

10

BIOAVAILABILITY AND BIOEQUIVALENCE

Many drugs are marketed by more than one pharmaceutical manufacturer. The study of biopharmaceutics gives substantial evidence that the method of manufacture and the final formulation of the drug can markedly affect the bioavailability of the drug. Because of the plethora of drug products containing the same amount of active drug, physicians, pharmacists, and others who prescribe, dispense, or purchase drugs must select generic products that produce an equivalent therapeutic effect to the brand product. To facilitate such decisions, guidelines have been developed by the United States Food and Drug Administration (FDA). The guidelines are available on the Internet (<http://www.fda.gov>). Some of the guidelines also appear in the United States Pharmacopeia/National Formulary (USP-NF). These guidelines and methods for determining drug availability are discussed in this chapter.

DEFINITIONS

- *Bioavailability*. Indicates a measurement of the rate and extent (amount) of therapeutically active drug that reaches the systemic circulation and is available at the site of action.
- *Bioequivalence requirement*. A requirement imposed by the Food and Drug Administration (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*. Bioequivalent drug products are pharmaceutical equivalents that have similar bioavailability (ie, are not significantly different with respect to rate and extent of absorption) when given in the same molar dose and studied under similar experimental conditions. Some drugs may be considered bioequivalent that are equal in the extent of absorption but *not* in the rate of absorption; this is possible if the difference in the rate of absorption is considered clinically insignificant, is not essential for the attainment of effective body drug concentrations on chronic use, and is reflected in the proposed labeling. For example, aspirin and acetaminophen are well-absorbed drugs, and small differences in the rate of absorption are of very little clinical consequence. 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. For some products, other *in vivo* tests may be appropriate, including comparative clinical trials or pharmacodynamic studies.

- **Brand name.** 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 competitors' products (eg, Tylenol, McNeil Laboratories).
- **Chemical name.** Name used by the organic chemist to indicate the chemical structure of the drug (eg, N-acetyl-*p*-aminophenol).
- **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 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).
- **Pharmaceutical equivalents.** Drug products that contain the same active drug ingredient (same salt, ester, or chemical form) and are identical in strength or concentration, dosage form, and route of administration (eg, diazepam, 5 mg oral tablets). *Chemical equivalents* are pharmaceutical equivalents. Pharmaceutical equivalent drug products must meet the identical standards (strength, quality, purity, and identity), but may differ in such characteristics as color, flavor, shape, scoring configuration, packaging, excipients, preservatives, expiration time, and (within certain limits) labeling.
- **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.
- **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 ex-

pected 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.* Therapeutic equivalents are drug products that contain the same therapeutically active drug that should give the same therapeutic effect and have equal potential for adverse effects under conditions set forth in the labels of these drug products. Therapeutic drug products may differ in certain characteristics, such as color, scoring, flavor, configuration, packaging, preservatives, and expiration date. Therapeutic equivalent drug products must be (1) safe and effective, (2) pharmaceutical equivalents, (3) bioequivalent, (4) adequately labeled, and (5) manufactured in compliance with current good manufacturing practices.
- *Therapeutic substitution.* The process of dispensing a therapeutic alternative in place of the prescribed drug product. For example, amoxicillin is dispensed for ampicillin or acetaminophen is dispensed for aspirin.

PURPOSE OF BIOAVAILABILITY STUDIES

Bioavailability studies are performed for both approved active drug ingredients or therapeutic moieties not yet approved for marketing by the FDA. New formulations of active drug ingredients or therapeutic moieties must be approved prior to marketing by the FDA. In approving a drug product for marketing, the FDA must ensure 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.

For unmarketed drugs which do not have full new drug application (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 performed also 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.

After the bioavailability and essential pharmacokinetic parameters of the active ingredient or therapeutic moiety are established, dosage regimens may be recommended in support of drug labeling.

In summary, clinical studies are useful in determining the safety and efficacy of the drug product. 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. 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 is placed into the systemic circulation. Therefore, the drug is considered to be completely available after IV administration. After oral administration of the 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 with the following equation:

$$\text{Relative availability} = \frac{[\text{AUC}]_A}{[\text{AUC}]_B} \quad (10.1)$$

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:

$$\text{Relative availability} = \frac{[\text{AUC}]_A/\text{dose } A}{[\text{AUC}]_B/\text{dose } B}$$

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:

$$\text{Percent relative availability} = \frac{[D_u]_A^\infty}{[D_u]_B^\infty} \times 100 \quad (10.2)$$

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). 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:

$$\text{Absolute availability} = \frac{[\text{AUC}]_{\text{PO}}/\text{dose}_{\text{PO}}}{[\text{AUC}]_{\text{IV}}/\text{dose}_{\text{IV}}} = \frac{F}{z} \quad (10.3)$$

Absolute availability using urinary drug excretion data can be determined by the following:

$$\text{Absolute availability} = \frac{[D_u]_{\text{PO}}^{\infty}/\text{dose}_{\text{PO}}}{[D_u]_{\text{IV}}^{\infty}/\text{dose}_{\text{IV}}} \quad (10.4)$$

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 the drug is completely absorbed. For all extravascular routes of administration, $F \leq 1$. F is usually determined by Equations 10.3 or 10.4.



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 hr after the dose and assayed for drug concentration. The average AUC values (0 to 48 hr) are given in the table below. From these data, calculate (1) the relative bioavailability of the drug from the tablet compared to the oral solution and (2) the absolute bioavailability of the drug from the tablet.

Drug Product	Dose (mg)	AUC($\mu\text{g hr/mL}$)	Standard Deviation
Oral tablet	200	89.5	19.7
Oral solution	200	86.1	18.1
IV bolus injection	50	37.8	5.7

Solution

The relative bioavailability of the drug from the tablet is estimated using Equation 10.1. No adjustment for dose is necessary.

$$\text{Relative bioavailability} = \frac{89.5}{86.1} = 1.04$$

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.

The absolute drug bioavailability from the tablet is calculated using Equation 10.3 and adjusting for the dose.

$$F = \text{absolute bioavailability} = \frac{89.5/200}{37.8/50} = 0.59$$

Because F , the fraction of dose absorbed from the tablet, was less than 1, the drug was not completely absorbed systemically either due to poor absorption or metabolism by first-pass effect. The relative bioavailability of the drug from the tablet was approximately 100% when compared to the oral solution. Results from bioequivalence studies may show that the relative bioavailability of the test oral product to be 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 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 10.1).

Plasma Drug Concentration

Measurement of drug concentrations in blood, plasma, or serum after drug administration is the most direct and objective data to determine systemic drug bioavailability. By appropriate blood sampling, an accurate description of the

TABLE 10.1 Methods for Assessing Bioavailability and Bioequivalence

Plasma Drug Concentration

- The time for peak plasma (blood) concentration ($t_{\max}$)
- The peak plasma drug concentration ($C_{\max}$)
- The area under the plasma drug concentration versus time curve (AUC)

Urinary Drug Excretion

- The cumulative amount of drug excreted in the urine (D_U)
- The rate of drug excretion in the urine (dD_U/dt)
- The time for maximum urinary excretion (t)

Acute Pharmacodynamic Effect

- Maximum pharmacodynamic effect ($E_{\max}$)
- Time for maximum pharmacodynamic effect
- Area under the pharmacodynamic effect versus time curve
- Onset time for pharmacodynamic effect

Clinical Observations

- Well-controlled clinical trials

In Vitro Studies

- Drug dissolution

plasma drug concentration versus time profile of the therapeutically active drug substance(s) can be obtained using a validated drug assay (see Appendix).

$t_{\max}$

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 (Fig. 10-5). 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 (Fig. 10-5). 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, $\mu\text{g/mL}$, ng/mL).

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.

$$[\text{AUC}]_0^\infty = \int_0^\infty C_p dt \quad (10.5)$$

$$[\text{AUC}]_0^\infty = \frac{FD_0}{\text{clearance}} = \frac{FD_0}{kV_D} \quad (10.6)$$

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, $\mu\text{g hr/mL}$).

For many drugs, the AUC is directly proportional to dose. For example, if a single dose of a drug is increased from 250 to 1000 mg, the AUC will also show a four-fold increase (Figs. 10-1 and 10-2).

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 (Fig. 10-3). Drug elimination includes the processes of metabolism and excretion. Drug metabolism is an en-

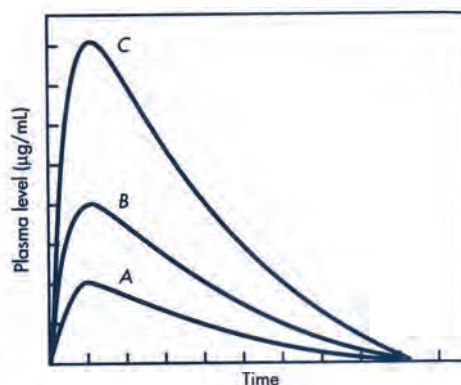


Figure 10-1. Plasma level-time curve following administration of single doses of **(A)** 250 mg, **(B)** 500 mg, and **(C)** 1000 mg of drug.

zyme-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 disproportionately 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.

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, timely urine samples must be collected and the total amount of urinary drug excretion must be obtained (see Chapter 3).

$$D_u^\infty$$

The cumulative amount of drug excreted in the urine, D_u^∞ is directly related to the total amount of drug absorbed. Experimentally, urine samples are collected periodically after administration of the drug product. Each urine specimen is analyzed for free drug with a specific assay. A graph is constructed relating the cumulative drug excreted to the collection time interval (Figure 10-4B).

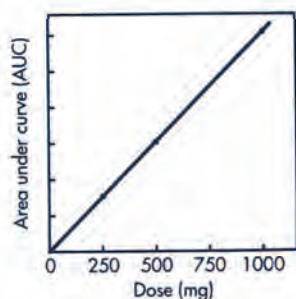


Figure 10-2. Linear relationship between AUC and dose (data from Fig. 10-1).

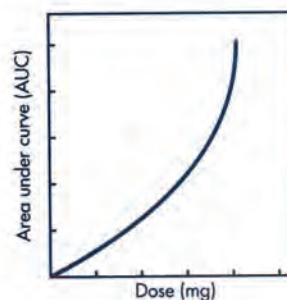


Figure 10-3. Relationship between AUC and dose when metabolism is saturable.

The relationship between the cumulative amount of drug excreted in the urine and the plasma level-time curve is shown in Figure 10-4. 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.

$$dD_u/dt$$

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 Figure 10-4, the maximum rate of drug excretion would be at point *B*, whereas the minimum rate of drug excretion would be 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 (Fig. 10-5).

$$t^\infty$$

The total time for the drug to be excreted is t^∞ . In Figures 10-4 and 10-5, 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 bio-equivalence studies that compare several drug products, as will be described later in this chapter.

Acute Pharmacodynamic Effect

In some cases, the quantitative measurement of a drug is not available, or it lacks sufficient accuracy and/or reproducibility. An acute pharmacodynamic effect—such as an effect on pupil diameter, heart rate, or blood pressure—can be used as an index of drug bioavailability. In this case, an acute pharmacodynamic effect-time curve is constructed. Measurements of the pharmacodynamic effect should be made with sufficient frequency to permit a reasonable estimate of the total area under

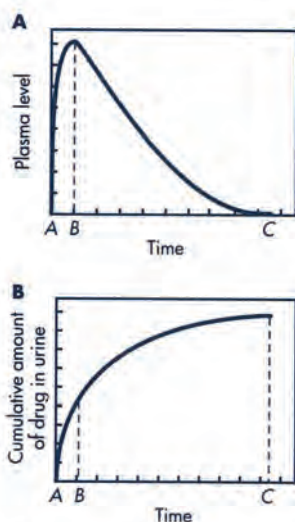


Figure 10-4. Corresponding plots relating the plasma level-time curve and the cumulative urinary drug excretion.

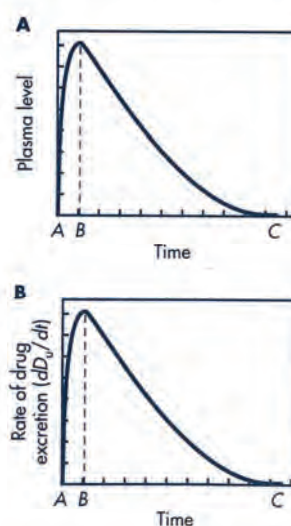


Figure 10-5. Corresponding plots relating the plasma level-time curve and the rate of urinary drug excretion.

the curve for a time period at least three times the half-life of the drug (Gardner, 1977).

The use of an acute pharmacodynamic effect to determine bioavailability generally requires demonstration of a dose-related response. Bioavailability is determined by characterization of the dose-response curve. Pharmacodynamic parameters that are obtained include the total area under the acute pharmacodynamic effect-time curve, peak pharmacodynamic effect, and time for peak pharmacodynamic effect. Onset time and duration of the pharmacokinetic effect may also be included in the analysis of the data.

Clinical Observations

Well-controlled clinical trials in humans that establish the safety and effectiveness of the drug product. The clinical trials approach is the least accurate, least sensitive and least reproducible of the general approaches for determining *in vivo* bioavailability. The FDA only considers this approach when analytical methods are not available to permit use of one of the approaches described above. An example of this approach is for the determination of the bioequivalence of two topical antifungal products by different manufacturers containing the same active antifungal agent (eg, ketoconazole)

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. 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*.

BIOEQUIVALENCE STUDIES

For many years, medical practitioners have observed either lack of response (therapeutic failure), good therapeutic response, or toxicity in patients receiving similar drug products.

Differences in the predicted clinical response may be due to differences in both the pharmacokinetic and/or pharmacodynamic behavior of the drug among individuals. 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 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 may affect the plasma drug level due to variable absorption in the presence of food or even a change in the metabolic clearance of the drug. Feldman and associates (1982) 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 hr), compared to patients on normal diets ($t_{1/2}$, 6.76 hr). 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 or decrease the bioavailability of the drug (Hendles et al, 1984).

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 material (usually the brand name product). In addition, statistical techniques used should be of sufficient sensitivity to detect differences in the rate and extent of absorption that are not attributable to subject variability.

A drug product that differs from the reference material in its rate of absorption, but not in its extent of absorption, may be considered bioavailable 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.

Criteria for Establishing a Bioequivalence Requirement

Bioequivalence requirements may be imposed by the FDA on the basis of the following:

1. Evidence from well-controlled clinical trials or controlled observations in patients that various drug products do not give comparable therapeutic effects.
2. Evidence from well-controlled bioequivalence studies that such products are not bioequivalent drug products.
3. Evidence that the drug products exhibit a narrow therapeutic ratio and minimum effective concentrations in the blood and that safe and effective use of the drug products requires careful dosage titration and patient monitoring.
4. Competent medical determination that a lack of bioequivalence would have a serious adverse effect on the treatment or prevention of a serious disease or condition.
5. Physicochemical evidence of the following:
 - a. The active drug ingredient has a low solubility in water (eg, less than 5 mg/mL).
 - b. The dissolution rate of one or more such products is slow (eg, less than 50% in 30 min when tested with a general method specified by the FDA).
 - c. The particle size and/or surface area of the active drug ingredient is critical in determining its bioavailability.
 - d. Certain structural forms of the active drug ingredient (eg, polymorphic forms, conformers, solvates, complexes, and crystal modifications) dissolve poorly, thus affecting absorption.

- e. Such drug products have a high ratio of excipients to active ingredients (eg, greater than 5 to 1).
 - f. Specific inactive ingredients (eg, hydrophilic or hydrophobic excipients and lubricants) either may be required for absorption of the active drug ingredient or of therapeutic moiety or may interfere with such absorption.
6. Pharmacokinetic evidence of the following:
- a. 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.
 - b. 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).
 - c. 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.
 - d. The therapeutic moiety is rapidly metabolized or excreted so that rapid dissolution and absorption are required for effectiveness.
 - e. 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.
 - f. 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.

Criteria for Waiver of Evidence of *In Vivo* Bioavailability

For certain drug products, the *in vivo* bioavailability of the drug product may be self-evident 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:

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.

The FDA has issued a guidance for establishing the bioequivalence of albuterol metered-dose inhalers using a pharmacodynamic effect study.

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.
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.

DESIGN AND EVALUATION OF BIOEQUIVALENCE STUDIES

In the past, generic drug products that were pharmaceutical equivalents did not always give identical therapeutic effects *in vivo* in patients. Thus, bioequivalence studies are performed to compare the bioavailability of the generic drug product to the brand name product. Once bioequivalence is established, then it is likely that both the generic and brand name dosage forms will produce the same therapeutic effect. The FDA publishes guidances (<http://www.fda.gov/cder/guidance>) for bioequivalence studies. These guidances should be consulted for specific drugs. Sponsors may also request a meeting with the FDA to review the study design.

Design

The design and evaluation of well-controlled bioequivalence studies require the 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 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 Table 10.2. Bioavailability studies for controlled-release dosage forms are discussed in Chapter 7.

For bioequivalence studies, both the test and reference drug formulations contain the pharmaceutical equivalent drug in the same dose strength, in similar dosage forms (eg, immediate release or controlled release), and both are given by the same route of administration. Both a single dose and/or a multiple dose (steady-state) study may be required. Prior to initiation of the study, the *Institutional Review Board* (IRB) of the clinical facility in which the study is to be performed must ap-

TABLE 10.2 Elements of a Bioavailability Study Protocol

I. Title	C. Inclusion/exclusion criteria
A. Principle investigator (study director)	1. Inclusion criteria
B. Project/protocol number and date	2. Exclusion criteria
II. Study objective	D. Restrictions/prohibitions
III. Study design	V. Clinical procedures
A. Design	A. Dosage and drug administration
B. Drug products	B. Biological sampling schedule and handling procedures
1. Test product(s)	C. Activity of subjects
2. Reference product	VI. Ethical considerations
C. Dosage regimen	A. Basic principles
D. Sample collection schedule	B. Institutional review board
E. Housing/confinement	C. Informed consent
F. Fasting/meals schedule	D. Indications for subject withdrawal
G. Analytical methods	E. Adverse reactions and emergency procedures
IV. Study population	VII. Facilities
A. Subjects	VIII. Data analysis
B. Subject selection	A. Analytical validation procedure
1. Medical history	B. Statistical treatment of data
2. Physical examination	IX. Drug accountability
3. Laboratory tests	X. Appendix

prove 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 that *no unnecessary human research be done*. Generally, the study is performed in normal, healthy male volunteers who have given informed consent to be in the study. The number of subjects in the study will depend upon the expected intersubject variability. Patient selection will be made according to certain established criteria for inclusion into, or exclusion from, the study. For example, the study might exclude any volunteers who are smokers, have known allergies to the drug, are overweight, or have taken any medication within a specified period (often 1 week) prior to the study. The subjects are generally fasted 10 to 12 hours (overnight) prior to drug administration and may continue to fast for a 2- to 4-hour period after dosing.

Reference Standard

In considering a bioequivalence study, one formulation of the drug is chosen as a reference standard against which all other formulations of the drug are compared. The reference material 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. The reference standard is generally a formulation currently marketed with a fully approved NDA for which there are valid scientific safety and efficacy data. The reference formulation is usually the innovator's or original manufacturer's brand name product.

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 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.

Study Designs

For many drug products, the FDA, Division of Bioequivalence, Office of Generic Drugs provides a guidance for the performance of in vitro dissolution and in vivo bioequivalence studies. Similar guidelines appear in the USP/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. Three other study designs have been proposed by FDA. For example, FDA published two draft guidelines in October and December of 1997 to consider the performance of individual bioequivalence 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. 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 prior (zero time) to the dose and at appropriate intervals after the dose to obtain an adequate description of the plasma drug concentration versus time profile. The subjects should be in the fasting state (overnight fast) prior to drug administration and should continue to fast for up to 4-hour period after dosing. No other medication is normally given to the subject for at least one week prior to the study.

Food Intervention Study

This study is a single dose, randomized, three-treatment, three period, six sequence, crossover, limited food effects study comparing equal doses of the test product given under fasting conditions with those of the test and reference products given immediately after a standard, high fat content breakfast. 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).

Multiple Dose (Steady-State) Study

A multiple dose, steady-state, randomized, two-treatment, two-way, crossover study comparing equal doses of the test and reference products in adult, healthy subjects is required for oral extended-release (controlled-release) drug products in addition to a single dose fasting study and a food intervention study. 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

TABLE 10.3 Latin Square Crossover Design for a Bioequivalence Study of 3 Drug Products in 6 Human Volunteers

SUBJECT	DRUG PRODUCT		
	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

human volunteers, comparing three different drug formulations (A, B, C) or four different drug formulations (A, B, C, D), are described in Tables 10.3 and 10.4. 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 carry-over 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 (Table 10.4). 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. In some cases, the measurement of drug in urine samples may be necessary.

TABLE 10.4 Latin Square Crossover Design for a Bioequivalency Study of 4 Drug Products in 16 Human Volunteers

SUBJECT	DRUG PRODUCT			
	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, sometimes referred to as a *two-legged* study, means that the study is performed on two different days (time periods) separated by a washout period during which most of the drug has been 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

R = reference; T = treatment.

Table 10.4 shows a design for three different drug treatments 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.

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 graphical form for evaluation. The plasma drug concentration versus 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 [$100 \times (C_{max} - C_{min}) / C_{min}$]. Proper statistical evaluation should be performed on the estimated pharmacokinetic parameters.

Statistical Evaluation of the Data

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 Appendix A). The distribution of many biological parameters such as C_{max} , AUC have a longer right tail than would be observed in a normal distribution (Midha et al, 1993). Moreover, the true distribution of these biological parameters may be difficult to ascertain due to 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 prior to statistical data evaluation for bioequivalence determination.

Analysis of Variance (ANOVA)

An *analysis of variance* (ANOVA) is a statistical procedure (Appendix A) used to test the data for difference 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_{0-24} , $AUC_{0-\infty}$, $t_{\max}$, 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 .05 probability ($P \leq 0.05$) that these results would have happened on the basis of chance alone. The term *probability*, or 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 an $\alpha = 0.2$ 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

This procedure is also referred to as the *confidence interval* approach (Schuirmann, 1987). 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 the reference drug product. The objective of the two one-sided tests statistical 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 $\pm 20\%$ 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 is used, the 90% confidence interval is set at 80 to 125%, since the log of 0.8 (except for sign) is 1.25 or 125%. These confidence limits have also been termed the *bioequivalence interval* (Midha et al, 1993). 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 $C_{\max}$. There should be no statistical dif-

TABLE 10.5 Bioavailability Comparison of a Generic (Test) and Brand (Reference) Drug Product^a

PARAMETER	TEST	REFERENCE	RATIO (%)T/R	90% CONFIDENCE LIMITS	ANOVA T vs R	POWER T vs R
AUC _{0-t} (μg hr/mL)	1466	1494	98.1	93.0–102.5	<i>P</i> < 0.78	>99.9%
AUC _{0-∞} (μg hr/mL)	1592	1606	99.1	94.5–104.1	<i>P</i> < 0.76	>99.7%
C _{max} (μg/mL)	11.6	12.5	92.8	88.5–98.6	<i>P</i> < 0.65	>87.9%
t _{max} (hr)	1.87	2.1	89.1			

^a The results were obtained from a two-way crossover, single-dose, fasting study in 24 healthy adult volunteers. Mean values are reported. No statistical differences were observed between AUC and C_{max} values for the test and reference products.

ferences 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 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 Table 10.5. As shown by the ANOVA, no statistical differences for the pharmacokinetic parameters AUC_{0-t}, AUC_{0-∞}, and C_{max} were observed between the test product compared to the brand product. The 90% confidence limits for the mean pharmacokinetic parameters of the test product were within the 0.80 to 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.

For a single dose, three-way crossover food/fasting study, the mean values for the AUC and C_{max} for the test product when administered with food should be within 20% from the respective mean values of the reference product when given with food. No ANOVA is required by FDA.

For multiple dose studies, an ANOVA should be performed on the log transformed AUC and C_{max}. The AUC and C_{max} parameters for the test (generic) product should be within 80 to 125% of the reference product using the 90% confidence interval.

Table 10.6 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 the solution. According to the ANOVA, the mean AUC values were not statistically different from each other or different from 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.

For illustrative purposes, consider a drug that has been prepared at the same dosage level in three formulations, formulations A, B, and C. These formulations

TABLE 10.6 Summary of the Results of a Bioavailability Study^a

DOSAGE FORM	C_{MAX} ($\mu\text{g/mL}$)	T_{MAX} (hr)	AUC_{0-24} ($\mu\text{g hr/mL}$)	F^b	90% CONFIDENCE INTERVAL FOR AUC
Solution	16.1 ± 2.5	1.5 ± 0.85	1835 ± 235		
Tablet A	10.5 ± 3.2^c	2.5 ± 1.0^c	1523 ± 381	81	74–90%
Tablet B	13.7 ± 4.1	2.1 ± 0.98	1707 ± 317	93	88–98%
Tablet C	14.8 ± 3.6	1.8 ± 0.95	1762 ± 295	96	91–103%

^a The bioavailability of a drug from 4 different formulations was studied in 24 healthy adult male subjects using a four-way Latin square crossover design. The results represent the mean $\pm$ standard deviation.

^b Oral bioavailability relative to the solution.

^c $P \leq .05$.

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 this data we can observe the relationship between plasma and urinary excretion parameters and drug bioavailability (Fig. 10-6). 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} of A is higher than B because the rate of drug absorption is more rapid.

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 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 from formulation A. The corresponding urinary excretion data confirm these observations.

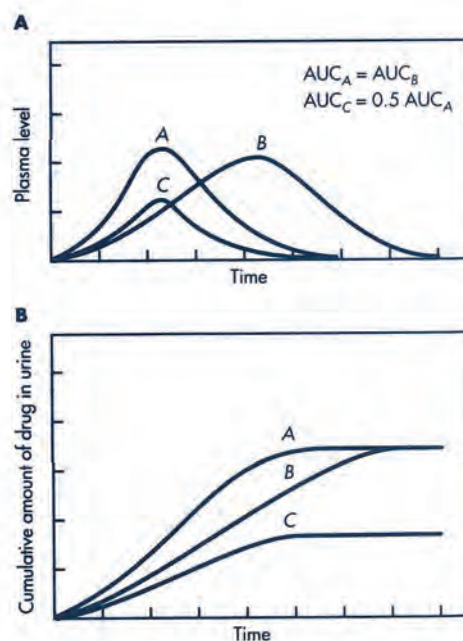


Figure 10-6. Corresponding plots relating plasma concentration and urinary excretion data.

TABLE 10.7 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
$[dD_u/dt]_{\max}^*$	Decrease	$[dD_u/dt]_{\max}^*$	Decrease
D_u^{∞}	Decrease	D_u^{∞}	Same

*Maximum rate of urinary drug excretion.

These relationships are summarized in Table 10.7. The table illustrates how bioavailability parameters for plasma and urine change when the extent and rate of bioavailability is changed, respectively.

Study Submission

An outline for the submission of a completed bioavailability study for submission to the FDA is shown in Table 10.8. Before the study is performed, 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). Moreover, the protocol for the study may be submitted to the FDA for discussion and approval prior to initiation of the investigation. After the study has been performed, the results are statistically and pharmacokinetically analyzed. These results, along with case reports and various data supporting the validity of the analytical method, are included in the submission. The Biopharmaceutics Branch of the FDA, after receiving the completed study, will review the study in detail according to the outline presented in Table 10.9. If necessary, an FDA investigator may inspect both the clinical and analytical facilities used for the study and audit the raw data used in support of the bioavailability study. The FDA then makes a decision to approve the bioavailability of the drug product.

Dissolution Testing in Lieu of Bioavailability Studies

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 the 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 proportionally 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, if the drug product is available in 50 mg, 75

TABLE 10.8 Proposed Format and Contents of an *In Vivo* Bioequivalence Study Submission and Accompanying *In Vitro* Data

Title Page	
Study title	Data on intraday precision and accuracy
Name of sponsor	Figure for standard curve(s) for low/high ranges
Name and address of clinical laboratory	Chromatograms of standard and quality control samples
Name of principal investigator(s)	Sample calculation
Name of clinical investigator	V. Pharmacokinetic Parameters and Tests
Name of analytical laboratory	Definition and calculations
Dates of clinical study (start, completion)	Statistical tests
Signature of principal investigator (and date)	Drug levels at each sampling time and pharmacokinetic parameters
Signature of clinical investigator (and date)	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
	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
	<i>Curricula vitae</i> of the investigators
	VIII. <i>In Vitro</i> Testing
	Dissolution testing
	Dissolution assay methodology
	Content uniformity testing
	Potency determination
	IX. Batch Size and Formulation
	Batch record
	Quantitative formulation
Table of Contents	
I. Study Resume	
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	

From Dighe and Adams, 1991, with permission.

mg, and 100 mg dose strengths, an *in vivo* study is performed on the 100 mg dose strength, and comparative dissolution studies are performed on the 50 mg and 75 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 Chapters 6 and 7.

TABLE 10.9 General Elements of a Biopharmaceutics Review

Introduction	Determination of detectable differences
Study design	Determination of power to detect a 20% difference at $\alpha = .05$
Study objective(s)	Comments
Assay description and validation	Deficiencies
Assay for individual samples checked	Recommendation
Summary and analysis of data	

From Purich (1980), with permission.

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 *Report by the Bioequivalence Task Force, 1988* 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 states that "clinical studies of effectiveness have difficulty detecting differences in dose 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 Westlake (1972), 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 intra-subject variability. It is theoretically possible that the excipients in one of the dosage forms tested may pose a problem in the 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.

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 (Table 10.10).

TABLE 10.10 Problems in Bioavailability and Bioequivalence

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

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 (Shah et al, 1996). The number of subjects required to demonstrate bioequivalence for these drug products may be excessive, requiring more than 48 subjects to meet the 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 (Shah et al, 1996).

For drugs with very long elimination half-lives or a complex elimination phase, a complete plasma drug concentration versus 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 versus time curve (AUC_{0-t}) may be more practical. The use of a truncated AUC allows for the measurement of peak absorption and would decrease 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 due to problems in analysis. Some drugs have active metabolites, which should be quantitated as well as the parent drug. Drugs such as thioridazine and selegiline 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. In contrast, some drugs, such as procainamide, have an active metabolite, N-acetylprocainamide. The acetylation of procainamide demonstrates genetic polymorphism with two groups of subjects consisting of either rapid acetylators or slow acetylators. To decrease intersubject variability, a bioequivalence study may be performed only on one phenotype, such as the rapid acetylators.

Some drugs (eg, benzocaine, hydrocortisone, antiinfectives, antacids) are intended for local effect; 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 bio-

TABLE 10.11 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
Antacids	Magnesium and aluminum hydroxide gel	Neutralization of acid
Topical antifungal	Ketoconazole	Drug uptake into stratum corneum

equivalence determination (Table 10.11). 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.

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 manufactures 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 hormonal replacement (estrogen, levothyroxine) or 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 be more appropriate to measure.

GENERIC SUBSTITUTION

To contain drug costs, most states have adopted generic substitution laws to allow the pharmacist 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 the pharmacist 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.

Due to public demand, the FDA Center for Drug Evaluation and Research publishes annually a listing of approved drug products, *Approved Drug Products with*

TABLE 10.12 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.

- 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.

Adapted from USP DI, vol 3, Approved Drug Products and Legal Requirements, 1998.

Therapeutic Equivalence Evaluations (commonly known as the “Orange Book”). This monograph is also reproduced in the *United States Drug Information* publication (USP DI, vol 3), entitled *Approved Drug Products and Legal Requirements*. The Orange Book is available on the Internet at <http://www.fda.gov/cder/drug.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 Table 10.12. 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.



FREQUENTLY ASKED QUESTIONS

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

2. What does sequence, washout period, and period mean in a crossover bioavailability study?
3. Why does FDA require a food intervention (food effect) study for some generic drug products prior to 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 (Fig. 10-7). Each drug product was given in the same dose as the others. 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?
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 Table 10.13 represent the average findings in antibiotic plasma samples taken from 10 humans (average weight 70 kg), tabulated in a four-way crossover design.

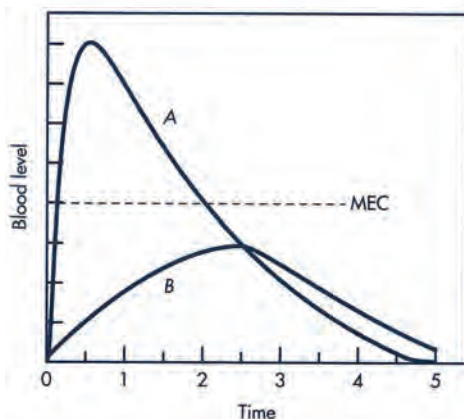
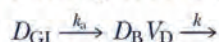


Figure 10-7. Blood level curves for two different oral dosage forms of a hypothetical antibiotic.

TABLE 10.13 Comparison of Plasma Concentrations of Antibiotic, as Related to Dosage Form and Time

TIME AFTER DOSE (hr)	PLASMA CONCENTRATION ($\mu\text{g/mL}$)			
	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
$\text{AUC } \left(\frac{\mu\text{g}}{\text{mL}} \times \text{hr} \right)$	29.0	145.0	116.0	116.0

- Which of the four drug products in Table 10.13 would be preferred as a reference standard for the determination of relative bioavailability? Why?
 - From which oral drug product is the drug absorbed more rapidly?
 - What is the absolute bioavailability of the drug from the oral solution?
 - What is the relative bioavailability of the drug from the oral tablet compared to the reference standard?
 - From the data in Table 10.13 determine:
 - Apparent V_D
 - Elimination $t_{1/2}$
 - First-order elimination rate constant k
 - Total body clearance
 - 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 assumes a one-compartment open model with first-order absorption and first-order elimination:



The drug was given in a single oral dose of 250 mg to a group of college students 21 to 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 is summarized in Table 10.14.

- The minimum effective concentration of Aphrodisia in plasma is $2.3 \mu\text{g/mL}$. What is the *onset* time of this drug?
- The minimum effective concentration of Aphrodisia in plasma is $2.3 \mu\text{g/mL}$. What is the *duration* of activity of this drug?
- What is the elimination half-life of Aphrodisia in college students?
- What is the time for peak drug concentration ($t_{\max}$) of Aphrodisia?
- What is the peak drug concentration ($C_{\max}$)?
- Assuming that the drug is 100% systemically available (ie, fraction of drug absorbed equals unity), what is the AUC for Aphrodisia?

TABLE 10.14 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

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 6 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 to 28 years, average weight 73 kg) in a four-way crossover design. The volunteers were fasted 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 10.15.
 - 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, SKF Lab Co), 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 the oral dose after 24 hours.
8. Define the term "*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 to 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

TABLE 10.15 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

TABLE 10.16 Blood Level Data Summary for Two Drug Products

KINETIC VARIABLE	UNIT	DRUG PRODUCT		STATISTIC
		A 4 × 250 MG TABLET	B 1000 MG TABLET	
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

tablet containing 1000 mg of drug (drug product *B*). Blood levels of the drug were obtained and the data are summarized in Table 10.16.

- 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 intravenous bolus and the AUC was computed as 20 μg hr/mL. Calculate the fraction of drug systemically absorbed from drug product *B* (1 × 1000 mg) tablet using the data in Table 10.13.
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 a greater bioavailability than the brand name drug product.
 - a. Would you approve marketing the generic drug product, claiming it was superior than 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 Welling and associates (1982):

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 × 4 Latin square design. Each treatment was separated by 1-week intervals. All subjects fasted overnight prior to receiving the tolazamide tablet the following morning. The tablet was given with 180 mL of water. Food intake was allowed at 5 hours post dose. Blood samples (10 mL) were taken just prior to the dose and periodically

TABLE 10.17 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 Welling et al. 1982, with permission.

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 Wagner and Nelson. 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* prior to taking 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 Table 10.17 *only*, from which tablet formulation would you expect the greatest bioavailability? *Why?*
 - e. From the data in Table 10.14, did the disintegration times correlate with the dissolution times? *Why?*
 - f. Do the data in Table 10.17 appear to correlate with the data in Table 10.18? *Why?*
 - g. Draw the expected cumulative urinary excretion versus 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 Table 10.18 for formulation A, calculate the elimination half-life ($t_{1/2}$) for tolazamide.
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 analysis 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?

TABLE 10.18 Mean Tolazamide Concentrations^a in Serum

TIME (hr)	TREATMENT, $\mu\text{g/mL}$				STATISTIC ^b
	A	B	C	D	
0	10.8 $\pm$ 7.4	1.3 $\pm$ 1.4	1.8 $\pm$ 1.9	3.5 $\pm$ 2.6	ADCB
1	20.5 $\pm$ 7.3	2.8 $\pm$ 2.8	5.4 $\pm$ 4.8	13.5 $\pm$ 6.6	ADCB
3	23.9 $\pm$ 5.3	4.4 $\pm$ 4.3	9.8 $\pm$ 5.6	20.0 $\pm$ 6.4	ADCB
4	25.4 $\pm$ 5.2	5.7 $\pm$ 4.1	13.6 $\pm$ 5.3	22.0 $\pm$ 5.4	ADCB
5	24.1 $\pm$ 6.3	6.6 $\pm$ 4.0	15.1 $\pm$ 4.7	22.6 $\pm$ 5.0	ADCB
6	19.9 $\pm$ 5.9	6.8 $\pm$ 3.4	14.3 $\pm$ 3.9	19.7 $\pm$ 4.7	ADCB
8	15.2 $\pm$ 5.5	6.6 $\pm$ 3.2	12.8 $\pm$ 4.1	14.6 $\pm$ 4.2	ADCB
12	8.8 $\pm$ 4.8	5.5 $\pm$ 3.2	9.1 $\pm$ 4.0	8.5 $\pm$ 4.1	CADB
16	5.6 $\pm$ 3.8	4.6 $\pm$ 3.3	6.4 $\pm$ 3.9	5.4 $\pm$ 3.1	CADB
24	2.7 $\pm$ 2.4	3.1 $\pm$ 2.6	3.1 $\pm$ 3.3	2.4 $\pm$ 1.8	CBAD
C_{max}^c , $\mu\text{g/mL}$	27.8 $\pm$ 5.3	7.7 $\pm$ 4.1	16.4 $\pm$ 4.4	24.0 $\pm$ 4.5	ADCB
t_{max}^d , hr	3.3 $\pm$ 0.9	7.0 $\pm$ 2.2	5.4 $\pm$ 2.0	4.0 $\pm$ 0.9	BCDA
AUC_{0-24}^e , $\mu\text{g hr/mL}$	260 $\pm$ 81	112 $\pm$ 63	193 $\pm$ 70	231 $\pm$ 67	ADCB

^a Concentrations $\pm$ 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 Welling et al (1982), with permission.

- 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 versus 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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