product is given with 240 mL (8 fluid ounces) of water. No food is allowed for at least 4 hours postdose. This study is required for all modified-release dosage forms and may be required for immediate-release dosage forms if the bioavailability of the active drug ingredient is known to be affected by food (eg, ibuprofen, naproxen). For certain extended-release capsules that contain coated beads, the capsule contents are sprinkled over soft foods such as apple sauce, which is taken by the fasted subject and the bioavailability of the drug is then measured. Bioavailability studies might also examine the affects of other foods and special vehicles such as apple juice.

Multiple-Dose (Steady-State) Study

In a few cases, a multiple-dose, steady-state, randomized, two-treatment, two-way crossover study comparing equal doses of the test and reference products may be performed in adult, healthy subjects. For these studies, three consecutive trough concentrations (C_{min}) on three consecutive days should be determined to ascertain that the subjects are at steady state. The last morning dose is given to the subject after an overnight fast, with continual fasting for at least 2 hours following dose administration. Blood sampling is performed similarly to the single-dose study.

Crossover Designs

Subjects who meet the inclusion and exclusion study criteria and have given informed consent are selected at random. A complete crossover design is usually employed, in which each subject receives the test drug product and the reference product. Examples of *Latin-square crossover designs* for a bioequivalence study in human volunteers, comparing three different drug formulations (A, B, C) or four different drug formulations (A, B, C, D), are described in and . The Latin-square design plans the clinical trial so that each subject receives each drug product only once, with adequate time between medications for the elimination of the drug from the body (). In this design, each subject is his own control, and subject-to-subject variation is reduced. Moreover, variation due to sequence, period, and treatment (formulation) are reduced, so that all patients do not receive the same drug product on the same day and in the same order. Possible carryover effects from any particular drug product are minimized by changing the sequence or order in which the drug products are given to the subject. Thus, drug product B may be followed by drug product A, D, or C (). After each subject receives a drug product, blood samples are collected at appropriate time intervals so that a valid blood drug level–time curve is obtained. The time intervals should be spaced so that the peak blood concentration, the total area under the curve, and the absorption and elimination phases of the curve may be well described.

Table 15.3 Latin-Square Crossover Design for a Bioequivalence Study of Three Drug Products in Six Human Volunteers

	Drug Product		
Subject	**Study Period 1**	**Study Period 2**	**Study Period 3**

1	A	B	C
2	B	C	A
3	C	A	B
4	A	C	B
5	C	B	A
6	B	A	C

Table 15.4 Latin-Square Crossover Design for a Bioequivalency Study of Four Drug Products in 16 Human Volunteers

	Drug Product			
Subject	**Study Period 1**	**Study Period 2**	**Study Period 3**	**Study Period 4**
1	A	B	C	D
2	B	C	D	A
3	C	D	A	B
4	D	A	B	C
5	A	B	D	C
6	B	D	C	A
7	D	C	A	B
8	C	A	B	D
9	A	C	B	D
10	C	B	D	A
11	B	D	A	C
12	D	A	C	B
13	A	C	D	B
14	C	D	B	A
15	D	B	A	C
16	B	A	C	D

Period refers to the time period in which a study is performed. A two-period study is a study that is performed on two different days (time periods) separated by a *washout period* during which most of the drug is eliminated from the body—generally about 10 elimination half-lives. A *sequence* refers to the number of different orders in the treatment groups in a study. For example, a two-sequence, two-period study would be designed as follows:

	Period 1	**Period 2**
Sequence 1	T	R
Sequence 2	R	T

where R = reference and T = treatment.

shows a design for three different drug treatment groups given in a three-period study with six different sequences. The order in which the drug treatments are given should not stay the same in order to prevent any bias in the data due to a residual effect from the previous treatment.

Replicated Crossover Design

Replicated crossover designs are used for the determination of individual bioequivalence, to estimate within-subject variance for both the Test and Reference drug products, and to provide an estimate of the subject-by-formulation interaction variance. Generally, a four-period, two-sequence, two-formulation design is recommended by the FDA.

	Period 1	**Period 2**	**Period 3**	**Period 4**
Sequence 1	T	R	T	R
Sequence 2	R	T	R	T

where R = reference and T = treatment.

The same reference and the same test are each given twice to the same subject. Other sequences are possible. In this

design, Reference-to-Reference and Test-to-Test comparisons may also be made.

Evaluation of the Data

Analytical Method

The analytical method for measurement of the drug must be validated for accuracy, precision, sensitivity, and specificity. The use of more than one analytical method during a bioequivalence study may not be valid, because different methods may yield different values. Data should be presented in both tabulated and graphic form for evaluation. The plasma drug concentration–time curve for each drug product and each subject should be available.

Pharmacokinetic Evaluation of the Data

For single-dose studies, including a fasting study or a food intervention study, the pharmacokinetic analyses include calculation for each subject of the area under the curve to the last quantifiable concentration (AUC_{0-t}) and to infinity ($AUC_{0-\infty}$), T_{max}, and C_{max}. Additionally, the elimination rate constant, k, the elimination half-life, $t_{1/2}$, and other parameters may be estimated. For multiple-dose studies, pharmacokinetic analysis includes calculation for each subject of the steady-state area under the curve, (AUC_{0-t}), T_{max}, C_{min}, C_{max}, and the percent fluctuation [100 x ($C_{max} - C_{min}$)/C_{min}]. Proper statistical evaluation should be performed on the estimated pharmacokinetic parameters.

Statistical Evaluation of the Data

Bioequivalence is generally determined using a comparison of population averages of a bioequivalence metric, such as AUC and C_{max}. This approach, termed *average bioequivalence,* involves the calculation of a 90% confidence interval for the ratio of averages (population geometric means) of the bioequivalence metrics for the Test and Reference drug products. To establish bioequivalence, the calculated confidence interval should fall within a prescribed bioequivalence limit, usually, 80–125% for the ratio of the product averages. Standard crossover design studies are used to obtain the data. Another approach proposed by the FDA and others is termed *individual bioequivalence*. Individual bioequivalence requires a replicate crossover design, and estimates within-subject variability for the Test and Reference drug products, as well as subject-by-formulation interaction. Presently, only average bioequivalence estimates are used to establish bioequivalence of generic drug products.

To prove bioequivalence, there must be no statistical difference between the bioavailability of the Test product and the Reference product. Several statistical approaches are used to compare the bioavailability of drug from the test dosage form to the bioavailability of the drug from the reference dosage form. Many statistical approaches (parametric tests) assume that the data are distributed according to a normal distribution or "bell-shaped curve" (see). The distribution of many biological parameters such as C_{max} and AUC have a longer right tail than would be observed in a normal distribution (). Moreover, the true distribution of these biological parameters may be difficult to ascertain because of the small number of subjects used in a bioequivalence study. The distribution of data that has been transformed to log values resembles more closely a normal distribution compared to the distribution of non-log-transformed data. Therefore, log transformation of the bioavailability data (eg, C_{max}, AUC) is performed before statistical data evaluation for bioequivalence determination.

Analysis of Variance (ANOVA)

An analysis of variance (ANOVA) is a statistical procedure () used to test the data for differences within and between treatment and control groups. A bioequivalent product should produce no significant difference in all pharmacokinetic parameters tested. The parameters tested usually include AUC_{0-t}, $AUC_{0-\infty}$, t_{max}, and C_{max} obtained for each treatment or dosage form. Other metrics of bioavailability have also been used to compare the bioequivalence of two or more formulations. The ANOVA may evaluate variability in subjects, treatment groups, study period, formulation, and other variables, depending on the study design. If the variability in the data is large, the difference in means for each pharmacokinetic parameter, such as AUC, may be masked, and the investigator might erroneously conclude that the two drug products are bioequivalent.

A statistical difference between the pharmacokinetic parameters obtained from two or more drug products is considered statistically significant if there is a probability of less than 1 in 20 times or 0.05 probability (p 0.05) that these results would have happened on the basis of chance alone. The probability, p, is used to indicate the level of statistical significance. If $p < 0.05$, the differences between the two drug products are not considered statistically significant.

To reduce the possibility of failing to detect small differences between the test products, a *power test* is performed to calculate the probability that the conclusion of the ANOVA is valid. The power of the test will depend on the sample size, variability of the data, and desired level of significance. Usually the power is set at 0.80 with a = 0.2 and a level of significance of 0.05. The higher the power, the more sensitive the test and the greater the probability that the conclusion of the ANOVA is valid.

Two One-Sided Tests Procedure

The two one-sided tests procedure is also referred to as the *confidence interval approach* (). This statistical method is used to demonstrate if the bioavailability of the drug from the Test formulation is too low or high in comparison to that of the Reference product. The objective of the approach is to determine if there are large differences (ie, greater

than 20%) between the mean parameters.
The 90% confidence limits are estimated for the sample means. The interval estimate is based on a Student's *t* distribution of the data. In this test, presently required by the FDA, a 90% confidence interval about the ratio of means of the two drug products must be within ±20% for measurement of the rate and extent of drug bioavailability. For most drugs, up to a 20% difference in AUC or C_{max} between two formulations would have no clinical significance. The lower 90% confidence interval for the ratio of means cannot be less than 0.80, and the upper 90% confidence interval for the ratio of the means cannot be greater than 1.20. When log-transformed data are used, the 90% confidence interval is set at 80–125%. These confidence limits have also been termed the *bioequivalence interval* (). The 90% confidence interval is a function of sample size and study variability, including inter- and intrasubject variability.
For a single-dose, fasting study, an analysis of variance (ANOVA) is usually performed on the log-transformed AUC and C_{max} values. There should be no statistical differences between the mean AUC and C_{max} parameters for the Test (generic) and Reference drug products. In addition, the 90% confidence intervals about the ratio of the means for AUC and C_{max} values of the Test drug product should not be less than 0.80 (80%) nor greater than 1.25 (125%) of that of the Reference product based on log-transformed data.
Bioequivalence Example
A simulated example of the results for a single-dose, fasting study is shown in and in . As shown by the ANOVA, no statistical differences for the pharmacokinetic parameters AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} were observed between the Test product and the brand-name product. The 90% confidence limits for the mean pharmacokinetic parameters of the Test product were within 0.80–1.25 (80–125%) of the reference product means based on log transformation of the data. The power test for the AUC measures were above 99%, showing good precision of the data. The power test for the C_{max} values was 87.9%, showing that this parameter was more variable.

Table 15.5 Bioavailability Comparison of a Generic (Test) and Brand-Name (Reference) Drug Products (Log-Normal Transformed Data)

		Geometric Mean						
Variable	**Units**	**Test**	**Reference**	**% Ratio**	**90% Confidence Interval (Lower Limit, Upper Limit)**	***p* Values for Product Effects**	**Power of ANOVA**	**ANOVA % CV**
C_{max}	ng/mL	344.79	356.81	96.6	(89.5,112)	0.3586	0.8791	17.90%
AUC_{0-t}	ng hr/mL	2659.12	2674.92	99.4	(95.1,104)	0.8172	1.0000	12.60%
AUC_{∞}		2708.63	2718.52	99.6	(95.4,103)	0.8865	1.0000	12.20%
T_{max}	hr	4.29	4.24	101				
K_{elim}	1/hr	0.0961	0.0980	98.1				
$t_{1/2}$	hr	8.47	8.33	101.7				

The results were obtained from a two-way, crossover, single-dose study in 36 fasted, healthy, adult male and female volunteers. No statistical differences were observed for the mean values between Test and Reference products.

Figure 15-8.

Bioequivalence of Test and Reference drug products: mean plasma drug concentrations.

shows the results for a hypothetical bioavailability study in which three different tablet formulations were compared to a solution of the drug given in the same dose. As shown in the table, the bioavailability from all three tablet formulations was greater than 80% of that of the solution. According to the ANOVA, the mean AUC values were not statistically different from each other nor different from that of the solution. However, the 90% confidence interval for the AUC showed that for tablet A, the bioavailability was less than 80% (ie, 74%), compared to the solution at the low-range estimate and would not be considered bioequivalent based on AUC.

Table 15.6 Summary of the Results of a Bioavailability Study[a]					
Dosage Form	***C*** **$_{max}$ (g/mL)**	***t*** **$_{max}$ (hr)**	**AUC$_{0–24}$ (g hr/mL)**	***F*** **[b]**	**90% Confidence Interval for AUC**
Solution	16.1 ± 2.5	1.5 ± 0.85	1835 ± 235		
Tablet A	10.5 ± 3.2[c]	2.5 ± 1.0[c]	1523 ± 381	81	74–90%
Tablet B	13.7 ± 4.1	2.1 ± 0.98	1707 ± 317	93	88–98%
Tablet C	14.8 ± 3.6	1.8 ± 0.95	1762 ± 295	96	91–103%

[a] The bioavailability of a drug from four different formulations was studied in 24 healthy, adult male subjects using a four-way Latin-square crossover design. The results represent the mean ± standard deviation.
[b] Oral bioavailability relative to the solution.

[c] p 0.05.
For illustrative purposes, consider a drug that has been prepared at the same dosage level in three formulations, A, B, and C. These formulations are given to a group of volunteers using a three-way, randomized crossover design. In this experimental design, all subjects receive each formulation once. From each subject, plasma drug level and urinary drug excretion data are obtained. With these data we can observe the relationship between plasma and urinary excretion parameters and drug bioavailability (). The rate of drug absorption from formulation A is more rapid than that from formulation B, because the t_{max} for formulation A is shorter. Because the AUC for formulation A is identical to the AUC for formulation B, the extent of bioavailability from both of these formulations is the same. Note, however, the C_{max} for A is higher than that for B, because the rate of drug absorption is more rapid.

Figure 15-9.
Corresponding plots relating plasma concentration and urinary excretion data.

The C_{max} is generally higher when the extent of drug bioavailability is greater. The rate of drug absorption from formulation C is the same as that from formulation A, but the extent of drug available is less. The C_{max} for formulation C is less than that for formulation A. The decrease in C_{max} for formulation C is proportional to the decrease in AUC in comparison to the drug plasma level data for formulation A. The corresponding urinary excretion data confirm these observations. These relationships are summarized in . The table illustrates how bioavailability parameters for plasma and urine change when only the extent and rate of bioavailability are changed, respectively. Formulation changes in a drug product may affect both the rate and extent of drug bioavailability.

Table 15.7 Relationship of Plasma Level and Urinary Excretion Parameters to Drug Bioavailability			
Extent of Drug Bioavailability Decreases		**Rate of Drug Bioavailability Decreases**	
Parameter	**Change**	**Parameter**	**Change**
Plasma data			
t_{max}	Same	t_{max}	Increase
C_{max}	Decrease	C_{max}	Decrease
AUC	Decrease	AUC	Same
Urine data			
t^{∞}	Same	t^{∞}	Increase
$[dD_u/dt]_{max}$ [a]	Decrease	$[dD_u/dt]_{max}$ [a]	Decrease
D^{∞}_u	Decrease	D^{∞}_u	Same