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Bioavailability and Bioequivalence

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8.1 Introduction

For systemically acting drugs, absorption is a prerequisite for therapeutic activity when drugs are administered extravascularly. Factors affecting drug absorption have been discussed in previous chapters. This chapter will cover general methods to evaluate bioavailability and bioequivalence. Scientific principles as well as regulatory perspectives related to these two topics will be discussed. Historically, the development of sensitive and precise bioanalytical methods in the 1960s and 1970s allowed for the first time the measurement of very low levels of drug concentrations in biological fluids. As a result, pharmacokinetic profiles of drugs, describing absorption, distribution, and clearance, could be determined. Regulations related to bioavailability and bioequivalence were put into place, considering the latest advances in the science. Currently, bioavailability and bioequivalence play a significant role in the discovery, development, and regulation of new drug products. Additionally, bioequivalence studies are a crucial component of abbreviated new drug applications (ANDAs), leading to market access of safe, effective, and low cost generic drugs.

8.2 Bioavailability and Bioequivalence

8.2.1 Bioavailability and its Utility in Drug Development and Regulation

The therapeutic action of a drug is usually correlated with the delivery of the active substance to the site or more accurately, sites, of pharmacological response. US Federal regulations (21 Code of Federal Regulations (CFR), 2006) define bioavailability (BA) as

"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".

Most drugs are systemically acting, meaning that they reach their sites of action through the systemic circulation. Thus, it is common for pharmaceutical scientists to evaluate the bioavailability of a drug product as the fraction of the dose reaching the systemic circulation. BA can depend on the physicochemical properties of the drug substance and the route of administration, in addition to drug product excipients and manufacturing process.

The BA of a drug is an important attribute that is investigated early in drug development and used throughout development. In many cases, it is the deciding factor for whether or not a drug candidate is selected for further development (Sun *et al.*, 2004). As stated in the FDA bioavailability and bioequivalence guidance (FDA, 2003a) BA studies help elucidate the process by which a drug is released from its dosage form and reaches the sites of action including the impact of presystemic metabolism and/or transporters. BA studies also provide information about the drug's pharmacokinetic properties such as dose proportionality, linearity, and effect of food on absorption. When multiple formulations are used in the clinical development program, relative bioavailability studies can link observations of safety and efficacy to drug exposure and provide a basis for labeling or formulation optimization. BA studies can also be used in establishing an exposure–response relationship (FDA Exposure–Response guidance, 2003b).

8.2.2 Bioequivalence and its Utility in Drug Development and Regulation

Federal regulations (21 CFR, 2006) define bioequivalence as:

> The absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study.

Bioequivalence (BE) studies are a major component of ANDAs. They verify that the active ingredient in a generic drug product will be absorbed into the body to the same extent and at the same rate as its corresponding reference listed drug (RLD) product. The significance of BE studies is that when two pharmaceutically equivalent products are shown to be bioequivalent, the two products are judged to be therapeutically equivalent. Therapeutically equivalent products are expected to have the same safety and efficacy profiles, when administered under the conditions listed in the product labeling. This is the basis for the approval and use of generic drug products.

BE studies are not only performed as part of the ANDA process, but also conducted by new drug manufacturers to confirm equivalence between formulations when it is necessary to make manufacturing and/or formulation changes. For example, often the marketed drug product is different in formulation or method of manufacture from the product used in the safety and efficacy clinical trials. These differences may be the result of formulation changes necessary to scale up

the product from a small (laboratory or pilot) scale size to a large scale (commercial) size. After approval, the New Drug Application or NDA sponsor may significantly modify the scale of product runs, equipment, manufacturing process, formulation and dosage forms, ingredient specifications, source of supplies, and method of synthesis of the active ingredient. In these cases, the marketed or reformulated product must demonstrate bioequivalence to the original formulation to link the safety and efficacy data of the original product to the new product.

8.2.3 Bioavailability and Bioequivalence Studies: General Approaches

There are several acceptable approaches for the determination of BA and BE. Title 21 of the Code of Federal Regulations (21 CFR, 2006) §320.24 lists the *in vivo* and *in vitro* methods of determining BA or BE for a drug product.

They are:

1. (a) An *in vivo* test in humans in which the concentration of the active ingredient or active moiety, and, when appropriate, its active metabolite(s), in whole blood, plasma, serum, or other appropriate biological fluid is measured as a function of time; (b) an *in vitro* test that has been correlated with and is predictive of human *in vivo* bioavailability data.
2. An *in vivo* test in humans in which the urinary excretion of the active ingredient or active moiety, and, when appropriate, its active metabolite(s), is measured as a function of time.
3. An *in vivo* test in humans in which an appropriate acute pharmacological effect of the active moiety, and, when appropriate, its active metabolite(s), is measured as a function of time if such effect can be measured with sufficient accuracy, sensitivity, and reproducibility.
4. Well-controlled clinical trials that establish the safety and effectiveness of the drug product, for purposes of measuring bioavailability, or appropriately designed comparative clinical trials, for purposes of demonstrating BE. This approach is the least accurate, sensitive, and reproducible of the general approaches for determining BA or BE.
5. A currently available *in vitro* test acceptable to the FDA (usually a dissolution rate test) that ensures human *in vivo* BA.
6. Any other approach deemed adequate by FDA to measure BA or establish BE.

For most systemically acting drugs, the active moiety can be detected and accurately measured in the plasma over time. Therefore, the first (pharmacokinetic) method (1) listed above is preferred. This pharmacokinetic method is generally considered as the most sensitive, accurate, and reproducible method for the assessment of BA and BE. Section 8.3 describes the conduct of these types of studies in detail.

For some drug products, there is sufficient understanding of the physicochemical properties and biological factors that affect BA that there is no need for *in vivo* BE studies. Section 8.4 describes the situations in which the *in vivo* studies can be waived.

Drugs that do not reach their sites of action through the systemic circulation are defined as locally acting drugs. For locally acting drugs, the bioequivalence method of choice is usually dependent on the attributes of the drug and drug product including physicochemical properties, BA, route of administration, site of action, and ability to detect/measure the active moiety. FDA recommends the most sensitive, accurate, and reproducible method for a particular product be used. Section 8.5 provides examples of the selection of BE methods for locally acting drugs

8.3 Pharmacokinetic Bioavailability and Bioequivalence Studies

8.3.1 Bioavailability Studies: General Guidelines and Recommendations

BA for systemically acting, orally administered drug products is usually determined by measuring the concentration of the active ingredient and, when appropriate, its active metabolites over time in samples collected from the systemic circulation. Figure 8.1 shows a typical concentration–time profile. The profile determines the following important parameters:

1. $C_{\max}$ is the maximum observed plasma concentration.
2. AUC_{0-t} is the area under the concentration–time curve. It is calculated using the trapezoid rule on the actual data points.
3. $T_{\max}$ is the time at which $C_{\max}$ is observed.
4. k_{e} is the terminal elimination rate constant determined from fitting the tail of the profile to a linear elimination model: $\mathrm{d}C/\mathrm{d}t = -k_{\mathrm{e}}\, C$.
5. $t_{1/2}$ is the terminal half-life. $t_{1/2} = 0.693/k_{\mathrm{e}}$. It is the time it takes for the concentration to be reduced by half due to drug elimination.
6. AUC_{∞} is the area under the concentration–time curve extrapolated to infinity. $\mathrm{AUC}_{\infty} = \mathrm{AUC}_{0-t} + C_{\mathrm{last}}/k_{\mathrm{e}}$. C_{last} is the last observed concentration.

$C_{\max}$ is usually correlated with the rate of absorption and AUC reflects the extent of absorption, and thus are considered the parameters most relevant to safety and efficacy.

In most cases, BA studies are conducted as a single dose comparison between a test product and a reference product. These studies are generally conducted using a cross-over design in which each subject receives both treatments in a random order. Treatments should be separated by a washout period exceeding five half-lives of the active moieties measured. Following administration of the test or reference product, blood samples are collected to obtain a profile of the time-course

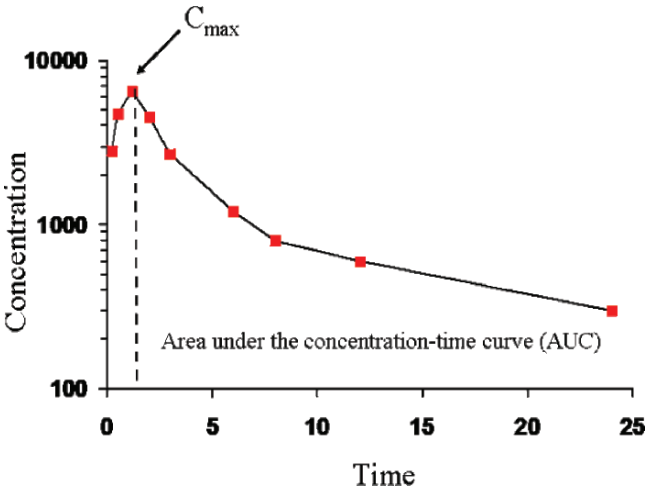

FIGURE 8.1. Example of a plasma-concentration time profile

of the drug for each subject. Drug or active metabolite concentrations in the urine may be used when they are not detectable in the blood or plasma. The sampling schedule, which may differ for each drug, should be of sufficient frequency to ensure precise estimation of the drug's pharmacokinetic parameters. It is generally recommended that samples should be collected over at least three times the elimination half-life of the drug.

As stated previously, a drug's BA can be impacted by its route of administration. By definition, a drug which is administered intravenously (i.v.) has 100% BA. However, BA generally decreases when the drug is administered by extravascular routes (e.g., oral, transdermal, etc.). This decrease is usually a function of incomplete absorption, and/or presystemic metabolism or degradation.

BA may be classified as absolute or relative. Absolute BA is the fraction of the administered dose that reaches the systemic circulation relative to an intravenous dose, while relative bioavailability is the fraction of the dose of a test product that reaches the systemic circulation relative to a non-i.v. reference product. For example, a tablet may have an absolute BA of 60% (or some other value); however, the same tablet would have a *relative* BA of 100%, if the drug in the tablet is absorbed to the same extent as an oral solution of the same drug. The relative BA of the tablet in this case is specific to the oral solution, and may differ relative to other dosage forms.

Absolute BA is calculated by taking the ratio of the dose-corrected AUC of the test product (oral) divided by AUC of the i.v. reference product. Mathematically, the relationship is expressed as

$$\frac{AUC_{PO} \times Dose_{IV}}{AUC_{IV} \times Dose_{PO}} \tag{8.1}$$

where AUC_{PO} and AUC_{IV} are the area under the concentration–time curve after oral and intravenous administration, and $Dose_{po}$ and $Dose_{iv}$ are the amount of dose given orally and i.v., respectively.

Similarly, relative BA can be measured using a test product and a reference (non-i.v.) drug, and calculated by

$$F = \frac{AUC_{test} \times Dose_{ref}}{AUC_{ref} \times Dose_{test}}. \tag{8.2}$$

8.3.2 Bioequivalence Studies: General Guidelines and Recommendations

It is generally recommended that BE studies be conducted using a single dose, cross-over design. Parallel and replicate designs are also acceptable, and may be more appropriate under certain circumstances. Treatments are usually administered to healthy subjects, representative of the general population. Samples of an accessible biologic fluid, usually blood or urine, are analyzed for drug concentrations. Pharmacokinetic parameters, such as AUC and C_{max}, are determined from the resulting concentration–time profiles.

8.3.2.1 Study Design

In the standard cross-over design for *in vivo* BE studies, subjects receive a single dose of test and reference products on separate occasions with random assignment to the two possible sequences of product administration. Treatments are separated by a washout period exceeding five half-lives of the active moieties measured. Parallel designs in which separate groups of subjects receive the test and reference products require larger numbers of subjects and are recommended only in special cases when the half life of the drug is so long that the cross-over design is not feasible. The use of replicate designs for highly variable drugs is discussed in Sect. 8.3.3.3. Single dose studies are recommended over multiple dose studies because single dose studies are generally more sensitive "in assessing release of the drug substance from the drug product into the systemic circulation. . ." (FDA BA/BE guidance, 2003a).

8.3.2.2 Dose

For a product with multiple strengths, the highest strength is usually recommended for use in a BE study. The pharmacokinetics of most drugs is well described by linear absorption, distribution, and clearance processes. The rate of linear processes increases proportionally to the amount of drug or the dose. Thus a bioequivalence conclusion for one of these drugs will be same at any dose.

For drugs with nonlinear pharmacokinetics, the dose used in the BE study should be the most sensitive to differences in formulation. The most common source of nonlinear pharmacokinetics is saturable metabolism, where the rate of metabolism reaches a maximum that is independent of drug concentration.

Other potential causes for nonlinear pharmacokinetics are solubility limited absorption or saturable uptake mediated by transporters. If there are safety concerns with administration of a single dose of the highest strength in healthy subjects, FDA will recommend use of a lower dose.

8.3.2.3 Subjects

Because determination of BE is dependent on statistical methods, the number of subjects in the study should be sufficient to ensure adequate power. The typical number of subjects is 24–36 with the minimum number of subjects in the study being 12. Healthy subjects are recommended for BE studies for two main reasons: patients are more variable and patients require continuous treatment that does not allow for a washout period. The greater variability observed in patients has a direct impact on the sensitivity of BE testing. Patients are generally used only when drug is not safe to administer in healthy subjects.

The physical processes of drug absorption for solid oral dosage forms are usually the same in patients as they are in healthy subjects. Given that BE studies compare the relative performance of two formulations, any conclusion drawn in healthy subjects will also apply to patients. This is true even for products where there is a known difference in BA between patients and healthy subjects.

8.3.2.4 Statistical Analysis of Bioequivalence

Pharmacokinetic parameters AUC and C_{max} are analyzed statistically because of the variability inherent in human subjects. This variability may be observed when the same subject receives the *same* drug product on two different occasions, i.e., the resulting plasma concentrations will not be exactly the same. Because of this inherent variability, an individual who takes two *different* products on separate occasions may show a measurable difference in the pharmacokinetic parameters. In this situation, it is not clear whether this difference is the result of a difference between the products, or the result of normal within subject variability. Thus, FDA recommends that ANDA or NDA applicants use statistical methods to estimate more accurately those differences in pharmacokinetics that result from the two product formulations. When considering the results from BE studies, it is important to understand what statistical tests are used and how FDA uses the results of these statistical tests to determine whether two products are bioequivalent. The following is a qualitative description, drawing on FDA's responses to citizen petitions related to BE (FDA, 2004). Details of the statistical calculations can be found in the FDA guidance statistical approaches to establishing bioequivalence (FDA, 2001b).

The mean is the average of all the differences in pharmacokinetic values observed in the small group of study subjects. For example, in a study the mean AUC of the test product might be 99% of the AUC of the reference product. The mean difference in this case would be 1% and the mean ratio would be 99%. However, if the same BE study is repeated in another small group of subjects, the second study's mean may be different from the first study's mean. Therefore, FDA

uses a statistical confidence interval to provide an estimated range that is likely to contain the mean if the drug were given to the entire population. In our example study with a mean ratio of 99%, the confidence interval might be 89–109%. This confidence interval shows that for the entire population, the ratio of the mean AUC between test and reference products is likely (with a 90% probability) to be between 90 and 118%. If the small study used a greater number of subjects to more accurately reflect the general population's results, then the 90% confidence interval would be smaller (i.e., a smaller range of the possible pharmacokinetic values in the general population, such as 93–105%).

FDA determines whether a study shows that two products are bioequivalent based on the confidence interval and not on the mean value of the study. The results of a study are expressed as a confidence interval for the ratio of test to reference products. To decide whether two products are bioequivalent, the calculated confidence interval is compared to an acceptance interval. The acceptance interval (also referred to as acceptance limits) is expressed as two numbers that provide upper and lower limits on the confidence interval. If the confidence interval is contained within this acceptance interval, then FDA concludes that the study demonstrates BE; if not, then the study does not demonstrate BE. The acceptance interval is a fixed standard, while the confidence interval is determined from the data in a particular study.

FDA considers two drug products equivalent when the 90% confidence intervals of the geometric mean ratio for C_{max} and AUC are entirely within 80–125% (see Fig. 8.2). The choice of the 80–125% acceptance limits is based on medical opinion and FDA experience which determined that a difference of 20% or less in drug exposure was not clinically significant for most drugs. Thus, the limits of 80–125% were set around a difference of less than 20% in geometric mean ratio (test/reference) for C_{max} and AUC, although in practice this difference rarely

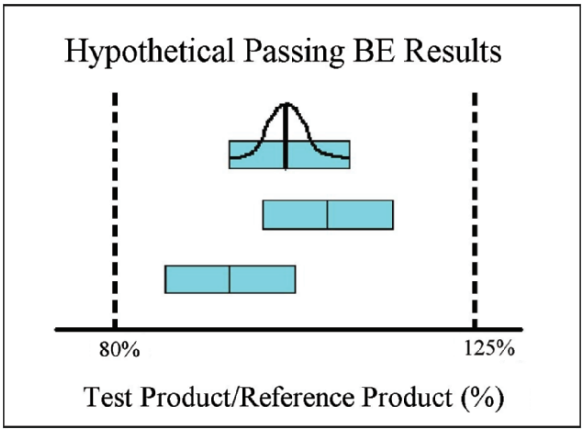

FIGURE 8.2. Hypothetical results from bioequivalence (BE) tests for approved generic drugs

exceeds 10%. FDA has not found any clinical problems resulting from the thousands of drug products approved with the current BE criteria.

As mentioned above, the 80–125% boundaries are acceptance limits for the confidence interval and not a judgment about the acceptable mean differences between test and reference products. The sample mean ratio of the pharmacokinetic values for the test and reference products lie at the center of the confidence interval. Because this confidence interval must fall within the 80–125% boundaries, these statistical criteria limit the acceptable range in which the mean values can stray from the 100% ratio. The actual mean differences FDA found for drugs tested and analyzed under this statistical procedure were much smaller than the 80–125% boundaries. In the 1980s, FDA reviewed 224 BE studies that passed the 80–125% criterion (Nightingale and Morrison, 1987). In these studies, the observed mean difference in AUC between the brand name and the generic product was approximately 3.5%. This analysis was repeated for the 127 BE studies conducted for generic drugs approved in 1997 (Henney, 1997). The average observed difference in AUC in these studies was approximately 3.3%. Recently, FDA surveyed the BE data for the 10-year period of 1996–2005. Results from more than 1,500 BE studies were analyzed. Once again, the mean difference in AUCs between generic products and their brand name counterparts averaged less than 4%.

Figure 8.2 graphically illustrates the relationship between the mean value obtained from a BE study, the 90% confidence interval for that BE study, and

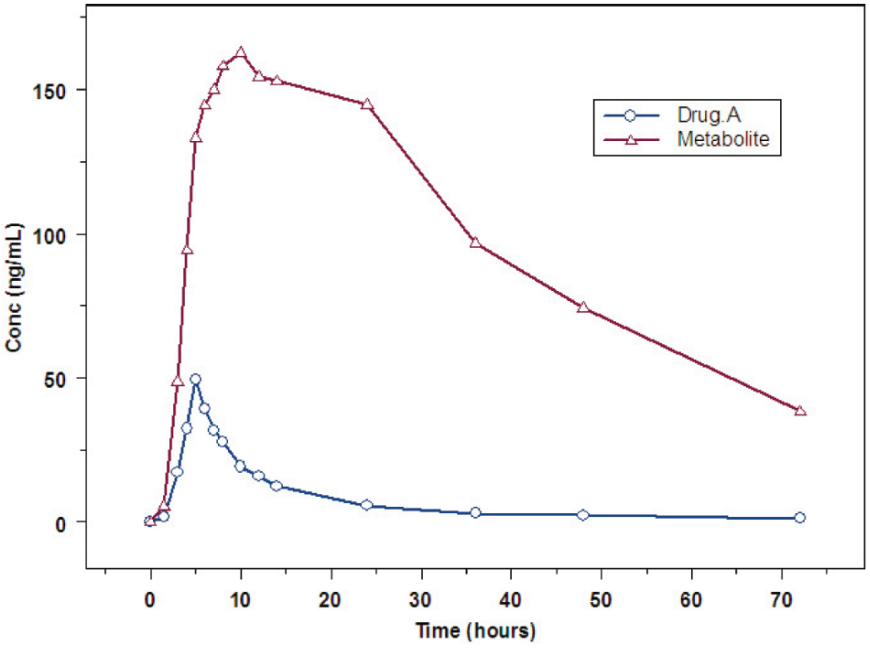

FIGURE 8.3. Plasma concentrations over time for parent drug and major metabolite, for a drug (Drug A) with extensive presystemic metabolism

FDA's acceptance limits of 80–125%. The center of each box is the mean value from a BE study, while the entire box represents the confidence interval from the same BE study. Because the 80–125% acceptance limits are bounds on the confidence intervals, the mean values from passing BE studies must be closer to 100%. As can be seen in Fig. 8.2, the actual mean differences between test and reference listed products will be much smaller than FDA's BE acceptance limits of 80–125%.

8.4 Bioequivalence: Challenging Topics

Some situations where the evaluation of BE presents a challenge include drugs with active metabolites, enantiomers, endogenous substances, and highly variable drugs. Each of these conditions will be discussed in some detail below.

8.4.1 Drugs with Active Metabolites

Following administration, drugs generally undergo biotransformation or metabolism to facilitate their elimination from the body. Metabolism can be systemic or presystemic. In systemic metabolism, drug in circulating blood is exposed to metabolic enzymes as it passes through the liver and other tissue. Presystemic metabolism occurs when the drug is exposed to metabolic enzymes found in the gut wall, skin, or other absorption sites. Additionally, presystemic metabolism occurs when the drug is metabolized by the liver immediately after oral absorption, prior to reaching the site of action (hepatic first pass effect). The relevance of this distinction to BE is that systemic metabolism is determined by the concentration of drug in the systemic circulation, while the presystemic metabolism can be affected by the rate and extent of the release of the absorption of the drug and its rate of release from the drug product.

The biotransformation of drugs can lead to the formation of compounds (metabolites) which may be active or inactive pharmacologically. The metabolites' activity may impact the efficacy of the drug or its side effects. The guidelines for using metabolites in BA or BE studies differ depending on the circumstances, i.e., whether the studies are part of a drug development program supporting an NDA, or they are BE studies supporting an ANDA for a generic product.

During drug development (investigational new drug (IND) or NDA), the objective is to learn as much as possible about a new compound that may become a marketed drug product. Thus, the recommendations for BA studies include measurement of the parent drug and all active metabolites. Determination of the activity of each metabolite relative to the parent is also desired.

On the other hand, by the time a patent expires on a drug and it becomes a candidate for generic competition, much is known about the drug's attributes and clinical performance. As a consequence, the objectives of a BE study supporting an ANDA are different from those for an NDA. For example, the goal of a BE study in the ANDA is to evaluate formulation performance of a generic candidate

relative to that of the RLD. Given that the concentration–time profile of the parent drug is generally more sensitive to differences in formulation performance, it is recommended that only concentrations of parent drug released from the dosage form be measured. This is true even if the drug has active metabolites.

The BA/BE Guidance (FDA, 2003a), however, does provide situations where metabolites should be measured in a BE study. These are quoted below:

1. Measurement of a metabolite may be preferred when parent drug levels are too low to allow reliable analytical measurement in blood, plasma, or serum for an adequate length of time. We recommend that the metabolite data obtained from these studies be subject to a confidence interval approach for BE demonstration. If there is a clinical concern related to efficacy or safety for the parent drug, we also recommend that sponsors and/or applicants contact the appropriate review division to determine whether the parent drug should be measured and analyzed statistically.
2. A metabolite may be formed as a result of gut wall or other presystemic metabolism. If the metabolite contributes meaningfully to safety and/or efficacy, we also recommend that the metabolite and the parent drug be measured. When the relative activity of the metabolite is low and does not contribute meaningfully to safety and/or efficacy, it does not have to be measured. We recommend that the parent drug measured in these BE studies be analyzed using a confidence interval approach. The metabolite data can be used to provide supportive evidence of comparable therapeutic outcome.

In the first case, a metabolite is measured in a BE study when levels of the parent drug are too low for accurate measurement, as is the case with some prodrugs. The statistical criteria for BE determination is applied to the metabolite(s) in this case. For example, the 90% confidence interval of the geometric mean ratio of test/reference AUC and C_{max} of the metabolite must fall within 80–125%. This may be the only situation where the confidence interval approach is used with the metabolite for the demonstration of bioequivalence.

In the second case, measurement of the active metabolite is recommended when there is evidence of hepatic first pass metabolism and/or gut presystemic formation of the metabolite. The active metabolite must also contribute significantly to the efficacy and/or safety profile of the drug. Unlike BE studies involving prodrugs, metabolite concentrations in this case are not subject to the BE statistical criteria (confidence interval approach), but summary statistics of the PK parameters serve as supporting evidence of bioequivalence. The parent drug, however, is evaluated statistically using confidence intervals as it would be in studies that do not include measurement of the metabolite(s).

To apply the second case to a particular drug product, there must be presystemic metabolism. One indicator suggesting presystemic metabolism is the early appearance of metabolite levels in the plasma, usually preceding parent drug levels. Plasma levels of the metabolite may also be significantly higher, relative to the parent. Figure 8.3 illustrates the early appearance, as well as higher levels, of

a major metabolite relative to that of the parent, for a sample drug (Drug "A") that has significant presystemic metabolism.

8.4.2 Enantiomers vs. Racemates

Enantiomers are stereoisomers, i.e., molecules that are nonsuperimposable mirror images of each other, with identical chemical and physical properties (Wade, 2003). A mixture of equal parts of an optically active isomer and its enantiomer is called a "racemate."

Enantiomers may differ in pharmacological activity and pharmacokinetic properties. This may be related to their three-dimensional fit within cell receptors or enzymes, leading to possible differences in pharmacological responses and potencies, as well as differences in absorption and clearance.

It is generally recommended that chiral assays (which can distinguish individual enantiomers) be used in BA studies during drug development. As stated above, enantiomers could potentially have different pharmacokinetics and pharmacological activities. This recommendation, however, does not extend to BE studies supporting ANDAs. The reason is, racemate levels of the parent drug, or in some cases the metabolite, may be adequate at detecting differences in formulation performance. There are exceptions, however, to this general rule.

The BA/BE Guidance lists four conditions, all of which have to be met, before measurement of the individual enantiomers are needed in BE studies. They are (1) the enantiomers exhibit different pharmacodynamic properties, (2) the enantiomers exhibit different pharmacokinetic properties, (3) the primary safety and efficacy resides in the minor enantiomer (one with the lowest concentration), and (4) there is evidence of nonlinear absorption for at least one of the enantiomers. In rare cases where a drug product meets all of the above four conditions, then a chiral assay is recommended to measure the concentration of individual enantiomers in a BE study supporting an ANDA.

8.4.3 Endogenous Substances

Drug products whose active ingredient is an endogenous substance (one that naturally occurs in the body) present a challenge to evaluating bioequivalence because a measurement of plasma concentration would include both the endogenous concentration plus the amount added by administration of the drug product (exogenous source). This may act to bias the results of a BE study.

To illustrate the potential error we use a model drug where B is the baseline level, R is the change in drug level above the baseline due to the reference product, T is the change in drug level above the baseline due to the test product. The apparent test to reference ratio is

$$\frac{B+T}{B+R}, \tag{8.3}$$

which should be compared to the true test to reference ratio of T/R. To estimate the error in this case let $B = 200$, $R = 200$, $T = 150$, and $T/R = 0.75$. The apparent ratio (unadjusted for baseline) is 0.875 compared to a true ratio (adjusted for baseline) of 0.75. This is a significant reduction in the ability to identify product differences.

One approach that has been successful for some drug products is a baseline correction method. In this approach, the measured predose concentration of the endogenous substance is subtracted from the measured concentration profiles after administration of the drug product. The remaining concentration should better reflect the amount delivered by the drug product.

When using this approach it should be noted that production of many endogenous substances is under feedback control and may be altered by the administered dose and thus there may be a nonlinear dependence on the external dose. Potassium chloride is an example drug for which the feedback control of the endogenous drug concentration is effective to the extent that there is no significant change in potassium concentration after administration of normal doses. For this drug, FDA recommends a urinary recovery study (FDA, 2002).

8.4.4 Highly Variable Drugs

The BE of highly variable drugs and drug products has been discussed in many conferences and meetings, nationally and internationally (Blume and Midha, 1993; Shah *et al.*, 1996). Highly variable drugs are generally defined as drugs or drug products which exhibit within subject variability of 30% or greater.

Drugs with high within subject variability can present challenges in BE studies because of impact on sample size. For example, when comparing the BA of a highly variable reference product with itself, the sample size needed to demonstrate BE can exceed 100 subjects, although there are no true differences in BA between test and reference in this case. Table 8.1 (adapted from Patterson *et al.*, 2001) provides more precise information about the association between within subject variability and sample size needed to achieve adequate statistical power.

Evaluating the BE of highly variable drugs using the standard criteria may present ethical concerns, i.e., unnecessary human testing, in addition to the practical difficulties of large BE studies. For this reason, the FDA has been evaluating different approaches for determining BE that would decrease sample size, without increasing patient risk. Several papers have been published on this topic. Some of the methods studied are summarized below.

8.4.4.1 Static Expansion of the BE Limits

Sample size in BE studies is generally determined by the BA parameter with the highest within subject variability. In most cases, this parameter is $C_{\max}$. The greater variability observed with $C_{\max}$ may result from the fact that this parameter is a single point measurement, which is highly dependent on the sampling time/frequency and the elimination rate of the drug. Arbitrary widening of the BE

TABLE 8.1. Sample sizes providing 90% power in two-way, cross over bioequivalence studies

CV_w%	% difference in true BA ratio	Number of subjects needed
30	0	40
	5	54
	10	112
45	0	84
	5	112
	10	230
60	0	140
	5	184
	10	384
75	0	200
	5	264
	10	554

CV_w%, within subject coefficient of variation; BA, bioavailability

limits for C_{max} has been proposed as one approach to reduce sample size when evaluating the BE of highly variable drugs. This entails increasing the 90% confidence interval limits for C_{max} from 80–125% (current FDA criterion) to 75–133%, or even 70–143%.

8.4.4.2 Expansion of Bioequivalence Limits Based on Fixed Sample Size

The basis of this approach is the belief that only a reasonable number of subjects should be used in BE studies (Boddy *et al.*, 1995). To conduct a study with the above method, a fixed number of subjects, e.g., 24, is used in a standard two-period, cross-over design comparing the reference product with itself. For a highly variable drug, this study is likely to fail the 80–125% criteria, because of low power with only 24 subjects. However, the 90% confidence interval obtained from the reference product would become the new "goalpost," or criteria, for subsequent studies comparing a test product (proposed generic) with the reference product (RLD), using the same number of subjects (24).

According to Boddy *et al.* (1995), a drawback of this method is that the wider acceptance limits are based on controlling the sample size, instead of a meaningful measure of formulation differences (Boddy *et al.*, 1995). Additionally, when the test product differs from the RLD by a small measure, there is no guarantee that the confidence interval for the test vs. reference product will fall within the "goalposts" set by the first study, where the reference is compared with itself.

8.4.4.3 Scaled Average Bioequivalence

Finally, a third proposed approach that is currently favored by the FDA is scaled average BE. This method entails widening of the BE limits as a function of the within subject variability of the reference product. In this case, instead of using fixed limits, i.e., 80–125%, to determine if a test product is BE to the reference product, the limits expand as within subject variability of the reference increases.

The variability of the reference is determined in a replicate or partial replicate design, where the reference product is administered twice to the same subject at different periods. Mathematically, this approach may be described by

$$\text{BE limits, upper, lower} = \exp\left(\pm\frac{0.223}{\sigma_{w0}}\sigma_{wr}\right), \tag{8.4}$$

where σ_{wr} is the within subject standard deviation of the reference, and σ_{w0} is a constant set by the regulatory agency.

One concern about the use of average scaling for BE purposes is the lack of sensitivity of this method to differences in the point estimate, or the test/reference ratio of the geometric means. At least in theory, the lack of sensitivity may lead two products with unacceptably large differences in formulation performance to be declared BE. For this reason, the FDA has proposed the use of point estimate constraints in conjunction with scaled average BE. For example, if a BE study passes the confidence interval criteria (scaling), but the mean ratio between test and reference exceeds a predefined limit (e.g., ± 20), then the two products may not be judged BE for regulatory purposes.

In a simulation-based study conducted at the FDA, the impact of scaled average BE on power was evaluated, and compared to the power of average BE (traditional criteria). Using a sample size of 36 subjects, one million studies were simulated for each variable tested. As can be seen in Fig. 8.4, the scaled average BE can have a significant impact on power even when the point estimate constraint is applied. For example, at within subject variability of 60%, the power of the study using average BE is about 24%, when the test and reference have zero differences in BA. Applying scaled average BE with point estimate constraint under the same test conditions, the power increases to $>90\%$. Thus, scaled average BE appears to have a great practical advantage over traditional BE methods, when it comes to highly variable drugs.

8.5 Biowaivers

For some drug products, there is sufficient understanding of the physicochemical properties and biological factors that affect BA that there is no need for *in vivo* BE studies. Sponsors may request waivers of BE studies (biowaivers) for solutions, products with a range of strengths, and biopharmaceutical classification system (BCS) Class 1 drugs.

8.5.1 Solutions

In vivo BA/BE is self-evident for certain drug products, such as topical solutions, solution nasal spray, oral solutions, elixirs, syrups, tinctures, or other solubilized forms of the drug. For these products, *in vivo* BA/BE can be waived, according to 21 CFR 320.22(b).

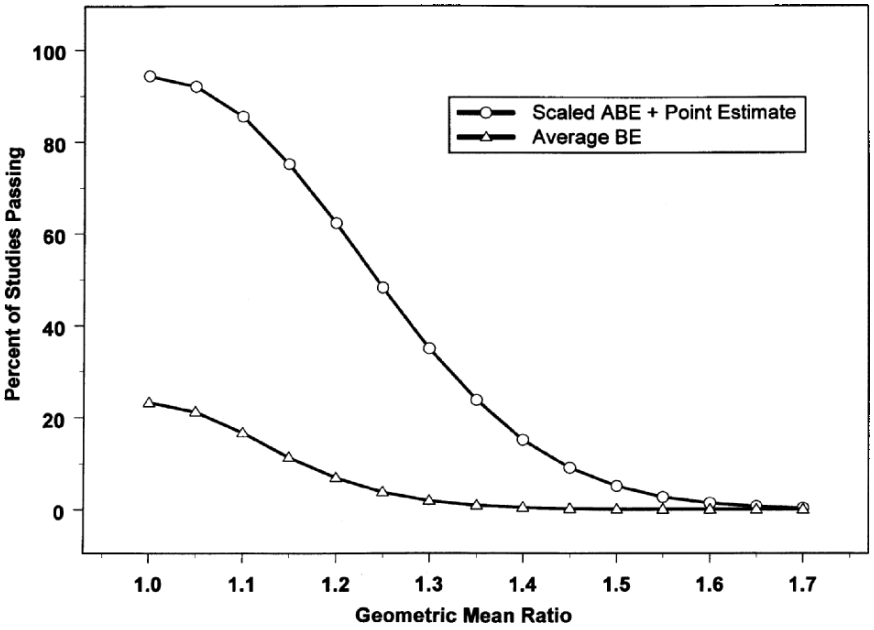

FIGURE 8.4. The difference in power, or percent of studies passing BE, between scaled average BE with a point estimate constraint and average BE (traditional criteria). A million BE studies with a sample size of 36 subjects were simulated. The geometric mean ratio reflects differences between the test and reference products

This waiver assumes that release of the drug substance from the drug product is self-evident and that the solutions do not contain any excipient that significantly affects drug absorption (21 CFR 320.22(b)(3)(iii)). The FDA can deny a biowaiver request if differences in excipients have the potential to change BE. For example, xylitol, sorbitol, and mannitol are commonly used formulation excipients for drug products (Fassihi *et al.*, 1991; Fukahori *et al.*, 1998). They are also used as artificial sweeteners in the food industry. These excipients are not well absorbed in the gastrointestinal (GI) tract. Additionally, they increase the osmotic pressure in the intestine, which changes the flux of water in the GI tract. This osmotic stress can change the gastric emptying time and the intestinal transit times through both the upper and lower parts of the intestine. Transit times in the GI can impact drug absorption. The total amount of drug absorbed depends on the rate of absorption from the intestine and the total time that the drug is present in the intestine.

When transit or emptying times are decreased, there is less time available for drug molecules in solution to be absorbed and thus, the total absorption may be decreased. Scintigraphic evidence suggests that osmotic agents can have minor effects on the residence time in the upper intestinal tract, but significantly reduce the residence time in the lower intestinal tract (Adkin *et al.*, 1995; Kruger *et al.*, 1992). The osmotic pressure changes may also affect the rate of transport across

the intestinal wall in addition to changing transit times, which could lead to changes in absorption of low permeability drugs (Polli *et al.*, 2004). As an example of this effect, Chen *et al.* (2007) measured the pharmacokinetics of ranitidine in a four way cross-over study of ranitidine oral solution dosed with various amounts of sorbitol (Chen *et al.*, 2007). Doses of sorbitol greater than 1.25 g significantly reduced the BA of ranitidine from an oral solution.

Another example of a pharmaceutical excipient with a demonstrated effect on drug absorption is polyethylene glycol 400 (PEG 400). Several studies investigated the effect of PEG 400 on the absorption characteristics of ranitidine from the gastrointestinal tract (Basit *et al.*, 2002; Schulze *et al.*, 2003). These studies show that there is no significant effect of PEG 400 on gastric emptying; however, the presence of PEG 400 reduced the mean small intestinal transit times of the ranitidine solutions containing PEG 400. This resulted in changes in drug absorption that depended upon the amount of PEG 400. Low concentrations of PEG 400 increased the absorption of ranitidine, presumably due to changes in intestinal permeability of ranitidine, whereas high concentrations of PEG 400 reduced ranitidine absorption possibly due to shorter small intestinal transit time.

8.5.2 Lower Strength

Waiver of *in vivo* studies for different strengths of a drug product can be granted under §320.22(d)(2) when (1) the drug product is in the same dosage form, but in a different strength; (2) this different strength is *proportionally similar* in its active and inactive ingredients to the strength of the product for which the same manufacturer has conducted an appropriate *in vivo* study; and (3) the new strength meets an appropriate *in vitro* dissolution test. The FDA guidance (FDA, 2003a) defines *proportionally similar* in the following ways:

1. All active and inactive ingredients are in exactly the same proportion between different strengths (e.g., a tablet of 50-mg strength has all the inactive ingredients, exactly half that of a tablet of 100-mg strength, and twice that of a tablet of 25-mg strength).
2. Active and inactive ingredients are not in exactly the same proportion between different strengths as stated above, but the ratios of inactive ingredients to total weight of the dosage form are within the limits defined by the SUPAC-IR (FDA, 1995) and SUPAC-MR (FDA, 1997a) guidances up to and including Level II changes.
3. For high potency drug substances, where the amount of the active drug substance in the dosage form is relatively low, the total weight of the dosage form remains nearly the same for all strengths (within $\pm 10\%$ of the total weight of the strength on which a biostudy was performed), the same inactive ingredients are used for all strengths, and the change in any strength is obtained by altering the amount of the active ingredients and one or more of the inactive ingredients. The changes in the inactive ingredients are within the limits defined by the SUPAC-IR (FDA, 1995) and SUPAC-MR (FDA, 1997a) guidances up to and including Level II changes.

8.5.3 *Biopharmaceutical Classification System*

The BCS is a scientific framework for classifying a drug substance based on its aqueous solubility and intestinal permeability (Amidon *et al.*, 1995). The solubility classification of a drug in the BCS is based on the highest dose strength in an IR product. A drug substance is considered highly soluble when the highest strength is soluble in 250 ml or less of aqueous media over the pH range of 1.0–7.5. Otherwise, the drug substance is considered poorly soluble. The volume estimate of 250 ml is derived from typical BE study protocols that prescribe administration of a drug product to fasting human volunteers with a glass (about 8 oz) of water.

The permeability classification is based on the extent of intestinal absorption of a drug substance in humans. The BCS guidance indicates for permeability that "In the absence of evidence suggesting instability in the gastrointestinal tract, a drug substance is considered to be highly permeable when gastrointestinal absorption in humans is determined to be 90% or more of an administered dose based on a mass balance determination or in comparison to an intravenous reference dose." The BCS guidance also provides for high permeability to be determined in *in-vitro* epithelial cell culture studies using suitable high and low permeability reference standards. Otherwise, the drug substance is considered to be poorly permeable. Solubility and permeability classifications result in four classes of drug substance:

BCS Class 1: highly soluble and highly permeable
BCS Class 2: poorly soluble and highly permeable
BCS Class 3: highly soluble and poorly permeable
BCS Class 4: poorly soluble and poorly permeable

An IR drug product is considered rapidly dissolving when not less than 85% of the labeled amount of the drug substance dissolves within 30 min using USP Apparatus I at 100 rpm or USP Apparatus II at 50 rpm in a volume of 900 ml or less of each of the following media: (a) acidic media, such as 0.1N HCl or USP simulated gastric fluid without enzymes (SGF); (b) a pH 4.5 buffer; and (c) a pH 6.8 buffer or USP simulated intestinal fluid without enzymes (SIF). Otherwise, the drug product is considered to be a slow dissolution product. When combined with the *in vitro* dissolution characteristics of the drug product, the BCS takes into account three major factors: solubility, intestinal permeability, and dissolution rate. These factors govern the rate and extent of oral drug absorption from IR solid oral dosage forms (FDA, 2001a).

The FDA BCS guidance indicates that sponsors of NDAs and ANDAs may request biowaivers for highly soluble and highly permeable drug substances (Class 1) formulated in IR solid oral dosage forms that exhibit rapid and similar *in vitro* dissolution. Rapid and similar dissolution is demonstrated by

1. Both drug products do not have less than 85% dissolution in 30 min in 900 ml at pHs of 1.2, 4.5, and 6.8

2. Similarity is demonstrated by an f2 comparison in all three pH conditions (the f2 test is not necessary if both products have 85% dissolution in 15 min or less).

Other conditions that should be met to qualify for a biowaiver are (a) the drug must be stable in the gastrointestinal tract, (b) excipients used in the IR solid oral dosage forms have no significant effect on the rate and extent of oral drug absorption, (c) the drug must not have a narrow therapeutic index, and (d) the product is designed not to be absorbed in the oral cavity.

The BCS guidance is generally considered to be conservative with respect to the class boundaries of solubility and permeability, and the dissolution criteria. Thus, the possibility of modifying these boundaries and criteria to allow waivers of *in vivo* BE studies, i.e., biowaivers, for additional drug products has received increasing attention. There are possible opportunities to expand the BCS-based biowaivers to drugs that are not BCS Class 1.

8.5.3.1 Biowaivers for BCS Class 2 Drugs with pH Dependent Solubility

Some drugs (weak bases) are classified as BCS Class 2 because they are highly soluble at low pH, but fail to meet the BCS solubility limit at higher pH. For these BCS Class 2 drugs, their absorption is complete before they reach a pH where their solubility is decreased significantly. For other BCS Class 2 drugs (weak acids), limited solubility at low pH (acid) may not be physiologically relevant and the solubility at higher pH (e.g., pH $>$ 5) is more appropriate. This may be true because most drugs are absorbed in the intestinal region (Yazdanian *et al.*, 2004; Rinaki *et al.*, 2004). It was questioned by Polli *et al.* (2004) that a solubility of the highest strength in 250 ml over the range pH 1–7.5 is conservative and solubility may need to be conducted only between pH 4.5 and 6.8 considering the pH range of the small intestine. Yu *et al.* (2002) suggest a potential intermediate solubility class for drugs that are soluble either in the stomach or in the intestine, because BCS Class 2 drugs would be absorbed in the intestine due to high permeability as long as the drugs are dissolved before or at the time when they reach the absorbing region of the intestine.

8.5.3.2 Biowaivers for BCS Class 3 Drugs

For rapidly dissolving dosage forms of BCS Class 3 drugs (high solubility, low permeability), intestinal permeability is considered to be the major rate-controlling step in oral drug absorption. Thus, these rapidly dissolving BCS Class 3 drug products are expected to behave like an oral solution. Drug dissolution and other formulation differences are unlikely to have effect on the rate and extent of drug absorption, as long as excipients do not alter intestinal permeability or intestinal residence time of the drug. To ensure rapid dissolution *in vivo* using *in vitro* dissolution, Yu *et al.* (2002) and Polli *et al.* (2004) suggest a more rapid *in vitro* dissolution criterion (not less than 85% within 15 min), because the sink condition common in *in-vitro* dissolution may not exist *in vivo*.

8.6 Locally Acting Drugs

Locally acting drug products require exploration of alternative bioequivalence methods because plasma concentration profiles of these products are not always appropriate surrogates of pharmacological activity. Examples of locally acting products include topical dermatological products, inhalation and nasal products. For many of these products, FDA recommends a BE study with clinical endpoints.

A BE study with clinical endpoints will use a product-specific clinical indication recommended by FDA. Patients in the study would be given the test product, the reference product, and/or a placebo. The placebo arm ensures that the study and its conduct are sufficiently sensitive to differences between treatments. If the reference product is labeled for multiple indications, then the indication that is most sensitive to difference in local delivery of drug is usually preferred.

Most clinical endpoint BE studies have a dichotomous endpoint; the treatment either succeeds or fails. To decide if the test product is bioequivalent to the reference, the success proportion for each treatment is calculated, and if the 90% confidence interval for the difference in success is within -20% to $+20\%$, then the test product passes. For dichotomous endpoints, there is no meaning to between-subject variability and all studies must enroll approximately 200–600 subjects to ensure sufficient power.

Some clinical endpoints are continuous variables or can be treated as such. For example, a reduction in a symptom score is a categorical endpoint, but for equivalence purposes may be treated as continuous data if certain assumptions are made. For these studies, the 90% confidence interval of test/reference ratio must be within 80–125%. The number of subjects required will depend on the between subject variability of the particular clinical endpoint.

Because clinical endpoint BE studies can be larger than studies conducted in support of the initial NDA and the insensitivity of some clinical endpoints to formulation differences, there is much interest in developing new BE methods that are more efficient and more sensitive at detecting product differences. A recent addition to the Federal Food Drug and Cosmetic Act at Section 505(j)(8)(A)(ii) indicates that "For a drug that is not intended to be absorbed into the bloodstream, the Secretary may assess bioavailability by scientifically valid measurements intended to reflect the rate and extent to which the active ingredient or therapeutic ingredient becomes available at the site of drug action." In the sections below, we will identify some alternative approaches to BE that have been employed, and point out some scientific challenges in developing new BE methods for locally acting products.

8.6.1 Topical Dermatological Products

Topical dermatological products are intended to treat conditions of the skin by direct application of the drug product to the skin. The skin consists of multiple layers beginning with the approximately 10 μm thick stratum corneum, the ≈100 μm thick living epidermis, and the ≈1,000 μm thick dermis. Depending on the drug,

topical dermatological products can act at any of these layers, but because the drug is applied to the surface of the skin it must diffuse across each layer sequentially. Thus drug can reach at sites of action without ever being systemically absorbed. This definition excludes products such as transdermal delivery systems that are intended to deliver drug systemically.

There are a variety of BE approaches that are or can be used for topical dermatological products:

1. Biowaivers (topical solutions)

This is discussed in the section on biowaivers. For topical products, a biowaiver usually requires that the test and reference product contain equivalent amounts of the same inactive ingredients. This requirement is necessary because many excipients in topical products are penetration enhancers that can alter the permeability of the skin. Differences in excipients can also change how a topical product spreads or adheres to the skin, which can alter its efficacy.

2. *In vitro* tests (no current topical dermatological products qualify)

The SUPAC-SS (FDA, 1997b) guidance discusses the role of *in vitro* release testing for semisolid dosage forms. The guidance states that "An *in vitro* release rate can reflect the combined effect of several physical and chemical parameters, including solubility and particle size of the active ingredient and rheological properties of the dosage form. In most cases, *in vitro* release rate is a useful test to assess product sameness between pre-change and post-change products" (FDA, 1997b). The use of *in vitro* release tests is currently limited to evaluating changes in manufacturing process, or scale-up by the same manufacturer and is not used for BE. However, when the test and the reference product have identical compositions, the only differences are in manufacturing and process scale.

3. *In vivo* pharmacodynamic studies (topical corticosteroids)

For topical corticosteroids, there is an FDA guidance (1995b) that describes a pharmacodynamic BE study. As in all pharmacodynamic BE studies, it is necessary to establish sufficient sensitivity in the dose–response curve to detect differences between products. For the skin blanching study, the dose is varied by changing the amount of time the topical product is applied to the skin. A pilot study using a range of application times of the reference product is used to identify patients in whom skin blanching is sensitive to differences in application time. Then, these patients are used to compare the test and reference products.

4. *In vivo* pharmacokinetic studies (some topical anesthetics)

For some topical dermatological products such as lidocaine/prilocaine cream,[1] a pharmacokinetic BE study has been the only recommended study. For other topical products, a pharmacokinetic study is requested in addition to other studies

[1] Publicly available at http://www.fda.gov/cder/foi/nda/2003/076453.pdf

TABLE 8.2. Examples of study results[a] using a clinical endpoint to demonstrate BE

N	% cure test	% cure ref	90% CI
728	50	48	[−12, +16]
453	46	40	[−8, +20]
447	29	27	[−9, +13]

[a] All of these studies had three arms and used the difference in cure rate as the endpoint with an acceptable 90% confidence interval of −20 to +20 percentage points

to evaluate whether the test and reference products provide equivalent systemic exposure. This additional BE study is usually requested because there are known safety issues related to systemic exposure.

5. Clinical endpoint BE studies (most other products)

As mentioned before, most other topical products establish BE through a clinical endpoint study. Table 8.2 provides some example results of clinical studies used in support of ANDAs (Lionberger, 2004). All of these studies had three arms and used the difference in cure rate as the endpoint with an acceptable 90% confidence interval of −20 to +20 percentage points. The results show that even with relatively large numbers of patients in each study, the confidence intervals were close to the limits defined by FDA. This suggests that these studies were at a high risk of failure.

8.6.2 Locally Acting Nasal and Oral Inhalation Drug Products

Locally acting nasal and inhalation drug products present significant BE challenges that have limited generic competition in these product categories. FDA has published a draft BE guidance for nasal spray products (FDA, 2003c) and approved one suspension nasal spray product following the publication of this guidance. Currently (2006), there is no BE guidance for inhalation products such as metered dose inhalers (MDI) and dry powder inhalers (DPI).

Given that the performance of these products is determined by the properties of both the formulation and the delivery device, the general approach to BE includes demonstration of:

1. Qualitatively the same, and quantitatively essentially the same, formulations
2. Container and closure systems that are as close as possible in critical attributes
3. Equivalent drug product performance (through *in vitro* tests)
4. Equivalent local delivery (through clinical bioequivalence or pharmacodynamic studies)
5. Equivalent systemic exposure (through a pharmacokinetic study)

8.6.2.1 Nasal Spray Products

For nasal spray solutions that are qualitatively and quantitatively the same as the reference product, and for which the container and closure system are as close as possible in critical attributes including metering chamber volume, the local delivery, and systemic exposure tests can be waived and BE can be demonstrated through equivalent *in vitro* drug product performance. Nasal spray suspension must demonstrate equivalence in three categories listed above (local delivery, systemic exposure, and device performance).

There are six measurable properties identified for use in comparing the drug product-device performance of nasal spray products:

1. Single actuation content (SAC) through container (product) life
2. Droplet size distribution by laser diffraction
3. Drug in small particles/droplets, or particle/droplet size distribution by cascade impactor
4. Spray pattern
5. Plume geometry
6. Priming and repriming

The tests require the use of 10 units from each of three lots of test and three lots of reference products. The FDA guidance (FDA, 2003c) did not specify the criteria for establishing equivalence. Prior to August 2005, FDA had evaluated *in vitro* studies on nasal sprays based on the ratios of geometric means of the test and reference products falling between acceptance limits of 90–111% and evidence for comparable variability of the test and reference products. Since 2005, FDA has been using population bioequivalence (PBE) to compare test and reference products. Information regarding the PBE methodology has been posted on the Agency Web site since April 11, 2003. Inherent in the PBE method is the principle that the BE acceptance limits depend upon the relative variability of the test and reference products observed in the study. This ensures that the acceptance limits are appropriate for the specific products being compared and are based on the characteristics of the approved RLD. In the case of low variability data for the reference product, the acceptance limits narrow toward the 90–111% criteria used in the previous geometric mean method, enabling only test products with comparable variability to meet the acceptance criteria. Conversely, in the case of a high variability reference product, the acceptance limits might be wider.

Usually local delivery BE studies of inhalation products require either pharmacodynamic or clinical studies with a demonstrated dose–response. However, nasal sprays for treatment of seasonal allergic rhinitis (SAR), perennial allergic rhinitis (PAR), and perennial nonallergic rhinitis (PNAR) indications have very limited dose–response. For these indications, equivalent local delivery is assumed to occur when products meet the formulation and device recommendations listed above, and when drug-device performance demonstrates equivalence by showing similar effectiveness in a comparative clinical trial of test and reference products. This clinical trial involves a large number of subjects, which may range from 500 to

1,000 patients. The lowest dose is usually recommended to increase the sensitivity of the study to potential differences between test and reference products, assuming an E_{max} model, as described later in this section. The test product demonstrates equivalent systemic exposure in a pharmacokinetic study in healthy subjects.

Because locally acting nasal spray products are not designed to deliver drug systemically, the conduct of the pharmacokinetic study can be challenging due to very low plasma concentrations. Significant analytical development effort may be required to develop a sensitive method to quantify the low plasma concentrations.

8.6.2.2 Oral Inhalation Products

BE of MDI and DPI products follow the same general approach recommended for nasal spray products (equivalent *in vitro* drug product performance, local delivery, and systemic exposure). As opposed to solution formulation nasal sprays for which *in vivo* studies are not deemed necessary to establish BE, *in vivo* studies are recommended for both solution and suspension MDIs, as well as DPIs.

For the drug product performance tests, a complete list of required tests is not available. However, the critical product quality attributes of MDI and DPI products have been discussed in the scientific literature and in product labeling.

Almost all DPIs currently approved in the United States are breath-actuated so patients' aspiratory effort provides energy for delivery of the powder formulation. There is population variation in the ability of asthma patients to induce flow through the device (Frijlink and De Boer, 2004). A generic sponsor should try to match the performance of the reference product. Localization of delivery in the lung is clearly related to the distribution of particle sizes emitted from the device. *In vitro* tests utilizing a cascade impactor can measure the aerodynamic particle size distribution in a way that is related to deposition in the lung, although it may not be predictive of *in vivo* deposition (Mitchell and Nagel, 2003).

All of these aspects of product performance are affected by the properties of the particles in the formulation. Part of the challenge in designing a DPI is that performance also depends on the device used and the interactions of the particles in the formulation with the device. The aerosol produced by a given patient will depend on the design of the device (its resistance to airflow). The patient effort results in a velocity gradient applied to the dry powder formulation. The effect of a specific velocity gradient on a powder formulation (how much aerosol will be created) will depend on the particle properties. Thus, manufacturing of the powders for DPI may involve critical process parameters to control particle size and shape and surface properties (Telko and Hickey, 2005).

Local delivery studies in asthma patients are challenging because many asthma drugs have a very shallow dose–response curve (the difference between one puff and two puffs may not be clinically detectable) and high between-subject variability, which requires a very large number of subjects to be used in a PD equivalence test. This is particularly true of dose–response for inhaled corticosteroids, as described below. For the nasal spray products, the establishment of dose–response in the clinical endpoint was not required, but because of the more complex nature

of particle delivery to the lung, FDA considers establishing the dose sensitivity of the local delivery study of inhalation products essential to establishment of equivalence.

Equivalence of local delivery for a test orally inhaled product relative to the reference orally inhaled product may be evaluated using a "dose-scale" method. The relative BA is determined in terms of "delivered" dose of the test formulation required to produce a PD response of the same magnitude as exhibited by the reference formulation, and its calculation takes into consideration the within-study dose–response. In the dose-scale method, the PD response (E_R) to varying doses of the reference product is fit to an E_{max} model to determine the function, ϕ_R,

$$\phi_R = E_{0R} + \frac{E_{max\,R}\text{Dose}_R}{ED_{50R} + \text{Dose}_R}. \tag{8.5}$$

The relative BA "F" of a dose of the test product relative to that of the reference product can be calculated by applying the inverse of ϕ_R to the mean of the response data of the PD response (E_T) of test product:

$$F = \phi_R^{-1}(E_T)/\text{Dose}_T. \tag{8.6}$$

The Office of Generic Drugs (OGD) has previously used this approach for statistical evaluation of PD BE studies in ANDAs for albuterol MDIs. The applications contained studies based on bronchoprovocation model (PD measure: Histamine PC_{20}) or the bronchodilatation model (PD Measures: AUEC-FEV1 and $FEV1_{max}$).

The number of subjects needed to establish BE of a product using the dose-scale method is a function of the slope of the dose–response curve and the variability in the pharmacodynamic response. As the ratio of the variance relative to the slope decreases, fewer subjects are needed to determine the relative BA. BE using a histamine challenge study has been demonstrated for an albuterol inhalation aerosol with 24 subjects (Stewart *et al.*, 2000). The number of subjects required estimating relative BA of an inhaled corticosteroid (ICS) is large; however, estimates suggest that hundreds of subjects would be needed to establish BE for a parallel study design of an ICS. Traditionally, a cross-over study design could not be used for BE studies of ICS due to the long washout period required between treatments. In the literature, an asthma stability model has been suggested as a method for comparing local delivery of ICS using an FEV1 endpoint in a cross-over design. This is estimated to require many fewer subjects to meet the BE requirements (Ahrens *et al.*, 2001).

Another challenge to the design of local delivery studies is that several inhalation products contain two active ingredients, a short acting ICS and long acting beta-agonist. To demonstrate BE, local delivery of both components must be equivalent. However, the FEV1 endpoint used in the asthma stability model is affected by both components. Exhaled nitric oxide (eNO) has been suggested as a pharmacodynamic endpoint for ICS (Silkoff *et al.*, 2001), as eNO levels appear to be unaffected by concomitant administration of beta-agonists. This endpoint, combined with an FEV1 endpoint for the beta-agonist, may enable equivalence of both components to be established.

8.7 Conclusions

Evaluation of BE for systemically acting drugs using pharmacokinetics is well established. Unusual cases such as endogenous substances and highly variable drugs sometimes require new study designs and new statistical analysis procedures. The knowledge available about formulation development and formulation performance for oral dosage forms has allowed the FDA to determine that *in vitro* testing in some cases can provide adequate evidence of BE. Drug companies can now request waivers of *in vivo* BE studies (biowaivers) for some of their products, greatly reducing the cost of such studies. Opportunities for future expansion of biowaivers have been identified and discussed above.

Locally acting drugs are more complex in terms of BA/BE. An appropriate BE method often needs to be established based on a scientific analysis of each drug product. As illustrated in the case studies, all of the following types of studies have been used by FDA to evaluate bioequivalence of locally acting drugs:

1. Clinical endpoint BE study
2. Pharmacodynamic endpoint BE study
3. Pharmacokinetic BE study
4. *In vitro* BE study

The development of generic versions of topical dermatological, nasal, and inhalation products can be significantly impacted by the required BE testing. Development of new and more efficient methods for evaluating BE of locally acting drugs could lead to faster development of generic drugs and facilitate formulation and manufacturing changes.

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