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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:

$$\text{Relative availability} = \frac{[\text{AUC}]_{\text{A}}}{[\text{AUC}]_{\text{B}}} \qquad (15.1)$$

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:

$$\text{Relative availability} = \frac{[\text{AUC}]_{\text{A}}/\text{dose A}}{[\text{AUC}]_{\text{B}}/\text{dose B}} \qquad (15.2)$$

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:

$$\text{Percent relative availability} = \frac{[D_{\text{u}}]_{\text{A}}^{\infty}}{[D_{\text{u}}]_{\text{B}}^{\infty}} \times 100 \qquad (15.3)$$

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:

$$\text{Absolute availability} = F = \frac{[\text{AUC}]_{\text{PO}}/\text{dose}_{\text{PO}}}{[\text{AUC}]_{\text{IV}}/\text{dose}_{\text{IV}}} \qquad (15.4)$$

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

$$\text{Absolute availability} = \frac{[D_{\text{u}}]_{\text{PO}}^{\infty}/\text{dose}_{\text{PO}}}{[D_{\text{u}}]_{\text{IV}}^{\infty}/\text{dose}_{\text{IV}}} \qquad (15.5)$$

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 (μg 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.

$$\text{Relative bioavailability} = \frac{89.5}{86.1} = 1.04 \quad \text{or} \quad 104\%$$

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.

$$F = \text{absolute bioavailability} = \frac{89.5/200}{37.8/50} = 0.592 \quad \text{or} \quad 59.2\%$$

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.

$$[\mathrm{AUC}]_0^\infty = \int_0^\infty C_\mathrm{p}\,dt \qquad (15.6)$$

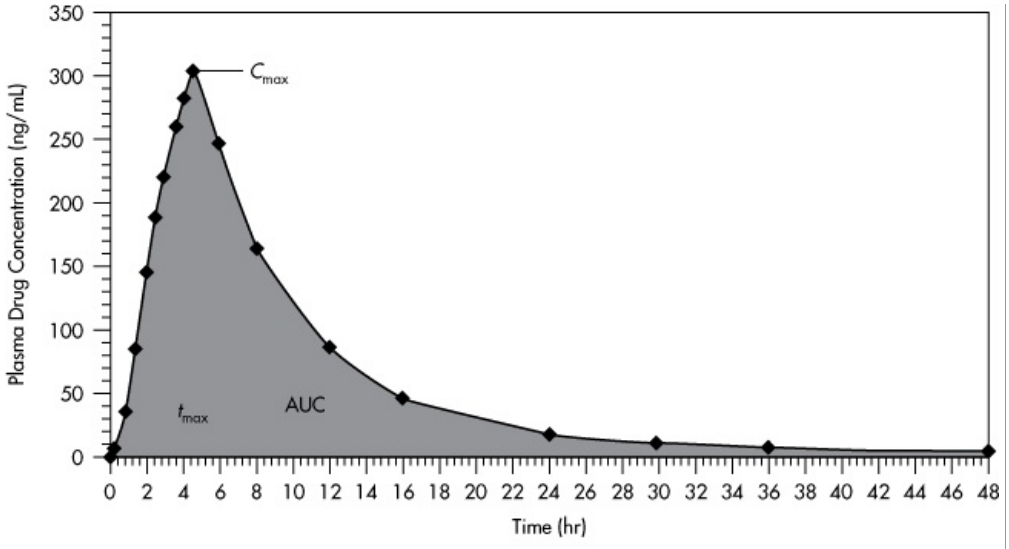
$$[\mathrm{AUC}]_0^\infty = \frac{FD_0}{\text{clearance}} = \frac{FD_0}{kV_\mathrm{D}} \qquad (15.7)$$

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, µg 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).

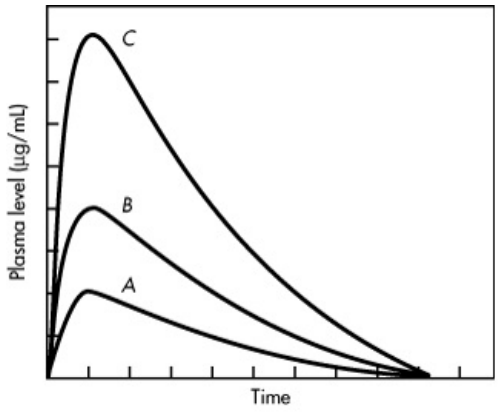
In some cases, the AUC is not directly proportional to the administered dose for all dosage levels. For example, as the dosage of drug is increased, one of the pathways for drug elimination may become saturated (). Drug elimination includes the processes of metabolism and excretion. Drug metabolism is 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.



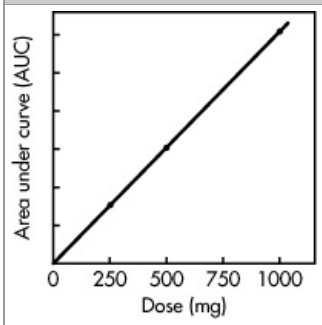
Plasma drug concentration–time curve.

Figure 15-2.



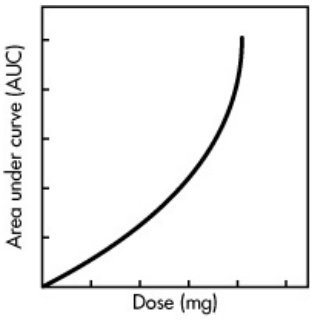
Plasma level–time curve following administration of single doses of **(A)** 250 mg, **(B)** 500 mg, and **(C)** 1000 mg of drug.

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).

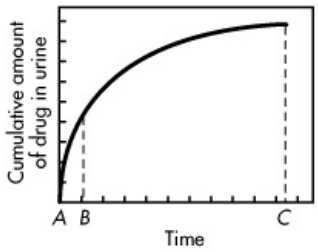
D $^{\infty}$ ***u*.** The *cumulative amount of drug excreted in the urine*, D^{∞}_{u}, is related directly to the total amount of drug absorbed. Experimentally, urine samples are collected periodically after administration of a drug product. Each urine specimen is analyzed for free drug using a specific 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 ().

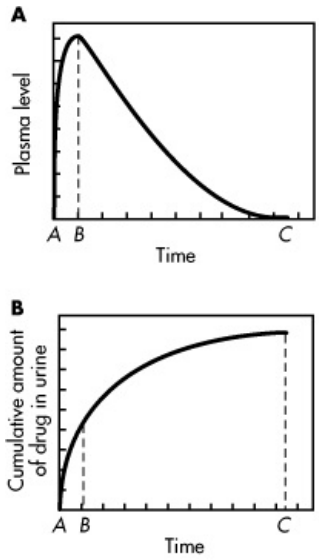
t $^{\infty}$**.** The *total time for the drug to be excreted*. In and , the slope of the curve segment *A*–*B* is related to the rate of drug absorption, whereas point *C* is related to the total time required after drug administration for the drug to be absorbed and completely excreted $t = \infty$. 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.

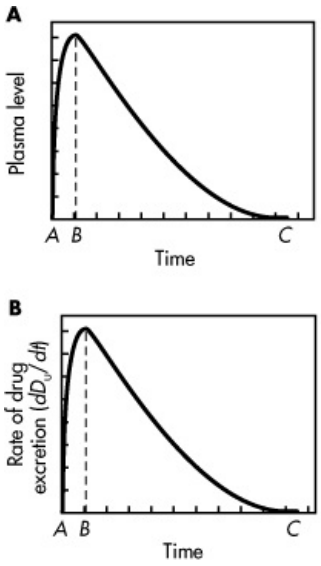
Figure 15-6.



Source: Shargel S, Wu-Pong S, Yu ABC: *Applied Biopharmaceutics & Pharmacokinetics*, 5th Edition: http://www.accesspharmacy.com

Corresponding plots relating the plasma level–time curve and the cumulative urinary drug excretion.

Figure 15-7.



Source: Shargel S, Wu-Pong S, Yu ABC: *Applied Biopharmaceutics & Pharmacokinetics*, 5th Edition: http://www.accesspharmacy.com

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
A. Analytical validation procedure
B. Statistical treatment of data
IX. Drug accountability
X. Appendix

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 ($p \leq 0.05$) 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 $\beta = 0.2$ 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

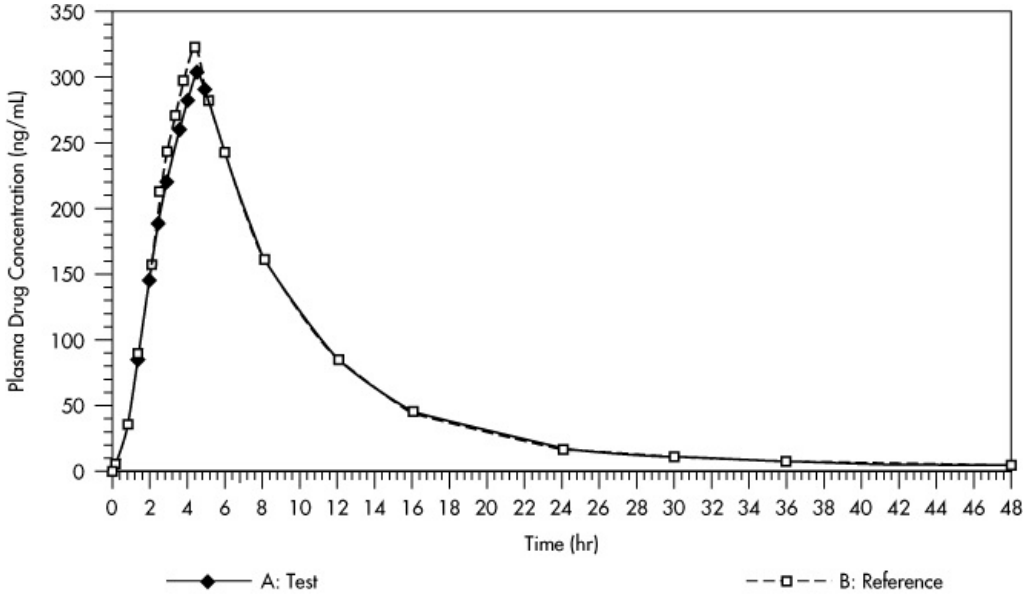
A simulated example of the results for a single-dose, fasting study is shown in and in . As shown by the ANOVA, no statistical differences for the pharmacokinetic parameters AUC_{0-t} , $AUC_{0-\infty}$, and C_{max} were observed between the Test product and the brand-name product. The 90% confidence 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.



Source: Shargel S, Wu-Pong S, Yu ABC: *Applied Biopharmaceutics & Pharmacokinetics*, 5th Edition: http://www.accesspharmacy.com

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} (μg/mL)	t_{max} (hr)	AUC_{0-24} (μg 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%

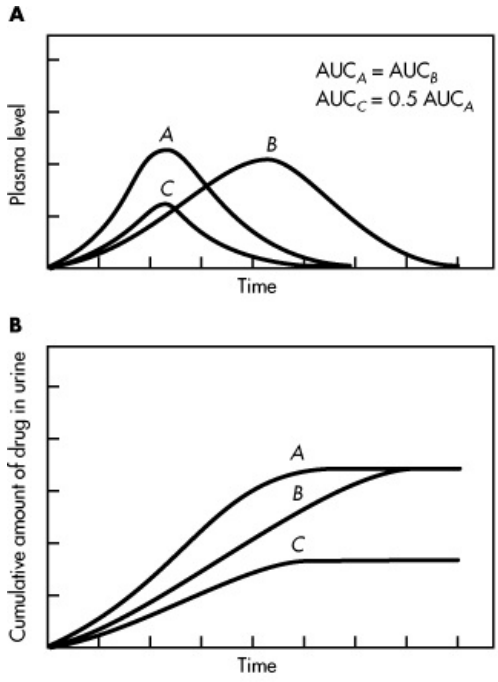
[a] The bioavailability of a drug from four different formulations was studied in 24 healthy, adult male subjects using a four-way Latin-square crossover design. The results represent the mean ± standard deviation.

[b] Oral bioavailability relative to the solution.

[c] $p \leq 0.05$.

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

[a] Maximum rate of urinary drug excretion.

STUDY SUBMISSION AND DRUG REVIEW PROCESS

The contents of New Drug Applications (NDAs) and Abbreviated New Drug Applications (ANDAs) are similar in terms of the quality of manufacture (). The submission for a NDA must contain safety and efficacy study as provided by animal toxicology studies, clinical efficacy studies, and pharmacokinetic/bioavailability studies. For the generic drug manufacturer, the bioequivalence study is the pivotal study in the ANDA that replaces the animal, clinical, and pharmacokinetic studies.

Table 15.8 NDA versus ANDA Review Process	
Brand–Name Drug NDA Requirements	**Generic Drug ANDA Requirements**
1. Chemistry	1. Chemistry
2. Manufacturing	2. Manufacturing
3. Controls	3. Controls
4. Labeling	4. Labeling
5. Testing	5. Testing
6. Animal studies	6. Bioequivalence
7. Clinical studies 8. Bioavailability	

Source: Center for Drug Evaluation & Research, U.S. Food & Drug Administration.

An outline for the submission of a completed bioavailability study for submission to the FDA is shown in . The investigator should be sure that the study has been properly designed, the objectives are clearly defined, and the method of analysis has been validated (ie, shown to measure precisely and accurately the plasma drug concentration). The results are analyzed both statistically and pharmacokinetically. These results, along with case reports and various data supporting the validity of the analytical method, are included in the submission. The FDA reviews the study in detail according to the outline presented in . If necessary, an FDA investigator may inspect both the clinical and analytical facilities used in the study and audit the raw data used in support of the bioavailability study. For ANDA applications, the FDA Office of Generic Drugs reviews the entire ANDA as shown in . If the application is incomplete, the FDA will not review the submission and the sponsor will receive a Refusal to File letter.

Table 15.9 Proposed Format and Contents of an *In-Vivo* Bioequivalence Study Submission and Accompanying *In-Vitro* Data
Title page
Study title
Name of sponsor Name and address of clinical laboratory
Name of principal investigator(s)
Name of clinical investigator
Name of analytical laboratory
Dates of clinical study (start, completion)
Signature of principal investigator (and date) Signature of clinical investigator (and date)
Table of contents
I. Study Résumé
Product information
Summary of bioequivalence study
Summary of bioequivalence data Plasma
Urinary excretion
Figure of mean plasma concentration–time profile
Figure of mean cumulative urinary excretion
Figure of mean urinary excretion rates
II. Protocol and Approvals
Protocol Letter of acceptance of protocol from FDA
Informed consent form
Letter of approval of Institutional Review Board
List of members of Institutional Review Board
III. Clinical Study
Summary of the study Details of the study
Demographic characteristics of the subjects

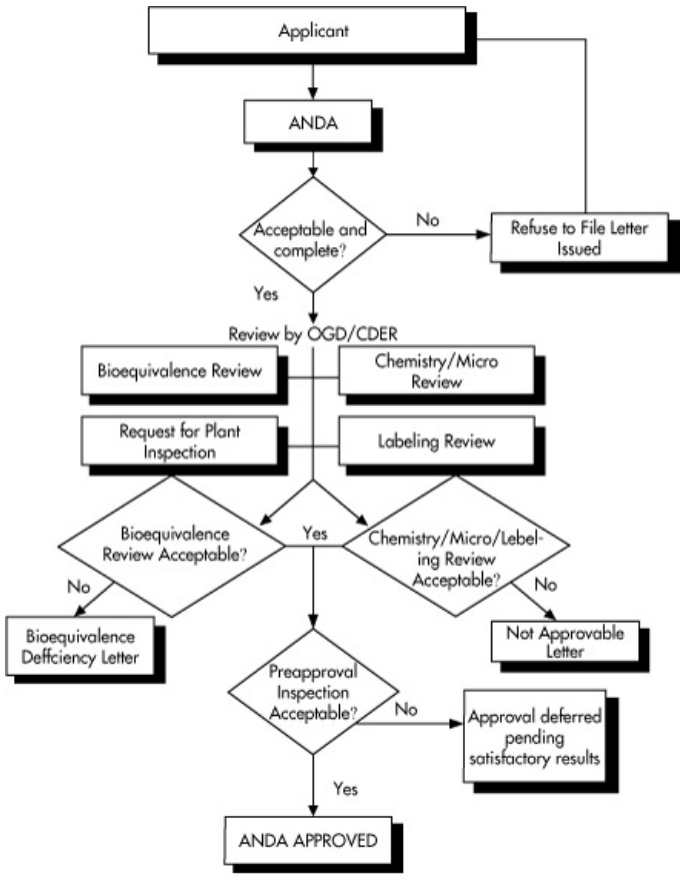
Subject assignment in the study
Mean physical characteristics of subjects arranged by sequence
Details of clinical activity
Deviations from protocol
Vital signs of subjects
Adverse reactions report
IV. Assay Methodology and Validation
Assay method description
Validation procedure
Summary of validation
Data on linearity of standard samples
Data on interday precision and accuracy
Data on intraday precision and accuracy
Figure for standard curve(s) for low/high ranges
Chromatograms of standard and quality control samples
Sample calculation
V. Pharmacokinetic Parameters and Tests
Definition and calculations
Statistical tests
Drug levels at each sampling time and pharmacokinetic parameters
Figure of mean plasma concentration–time profile
Figures of individual subject plasma concentration–time profiles
Figure of mean cumulative urinary excretion
Figures of individual subject cumulative urinary excretion
Figure of mean urinary excretion rates
Figures of individual subject urinary excretion rates
Tables of individual subject data arranged by drug, drug/period, drug/sequence
VI. Statistical Analyses
Statistical considerations
Summary of statistical significance
Summary of statistical parameters
Analysis of variance, least squares estimates and least-squares means
Assessment of sequence, period, and treatment effects
90% Confidence intervals for the difference between Test and Reference products for the log-normal-transformed parameters of AUC_{0-t} , $AUC_{0-\infty}$, and C_{max} should be within 80% and 125%
VII. Appendices
Randomization schedule
Sample identification codes
Analytical raw data
Chromatograms of at least 20% of subjects
Medical record and clinical reports
Clinical facilities description
Analytical facilities description
Curricula vitae of the investigators
VIII. *In-Vitro* Testing
Dissolution testing
Dissolution assay methodology
Content uniformity testing
Potency determination
IX. Batch Size and Formulation
Batch record
Quantitative formulation

Modified from Dighe and Adams (1991), with permission.

Table 15.10 General Elements of a Biopharmaceutics Review

Introduction	Summary and analysis of data
Study design Study objective(s)	Comments Deficiencies
Assay description and validation	Recommendation
Assay for individual samples checked	

Figure 15-10.



Source: Shargel S, Wu-Pong S, Yu ABC: *Applied Biopharmaceutics & Pharmacokinetics*, 5th Edition: http://www.accesspharmacy.com

Generic drug review process.

Source: Office of Generic Drugs, Center for Drug Evaluation & Research, U.S. Food & Drug Administration.

Waivers of *In-Vivo* Bioequivalence Studies (Biowaivers)

In some cases, *in-vitro* dissolution testing may be used in lieu of *in-vivo* bioequivalence studies. When the drug product is in the same dosage form but in different strengths, and is proportionally similar in active and inactive ingredients, an *in-vivo* bioequivalence study of one or more lower strengths can be waived based on the dissolution tests and an *in-vivo* bioequivalence study on the highest strength. Ideally, if there is a strong correlation between dissolution of the drug and the bioavailability of the drug, then the comparative dissolution tests comparing the test product to the reference product should be sufficient to demonstrate bioequivalence. For most drug products, especially immediate-release tablets and capsules, no strong correlation exists, and the FDA requires an *in-vivo* bioequivalence study. For oral solid dosage forms, an *in-vivo* bioequivalence study may be required to support at least one dose strength of the product. Usually, an *in-vivo* bioequivalence study is required for the highest dose strength. If the lower-dose-strength test product is substantially similar in active and inactive ingredients, then only a comparison *in-vitro* dissolution between the test and brand-name formulations may be used.

For example, an immediate-release tablet is available in 200-mg, 100-mg, and 50-mg strengths. The 100- and 50-mg-strength tablets are made the same way as the highest-strength tablet. A human bioequivalence study is performed on the highest or 200-mg strength. Comparative *in-vitro* dissolution studies are performed on the 100-mg and 50-mg dose strengths. If these drug

products have no known bioavailability problems, are well absorbed systemically, are well correlated with *in-vitro* dissolution, and have a large margin of safety, then arguments for not performing an *in-vivo* bioavailability study may be valid. Methods for correlation of *in-vitro* dissolution of the drug with *in-vivo* drug bioavailability are discussed in and . The manufacturer does not need to perform additional *in-vivo* bioequivalence studies on the lower-strength products if the products meet all *in-vitro* criteria.

Dissolution Profile Comparison

Comparative dissolution profiles are used as (1) the basis for formulation development of bioequivalent drug products and proceeding to the pivotal *in-vivo* bioequivalence study; (2) comparative dissolution profiles are used for demonstrating the equivalence of a change in the formulation of a drug product after the drug product has been approved for marketing (see SUPAC in); and (3) the basis of a biowaiver of a lower-strength drug product that is dose proportional in active and inactive ingredients to the higher-strength drug product.

A model-independent mathematical method was developed by to compare dissolution profiles using two factors, f_1 and f_2. The factor f_2, known as the *similarity factor*, measures the closeness between the two profiles:

$$f_2 = 50 \times \log\left\{\left[1 + \frac{1}{n}\sum_{t=1}^{n}(R_1 - T_1)^2\right]^{-5} \times 100\right\}$$

where n is the number of time points, R_1 is the dissolution value of the Reference product at time t, and T_1 is the dissolution value of the Test product batch at time t.

The Reference may be the original drug product before a formulation change (prechange) and the Test may be the drug product after the formulation was changed (postchange). Alternatively, the Reference may be the higher-strength drug product and the Test may be the lower-strength drug product. The f_2 comparison is the focus of several FDA guidances and is of regulatory interest in knowing the similarity of the two dissolution curves. When the two profiles are identical, $f_2 = 100$. An average difference of 10% at all measured time points results in a f_2 value of 50. The FDA has set a public standard for f_2 value between 50 and 100 to indicate similarity between two dissolution profiles.

In some cases, two generic drug products may have dissimilar dissolution profiles and still be bioequivalent *in-vivo*. For example, have shown that slow-, medium-, and fast-dissolving formulations of metoprolol tartrate tablets were bioequivalent. Furthermore, bioequivalent modified-release drug products may have different drug release mechanisms and therefore different dissolution profiles. For example, for theophylline extended-release capsules, the *United States Pharmacopeia* (USP) lists 10 individual drug release tests for products labeled for dosing every 12 hours. However, only generic drug products that are FDA approved as bioequivalent drug products and listed in the current edition of the *Orange Book* may be substituted for each other.

THE BIOPHARMACEUTICS CLASSIFICATION SYSTEM (BCS)

A theoretical basis for correlating *in-vitro* drug dissolution with *in-vivo* bioavailability was developed by . This approach is based on the aqueous solubility of the drug and the permeation of the drug through the gastrointestinal tract. The classification system is based on Fick's first law applied to a membrane:

$$J_w = P_w C_w$$

where J_w is the drug flux (mass/area/time) through the intestinal wall at any position and time, P_w is the permeability of the membrane, and C_w is the drug concentration at the intestinal membrane surface.

This approach assumes that no other components in the formulation affect the membrane permeability and/or intestinal transport. Using this approach, studied the solubility and permeability characteristics of various representative drugs and obtained a biopharmaceutic drug classification () for predicting the *in-vitro* drug dissolution of immediate-release solid oral drug products with *in-vivo* absorption.

Table 15.11 Biopharmaceutics Classification System

Class	Solubility	Permeability	Comments
Class 1	High	High	Drug dissolves rapidly and is well absorbed. Bioavailability problem is not expected for immediate release drug products.
Class 2	Low	High	Drug is dissolution limited and well absorbed. Bioavailability is controlled by the dosage form and rate of release of the drug substance.
Class 3	High	Low	Drug is permeability limited. Bioavailability may be incomplete if drug is not released and dissolved within absorption window.
Class 4	Low	Low	Difficulty in formulating a drug product that will deliver consistent drug bioavailability. An alternate route of administration may be needed.

From *FDA Guidance for Industry: Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate Release Solid Oral Dosage Forms Containing Certain Active Moieties/Active Ingredients Based on a Biopharmaceutics Classification System* (2000), and .

The FDA may waive the requirement for performing an *in-vivo* bioavailability or bioequivalence study for certain immediate-release solid oral drug products that meet very specific criteria, namely, the permeability, solubility, and dissolution of the drug. These characteristics include the *in-vitro* dissolution, of the drug product in various media, drug permeability information, and

assuming ideal behavior of the drug product, drug dissolution, and absorption in the GI tract. For regulatory purpose, drugs are classified according to the Biopharmaceutics Classification System (BCS) in accordance the solubility, permeability, and dissolution characteristics of the drug (*FDA Guidance for Industry*, 2000;).

Solubility

An objective of the BCS approach is to determine the equilibrium solubility of a drug under approximate physiologic conditions. For this purpose, determination of pH–solubility profiles over a pH range of 1–8 is suggested. The solubility class is determined by calculating what volume of an aqueous medium is sufficient to dissolve the highest anticipated dose strength. A drug substance is considered highly soluble when the highest dose strength is soluble in 250 mL or less of aqueous medium over the pH range 1–8. The volume estimate of 250 mL is derived from typical bioequivalence study protocols that prescribe administration of a drug product to fasting human volunteers with a glass (8 ounces) of water.

Permeability

Studies of the extent of absorption in humans, or intestinal permeability methods, can be used to determine the permeability class membership of a drug. To be classified as highly permeable, a test drug should have an extent of absorption > 90% in humans. Supportive information on permeability characteristics of the drug substance should also be derived from its physical-chemical properties (eg, octanol: water partition coefficient).

Some methods to determine the permeability of a drug from the gastrointestinal tract include: (1) *in-vivo* intestinal perfusion studies in humans; (2) *in-vivo* or *in-situ* intestinal perfusion studies in animals; (3) *in-vitro* permeation experiments using excised human or animal intestinal tissues; and (4) *in-vitro* permeation experiments across a monolayer of cultured human intestinal cells. When using these methods, the experimental permeability data should correlate with the known extent-of-absorption data in humans.

Dissolution

The dissolution class is based on the *in-vitro* dissolution rate of an immediate-release drug product under specified test conditions and is intended to indicate rapid *in-vivo* dissolution in relation to the average rate of gastric emptying in humans under fasting conditions. An immediate-release drug product is considered rapidly dissolving when not less than 85% of the label amount of drug substance dissolves within 30 minutes using USP Apparatus I (see) at 100 rpm or Apparatus II at 50 rpm in a volume of 900 mL or less in each of the following media: (1) acidic media such as 0.1 N HCl or Simulated Gastric Fluid USP without enzymes, (2) a pH 4.5 buffer, and (3) a pH 6.8 buffer or Simulated Intestinal Fluid USP without enzymes.

Drug Products for Which Bioavailability or Bioequivalence May Be Self-Evident

The best measure of a drug product's performance is to determine the *in-vivo* bioavailability of the drug. For some well-characterized drug products and for certain drug products in which bioavailability is self-evident (eg, sterile solutions for injection), *in-vivo* bioavailability studies may be unnecessary or unimportant to the achievement of the product's intended purposes. The FDA will waive the requirement for submission of *in-vivo* evidence demonstrating the bioavailability of the drug product if the product meets one of the following criteria (U.S. Code of Federal Regulations, 21 CFR 320.22). However, there may be specific requirements for certain drug products, and the appropriate FDA division should be consulted.

1. The drug product (a) is a solution intended solely for intravenous administration and (b) contains an active drug ingredient or therapeutic moiety combined with the same solvent and in the same concentration as in an intravenous solution that is the subject of an approved, full, New Drug Application.

2. The drug product is a topically applied preparation (eg, a cream, ointment, or gel intended for local therapeutic effect). The FDA has released guidances for the performance of bioequivalence studies on topical corticosteroids and antifungal agents. The FDA is also considering performing dermatopharmacokinetic (DPK) studies on other topical drug products. In addition, *in-vitro* drug release and diffusion studies may be required.

3. The drug product is in an oral dosage form that is not intended to be absorbed (eg, an antacid or a radiopaque medium). Specific *in-vitro* bioequivalence studies may be required by the FDA. For example, the bioequivalence of cholestyramine resin is demonstrated *in-vitro* by the binding of bile acids to the resin.

4. The drug product meets both of the following conditions:

a. It is administered by inhalation as a gas or vapor (eg, as a medicinal or as an inhalation anesthetic).

b. It contains an active drug ingredient or therapeutic moiety in the same dosage form as a drug product that is the subject of an approved, full, New Drug Application(NDA).

5. The drug product meets all of the following conditions:

a. It is an oral solution, elixir, syrup, tincture, or similar other solubilized form.

b. It contains an active drug ingredient or therapeutic moiety in the same concentration as a drug product that is the subject of an approved, full, New Drug Application.

c. It contains no inactive ingredient that is known to significantly affect absorption of the active drug ingredient or therapeutic moiety.

GENERIC BIOLOGICS

Biologics, in contrast to drugs that are chemically synthesized, are derived from living sources such as human, animal, or microorganisms. Many biologics are complex mixtures that are not easily identified or characterized and are manufactured by biotechnology. Other biological drugs, such as insulin and growth hormone, are proteins derived by biotechnology and have been well characterized.

Presently, there is no FDA regulatory pathway to establish the bioequivalence of a biotechnology-derived drug product. Scientifically, there are advocates for and against the feasibility for the manufacture of generic biotechnology-derived drug products (generic biologics) that are bioequivalent to the innovator or brand-drug product.

Those opposed to the development of generic biologics have claimed that generic manufacturers do not have the ability to fully characterize the active ingredient(s), that immunogenicity-related impurities may be present in the product, and that the manufacture of a biologic drug product is process dependent.

Many biologic drug products are given parenterally. The efficacy of the biologic may be affected by the development of antibodies to the active ingredient or to product-related impurities. The degree of immunogenicity and subsequent antibody formation to a foreign peptide or protein will alter the efficacy of the drug. Antibodies can increase bioavailability if they are not neutralizing, which would result in higher drug levels in the body. In contrast, antibodies can decrease bioavailability of the biologic drug by forming an antibody–protein complex that results in a change in drug distribution and a change in clearance.

Advocates for the manufacture of generic biologics argue that bioequivalent biotechnology-derived drug products can be made on a case-by-case basis. Currently, manufacturers of marketed biotechnology drugs may seek to make changes in the manufacturing process used to make a particular product for a variety of reasons, including improvement of product quality, yield, and manufacturing efficiency. These manufacturers have developed improvements in production methods, process and control test methods, and test methods for product characterization.

For example, a biologics manufacturer institutes a change in its manufacturing process, before FDA approval of its product but after completion of a pivotal clinical study. The FDA may not require the manufacturer to perform additional clinical studies to demonstrate that the resulting product is still safe, pure, and potent. Such manufacturing process changes, implemented before or after product approval, have included changes implemented during expansion from pilot-scale to full-scale production, the move of production facilities from one legal entity to another legal entity, and the implementation of changes in different stages of the manufacturing process such as fermentation, purification, and formulation. The manufacturer may be able to demonstrate product comparability between a biological product made after a manufacturing change ("new" product) and a product made before implementation of the change ("old" product) through different types of analytical and functional testing, with or without preclinical animal testing. The FDA may determine that two products are comparable if the results of the comparability testing demonstrate that the manufacturing change does not affect safety, identity, purity, or potency (*FDA Guidance Concerning Demonstration of Comparability of Human Biological Products, Including Therapeutic Biotechnology-Derived Products*, 1996). The FDA currently requires that manufacturers should carefully assess manufacturing changes and evaluate the product resulting from these changes for comparability to the preexisting product. Determinations of product comparability may be based on chemical, physical, and biological assays and, in some cases, other nonclinical data.

It is important to note that the FDA uses such terms as *comparable* and *similar* for approval of manufacturing changes of biologic drug products (*FDA Guidance,* 1996). In contrast, the FDA uses the term *bioequivalence* for approval of manufacturing changes of drug products that contain chemically derived active ingredients. Advocates for the manufacturer of generic biologics feel that the science and technology for the manufacture of certain bioequivalent biologic drug products are already available. Moreover, if the innovator manufacturer of a marketed biologic drug product can perform a manufacturing change and demonstrate the comparability of the "new" to the "old" marketed biologic drug product, then a generic manufacturer should be able to use similar techniques to demonstrate bioequivalence of the generic drug product.

CLINICAL SIGNIFICANCE OF BIOEQUIVALENCE STUDIES

Bioequivalence of different formulations of the same drug substance involves equivalence with respect to rate and extent of systemic drug absorption. Clinical interpretation is important in evaluating the results of a bioequivalence study. A small difference between drug products, even if statistically significant, may produce very little difference in therapeutic response. Generally, two formulations whose rate and extent of absorption differ by 20% or less are considered bioequivalent. The considered that differences of less than 20% in AUC and C_{max} between drug products are "unlikely to be clinically significant in patients." The Task Force further stated that "clinical studies of effectiveness have difficulty detecting differences in doses of even 50–100%." Therefore, normal variation is observed in medical practice and plasma drug levels may vary among individuals greater than 20%.

According to , a small, statistically significant difference in drug bioavailability from two or more dosage forms may be detected if the study is well controlled and the number of subjects is sufficiently large. When the therapeutic objectives of the drug are considered, an equivalent clinical response should be obtained from the comparison dosage forms if the plasma drug concentrations remain above the minimum effective concentration (MEC) for an appropriate interval and do not reach the minimum toxic concentration (MTC). Therefore, the investigator must consider whether any statistical difference in bioavailability would alter clinical efficiency.

Special populations, such as the elderly or patients on drug therapy, are generally not used for bioequivalence studies. Normal, healthy volunteers are preferred for bioequivalence studies, because these subjects are less at risk and may more easily endure the discomforts of the study, such as blood sampling. Furthermore, the objective of these studies is to evaluate the bioavailability of the drug from the dosage form, and use of healthy subjects should minimize both inter- and intrasubject variability. It is theoretically possible that the excipients in one of the dosage forms tested may pose a problem in a patient who uses the generic dosage form.

For the manufacture of a dosage form, specifications are set to provide uniformity of dosage forms. With proper specifications, quality control procedures should minimize product-to-product variability by different manufacturers and lot-to-lot variability with a single manufacturer (see).

SPECIAL CONCERNS IN BIOAVAILABILITY AND BIOEQUIVALENCE STUDIES

The general bioequivalence study designs and evaluation, such as the comparison of AUC, C_{max}, and t_{max}, may be used for systemically absorbed drugs and conventional oral dosage forms. However, for certain drugs and dosage forms, systemic bioavailability and bioequivalence are difficult to ascertain (). Drugs and drug products (eg, cyclosporine, chlorpromazine, verapamil, isosorbide dinitrate, sulindac) are considered to be highly variable if the intrasubject variability in bioavailability parameters is greater than 30% by analysis of variance coefficient of variation (). The number of subjects required to demonstrate bioequivalence for these drug products may be excessive, requiring more than 60 subjects to meet current FDA bioequivalence criteria. The intrasubject variability may be due to the drug itself or to the drug formulation or to both. The FDA has held public forums to determine whether the current bioequivalence guidelines need to be changed for these highly variable drugs ().

Table 15.12 Problems in Bioavailability and Bioequivalence

Drugs with high intrasubject variability	Inhalation
Drugs with long elimination half-life	Ophthalmic
Biotransformation of drugs	Intranasal
Stereoselective drug metabolism	Bioavailable drugs that should not produce peak drug levels
Drugs with active metabolites	Potassium supplements
Drugs with polymorphic metabolism	Endogeneous drug levels
Nonbioavailable drugs (drugs intended for local effect)	Hormone replacement therapy
Antacids	Biotechnology-derived drugs
Local anesthetics	Erythropoietin interferon
Anti-infectives	Protease inhibitors
Anti-inflammatory steroids	Complex drug substances
Dosage forms for nonoral administration	Conjugated estrogens
Transdermal	

For drugs with very long elimination half-lives or a complex elimination phase, a complete plasma drug concentration–time curve (ie, three elimination half-lives or an AUC representing 90% of the total AUC) may be difficult to obtain for a bioequivalence study using a crossover design. For these drugs, a truncated (shortened) plasma drug concentration–time curve (0–72 hr) may be more practical. The use of a truncated plasma drug concentration–time curve allows for the measurement of peak absorption and decreases the time and cost for performing the bioequivalence study.

Many drugs are stereoisomers, and each isomer may give a different pharmacodynamic response and may have a different rate of biotransformation. The bioavailability of the individual isomers may be difficult to measure because of problems in analysis. Some drugs have active metabolites, which should be quantitated as well as the parent drug. Drugs such as thioridazine and selegilene have two active metabolites. The question for such drugs is whether bioequivalence should be proven by matching the bioavailability of both metabolites and the parent drug. Assuming both biotransformation pathways follow first-order reaction kinetics, then the metabolites should be in constant ratio to the parent drug. Genetic variation in metabolism may present a bioequivalence problem. For example, the acetylation of procainamide to N-acetylprocainamide demonstrates genetic polymorphism, with two groups of subjects consisting of rapid acetylators and slow acetylators. To decrease intersubject variability, a bioequivalence study may be performed on only one phenotype, such as the rapid acetylators.

Some drugs (eg, benzocaine, hydrocortisone, anti-infectives, antacids) are intended for local effect and formulated as topical ointments, oral suspensions, or rectal suppositories. These drugs should not have significant systemic bioavailability from the site of administration. The bioequivalence determination for drugs that are not absorbed systemically from the site of application can be difficult to assess. For these nonsystemic-absorbable drugs, a "surrogate" marker is needed for bioequivalence determination (). For example, the acid-neutralizing capacity of an oral antacid and the binding of bile acids to cholestyramine resin have been used as surrogate markers in lieu of *in-vivo* bioequivalence studies.

Table 15.13 Possible Surrogate Markers for Bioequivalence Studies

Drug Product	Drug	Possible Surrogate Marker for Bioequivalence
Metered-dose inhaler	Albuterol	Forced expiratory volume (FEV_1)
Topical steroid	Hydrocortisone	Skin blanching
Anion-exchange resin	Cholestyramine	Binding to bile acids
Antacid	Magnesium and aluminum hydroxide gel	Neutralization of acid
Topical antifungal	Ketoconazole	Drug uptake into stratum corneum

Various drug delivery systems and newer dosage forms are designed to deliver the drug by a nonoral route, which may produce only partial systemic bioavailability. For the treatment of asthma, inhalation of the drug (eg, albuterol, beclomethasone

dipropionate) has been used to maximize drug in the respiratory passages and to decrease systemic side effects. Drugs such as nitroglycerin given transdermally may differ in release rates, in the amount of drug in the transdermal delivery system, and in the surface area of the skin to which the transdermal delivery system is applied. Thus, the determination of bioequivalence among different manufacturers of transdermal delivery systems for the same active drug is difficult. Dermatokinetics are pharmacokinetic studies that investigate drug uptake into skin layers after topical drug administration. The drug is applied topically, the skin is peeled at various time periods after the dose, using transparent tape, and the drug concentrations are measured in the skin.

Drugs such as potassium supplements are given orally and may not produce the usual bioavailability parameters of AUC, C_{max}, and t_{max}. For these drugs, more indirect methods must be used to ascertain bioequivalence. For example, urinary potassium excretion parameters are more appropriate for the measurement of bioavailability of potassium supplements. However, for certain hormonal replacement drugs (eg, levothyroxine), the steady-state hormone concentration in hypothyroid individuals, the thyroidal-stimulating hormone level, and pharmacodynamic endpoints may also be appropriate to measure.

GENERIC SUBSTITUTION

To contain drug costs, most states have adopted generic substitution laws to allow pharmacists to dispense a generic drug product for a brand-name drug product that has been prescribed. Some states have adopted a *positive formulary*, which lists therapeutically equivalent or interchangeable drug products that pharmacists may dispense. Other states use a *negative formulary*, which lists drug products that are not therapeutically equivalent, and/or the interchange of which is prohibited. If the drug is not in the negative formulary, the unlisted generic drug products are assumed to be therapeutically equivalent and may be interchanged.

Approved Drug Products with Therapeutic Equivalence Evaluations (*Orange Book*)

Due to public demand, the FDA Center for Drug Evaluation and Research publishes annually a listing of approved drug products, *Approved Drug Products with Therapeutic Equivalence Evaluations* (commonly known as the *Orange Book*). The Orange Book is available on the Internet at www.fda.gov/cder/orange/default.htm.

The Orange Book contains therapeutic equivalence evaluations for approved drug products made by various manufacturers. These marketed drug products are evaluated according to specific criteria. The evaluation codes used for these drugs are listed in . The drug products are divided into two major categories: "A" codes apply to drug products considered to be therapeutically equivalent to other pharmaceutically equivalent products, and "B" codes apply to drug products that the FDA does not at this time consider to be therapeutically equivalent to other pharmaceutically equivalent products. A list of therapeutic-equivalence-related terms and their definitions is also given in the monograph. According to the FDA, evaluations do not mandate that drugs be purchased, prescribed, or dispensed, but provide public information and advice. The FDA evaluation of the drug products should be used as a guide only, with the practitioner exercising professional care and judgment.

Table 15.14 Therapeutic Equivalence Evaluation Codes

A Codes
Drug products considered to be therapeutically equivalent to other pharmaceutically equivalent products
AA Products in conventional dosage forms not presenting bioequivalence problems
AB Products meeting bioequivalence requirements
AN Solutions and powders for aerosolization
AO Injectable oil solutions
AP Injectable aqueous solutions
AT Topical products
B Codes
Drug products that the FDA does not consider to be therapeutically equivalent to other pharmaceutically equivalent products
B* Drug products requiring further FDA investigation and review to determine therapeutic equivalence
BC Extended-release tablets, extended-release capsules, and extended-release injectables
BD Active ingredients and dosage forms with documented bioequivalence problems
BE Delayed-release oral dosage forms
BN Products in aerosol–nebulizer drug delivery systems
BP Active ingredients and dosage forms with potential bioequivalence problems
BR Suppositories or enemas for systemic use
BS Products having drug standard deficiencies
BT Topical products with bioequivalence issues
BX Insufficient data

Adopted from: *Approved Drug Products with Therapeutic Equivalence Evaluations* (Orange Book) (www.fda.gov/cder/orange/default.htm) 2003.

The concept of therapeutic equivalence as used to develop the Orange Book applies only to drug products containing the same active ingredient(s) and does not encompass a comparison of different therapeutic agents used for the same condition (eg,

propoxyphene hydrochloride versus pentazocine hydrochloride for the treatment of pain). Any drug product in the Orange Book that is repackaged and/or distributed by other than the application holder is considered to be therapeutically equivalent to the application holder's drug product even if the application holder's drug product is single source or coded as nonequivalent (eg, BN). Also, distributors or repackagers of an application holder's drug product are considered to have the same code as the application holder. Therapeutic equivalence determinations are not made for unapproved, off-label indications. With this limitation, however, 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 (www.fda.gov/cder/orange/default.htm).

Professional care and judgment should be exercised in using the Orange Book. Evaluations of therapeutic equivalence for prescription drugs are based on scientific and medical evaluations by the FDA. Products evaluated as therapeutically equivalent can be expected, in the judgment of the FDA, to have equivalent clinical effect and no difference in their potential for adverse effects when used under the conditions of their labeling. However, these products may differ in other characteristics such as shape, scoring configuration, release mechanisms, packaging, excipients (including colors, flavors, preservatives), expiration date/time, and, in some instances, labeling. If products with such differences are substituted for each other, there is a potential for patient confusion due to differences in color or shape of tablets, inability to provide a given dose using a partial tablet if the proper scoring configuration is not available, or decreased patient acceptance of certain products because of flavor. There may also be better stability of one product over another under adverse storage conditions, or allergic reactions in rare cases due to a coloring or a preservative ingredient, as well as differences in cost to the patient.

FDA evaluation of therapeutic equivalence in no way relieves practitioners of their professional responsibilities in prescribing and dispensing such products with due care and with appropriate information to individual patients. In those circumstances where the characteristics of a specific product, other than its active ingredient, are important in the therapy of a particular patient, the physician's specification of that product is appropriate. Pharmacists must also be familiar with the expiration dates/times and labeling directions for storage of the different products, particularly for reconstituted products, to assure that patients are properly advised when one product is substituted for another.

FREQUENTLY ASKED QUESTIONS

1. Why are preclinical animal toxicology studies and clinical efficacy drug studies in human subjects not required by the FDA to approve a generic drug product as a therapeutic equivalent to the brand-name drug product?

2. What do sequence, washout period, and period mean in a crossover bioavailability study?

3. Why does the FDA require a food intervention (food effect) study for some generic drug products before granting approval? For which drug products are food effect studies required?

4. What type of bioequivalence studies are required for drugs that are not systemically absorbed or for those drugs in which the C_{max} and AUC cannot be measured in the plasma?

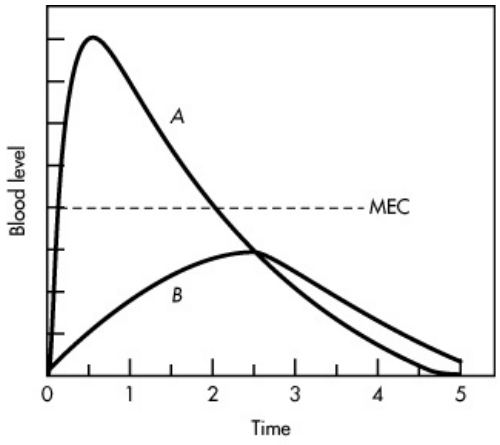
5. How does inter- and intrasubject variability affect the statistical demonstration of bioequivalence for a drug product?

6. Can chemically equivalent drug products that are not bioequivalent (ie, bioinequivalent) to each other have similar clinical efficacy?

LEARNING QUESTIONS

1. An antibiotic was formulated into two different oral dosage forms, A and B. Biopharmaceutic studies revealed different antibiotic blood level curves for each drug product (). Each drug product was given in the same dose as the other. Explain how the various possible formulation factors could have caused the differences in blood levels. Give examples where possible. How would the corresponding urinary drug excretion curves relate to the plasma level–time curves?

Figure 15-11.



Source: Shargel S, Wu-Pong S, Yu ABC: *Applied Biopharmaceutics & Pharmacokinetics*, 5th Edition: http://www.accesspharmacy.com

Blood-level curves for two different oral dosage forms of a hypothetical antibiotic.

2. Assume that you have just made a new formulation of acetaminophen. Design a protocol to compare your drug product against the acetaminophen drug products on the market. What criteria would you use for proof of bioequivalence for your new formulation? How would you determine if the acetaminophen was completely (100%) systemically absorbed?

3. The data in represent the average findings in antibiotic plasma samples taken from 10 humans (average weight 70 kg), tabulated in a four-way crossover design.

Table 15.15 Comparison of Plasma Concentrations of Antibiotic, as Related to Dosage Form and Time

	Plasma Concentration (μg/ml)			
Time after Dose (hr)	**IV Solution (2 mg/kg)**	**Oral Solution (10 mg/kg)**	**Oral Tablet (10 mg/kg)**	**Oral Capsule (10 mg/kg)**
0.5	5.94	23.4	13.2	18.7
1.0	5.30	26.6	18.0	21.3
1.5	4.72	25.2	19.0	20.1
2.0	4.21	22.8	18.3	18.2
3.0	3.34	18.2	15.4	14.6
4.0	2.66	14.5	12.5	11.6
6.0	1.68	9.14	7.92	7.31
8.0	1.06	5.77	5.00	4.61
10.0	0.67	3.64	3.16	2.91
12.0	0.42	2.30	1.99	1.83
$\text{AUC}\left(\frac{\mu g}{mL} \times hr\right)$	29.0	145.0	116.0	116.0

a. Which of the four drug products in would be preferred as a reference standard for the determination of relative bioavailability? Why?

b. From which oral drug product is the drug absorbed more rapidly?

c. What is the absolute bioavailability of the drug from the oral solution?

d. What is the relative bioavailability of the drug from the oral tablet compared to the reference standard?

e. From the data in , determine:

(1) Apparent V_D

(2) Elimination $t_{1/2}$

(3) First-order elimination rate constant k

(4) Total body clearance

f. From the data above, graph the cumulative urinary excretion curves that would correspond to the plasma concentration time curves.

4. Aphrodisia is a new drug manufactured by the Venus Drug Company. When tested in humans, the pharmacokinetics of the drug assume a one-compartment open model with first-order absorption and first-order elimination:

$$D_{GI} \xrightarrow{k_a} D_B V_D \xrightarrow{k}$$

The drug was given in a single oral dose of 250 mg to a group of college students 21–29 years of age. Mean body weight was 60 kg. Samples of blood were obtained at various time intervals after the administration of the drug, and the plasma fractions were analyzed for active drug. The data are summarized in .

Table 15.16 Data Summary of Active Drug Concentration in Plasma Fractions

Time (hr)	**C_p (μg/mL)**	**Time (hr)**	**C_p (μg/mL)**
0	0	12	3.02
1	1.88	18	1.86
2	3.05	24	1.12
3	3.74	36	0.40
5	4.21	48	0.14
7	4.08	60	0.05

9	3.70	72	0.02

a. The minimum effective concentration of Aphrodisia in plasma is 2.3 µg/mL. What is the onset time of this drug?

b. The minimum effective concentration of Aphrodisia in plasma is 2.3 µg/mL. What is the duration of activity of this drug?

c. What is the elimination half-life of Aphrodisia in college students?

d. What is the time for peak drug concentration (t_{max}) of Aphrodisia?

e. What is the peak drug concentration (C_{max})?

f. Assuming that the drug is 100% systemically available (ie, fraction of drug absorbed equals unity), what is the AUC for Aphrodisia?

5. You wish to do a bioequivalence study on three different formulations of the same active drug. Lay out a Latin-square design for the proper sequencing of these drug products in six normal, healthy volunteers. What is the main reason for using a crossover design in a bioequivalence study? What is meant by a "random" population?

6. Four different drug products containing the same antibiotic were given to 12 volunteer adult males (age 19–28 years, average weight 73 kg) in a four-way crossover design. The volunteers were fasted for 12 hours prior to taking the drug product. Urine samples were collected up to 72 hours after the administration of the drug to obtain the maximum urinary drug excretion, D_u^{∞}. The data are presented in .

Table 15.17 Urinary Drug Excretion Data Summary

Drug Product	Dose (mg/kg)	Cumulative Urinary Drug Excretion (D_u^{∞}), 0–72 hr (mg)
IV solution	0.2	20
Oral solution	4	380
Oral tablet	4	340
Oral capsule	4	360

a. What is the absolute bioavailability of the drug from the tablet?

b. What is the relative bioavailability of the capsule compared to the oral solution?

7. According to the prescribing information for cimetidine (Tagamet), following IV or IM administration, 75% of the drug is recovered from the urine after 24 hours as the parent compound. Following a single oral dose, 48% of the drug is recovered from the urine after 24 hours as the parent compound. From this information, determine what fraction of the drug is absorbed systemically from an oral dose after 24 hours.

8. Define *bioequivalence requirement*. Why does the FDA require a bioequivalence requirement for the manufacture of a generic drug product?

9. Why can we use the time for peak drug concentration (t_{max}) in a bioequivalence study for an estimate of the rate of drug absorption, rather than calculating the k_a?

10. Ten male volunteers (18–26 years of age) weighing an average of 73 kg were given either 4 tablets each containing 250 mg of drug (drug product A) or 1 tablet containing 1000 mg of drug (drug product B). Blood levels of the drug were obtained and the data are summarized in .

Table 15.18 Blood Level Data Summary for Two Drug Products

		Drug Product		
		A	B	
Kinetic Variable	**Unit**	**4 x 250-mg Tablet**	**1000-mg Tablet**	**Statistic**
Time for peak drug concentration (range)	hr	1.3 (0.7–1.5)	1.8 (1.5–2.2)	$p < 0.05$
Peak concentration (range)	µg/mL	53 (46–58)	47 (42–51)	$p < 0.05$
AUC (range)	µg hr/mL	118 (98–125)	103 (90–120)	NS
$t_{1/2}$	hr	3.2 (2.5–3.8)	3.8 (2.9–4.3)	NS

a. State a possible reason for the difference in the time for peak drug concentration ($t_{max,A}$) after drug product A compared to the $t_{max,B}$ after drug product B. (Assume that all the tablets were made from the same formulation—that is, the drug is in the same particle size, same salt form, same excipients, and same ratio of excipients to active drug.)

b. Draw a graph relating the cumulative amount of drug excreted in urine of patients given drug product A compared to the

cumulative drug excreted in urine after drug product B. Label axes!

c. In a second study using the same 10 male volunteers, a 125-mg dose of the drug was given by IV bolus and the AUC was computed as 20 μg hr/mL. Calculate the fraction of drug systemically absorbed from drug product B (1 x 1000 mg) tablet using the data in .

Table 15.19 Disintegration Times and Dissolution Rates of Tolazamide Tablets[a]

Tablet	Mean Disintegration Time[b] Min (Range)	Percent Dissolved in 30 Min[c] (Range)
A	3.8 (3.0–4.0)	103.9 (100.5–106.3)
B	2.2 (1.8–2.5)	10.9 (9.3–13.5)
C	2.3 (2.0–2.5)	31.6 (26.4–37.2)
D	26.5 (22.5–30.5)	29.7 (20.8–38.4)

[a] $N = 6$.

[b] By the method of USP-23.

[c] Dissolution rates in pH 7.6 buffer.

From , with permission.

11. After performing a bioequivalence test comparing a generic drug product to a brand-name drug product, it was observed that the generic drug product had greater bioavailability than the brand-name drug product.

a. Would you approve marketing the generic drug product, claiming it was superior to the brand-name drug product?

b. Would you expect identical pharmacodynamic responses to both drug products?

c. What therapeutic problem might arise in using the generic drug product that might not occur when using the brand-name drug product?

12. The following study is from :

Tolazamide Formulations. Four tolazamide tablet formulations were selected for this study. The tablet formulations were labeled A, B, C, and D. Disintegration and dissolution tests were performed by standard USP-23 procedures.

Subjects. Twenty healthy adult male volunteers between the ages of 18 and 38 (mean, 26 years) and weighing between 61.4 and 95.5 kg (mean, 74.5 kg) were selected for the study. The subjects were randomly assigned to 4 groups of 5 each. The four treatments were administered according to 4 x 4 Latin-square design. Each treatment was separated by 1-week intervals. All subjects fasted overnight before receiving the tolazamide tablet the following morning. The tablet was given with 180 mL of water. Food intake was allowed at 5 hours postdose. Blood samples (10 mL) were taken just before the dose and periodically after dosing. The serum fraction was separated from the blood and analyzed for tolazamide by high-pressure liquid chromatography.

Data Analysis. Serum data were analyzed by a digital computer program using a regression analysis and by the percent of drug unabsorbed by the method of (see). AUC was determined by the trapezoidal rule and an analysis of variance was determined by Tukey's method.

a. Why was a Latin-square crossover design used in this study?

b. Why were the subjects fasted before being given the tolazamide tablets?

c. Why did the authors use the Wagner–Nelson method rather than the Loo–Riegelman method for measuring the amount of drug absorbed?

d. From the data in only, from which tablet formulation would you expect the highest bioavailability? Why?

e. From the data in , did the disintegration times correlate with the dissolution times? Why?

f. Do the data in appear to correlate with the data in ? Why?

g. Draw the expected cumulative urinary excretion–time curve for formulations A and B. Label axes and identify each curve.

h. Assuming formulation A is the reference formulation, what is the relative bioavailability of formulation D?

i. Using the data in for formulation A, calculate the elimination half-life ($t_{1/2}$) for tolazamide.

Table 15.20 Mean Tolazamide Concentrations[a] in Serum

		Treatment (μg/mL)				
	Time (hr)	A	B	C	D	Statistic[b]
	0	10.8 ± 7.4	1.3 ± 1.4	1.8 ± 1.9	3.5 ± 2.6	$A\overline{DCB}$
	1	20.5 ± 7.3	2.8 ± 2.8	5.4 ± 4.8	13.5 ± 6.6	$AD\overline{CB}$
	3	23.9 ± 5.3	4.4 ± 4.3	9.8 ± 5.6	20.0 ± 6.4	$\overline{AD}CB$

	4	25.4 ± 5.2	5.7 ± 4.1	13.6 ± 5.3	22.0 ± 5.4	$\overline{AD}CB$
	5	24.1 ± 6.3	6.6 ± 4.0	15.1 ± 4.7	22.6 ± 5.0	$\overline{AD}CB$
	6	19.9 ± 5.9	6.8 ± 3.4	14.3 ± 3.9	19.7 ± 4.7	$\overline{AD}CB$
	8	15.2 ± 5.5	6.6 ± 3.2	12.8 ± 4.1	14.6 ± 4.2	$\overline{ADCB}$
	12	8.8 ± 4.8	5.5 ± 3.2	9.1 ± 4.0	8.5 ± 4.1	$\overline{CADB}$
	16	5.6 ± 3.8	4.6 ± 3.3	6.4 ± 3.9	5.4 ± 3.1	$\overline{CADB}$
	24	2.7 ± 2.4	3.1 ± 2.6	3.1 ± 3.3	2.4 ± 1.8	$\overline{CBAD}$
C_{max}, μg/mL[c]		27.8 ± 5.3	7.7 ± 4.1	16.4 ± 4.4	24.0 ± 4.5	$\overline{AD}CB$
t_{max}, hr[d]		3.3 ± 0.9	7.0 ± 2.2	5.4 ± 2.0	4.0 ± 0.9	$BC\overline{DA}$
AUC_{0-24}, μg hr/mL[e]		260 ± 81	112 ± 63	193 ± 70	231 ± 67	$\overline{AD}CB$

[a]Concentrations ± 1 SD, n = 20.

[b]For explanation see text.

[c]Maximum concentration of tolazamide in serum.

[d]Time of maximum concentration.

[e]Area under the 0–24-hr serum tolazamide concentration curve calculated by trapezoidal rule.

From , with permission.

13. If *in-vitro* drug dissolution and/or release studies for an oral solid dosage form (eg, tablet) does not correlate with the bioavailability of the drug *in-vivo*, why should the pharmaceutical manufacturer continue to perform *in-vitro* release studies for each production batch of the solid dosage form?

14. Is it possible for two pharmaceutically equivalent solid dosage forms containing different inactive ingredients (ie, excipients) to demonstrate bioequivalence *in-vivo* even though these drug products demonstrate differences in drug dissolution tests *in-vitro*?

15. For bioequivalence studies, t_{max}, C_{max}, and AUC, along with an appropriate statistical analyses, are the parameters generally used to demonstrate the bioequivalence of two similar drug products containing the same active drug.

a. Why are the parameters t_{max}, C_{max}, and AUC acceptable for proving that two drug products are bioequivalent?

b. Are pharmacokinetic models needed in the evaluation of bioequivalence?

c. Is it necessary to use a pharmacokinetic model to completely describe the plasma drug concentration–time curve for the determination of t_{max}, C_{max}, and AUC?

d. Why are log-transformed data used for the statistical evaluation of bioequivalence?

e. What is an add-on study?

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