

# Chapter 2

## Fundamentals of Bioequivalence

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### 2.1 Definition of Bioavailability and Bioequivalence

The US regulatory requirements for bioavailability (BA) and bioequivalence (BE) studies in drug applications originated from a report issued by the Congressional Office of Technology Assessment in 1974. Many recommendations in this report were adopted by the US Food and Drug Administration (FDA) and subsequently became the BA/BE regulations in 1977 (FDA 2013a). Statutory definitions for BA and BE are both expressed in terms of rate and extent of absorption, and thus they are interrelated to each other. Specifically, BA is defined in the regulations 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” (FDA 2013a). Similarly, BE is defined 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” (FDA 2013a). Both definitions describe the processes by which the drug substance is released from a dosage form followed by absorption and distribution to the site of action. As a result, similar approaches such as developing a systemic exposure profile by monitoring drug concentrations in plasma or serum over time have generally been applied to measure BA and demonstrate BE in drug applications.

The only difference between BA and BE definitions lies in the study goals, hence the study designs and statistical analysis of study outcome. BA studies can be employed to assess the pharmacokinetics and performance of a drug product related to the absorption, distribution, and elimination of the drug in vivo. In contrast, BE

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studies are primarily utilized for formulation comparisons, and thus data analysis focuses on the release of active ingredient (or moiety) from the drug product and subsequent absorption into the systemic circulation. Establishing BA is a benchmarking effort for drug products with a new molecular entity (NME), while demonstrating BE is a formal test that compares BA of various formulations with the same drug substance in the same dosage form, using specified criteria and acceptance limits for BE comparisons.

It is noteworthy that in the regulatory setting, BE can be established between drug products that are either pharmaceutical equivalents or pharmaceutical alternatives (Orange Book 2013). Drug products are considered as pharmaceutical equivalents when they are in identical dosage forms and contain identical amounts of the identical active drug ingredient. These products do not necessarily contain the same inactive ingredients (i.e., excipients) and they may differ in characteristics such as shape, scoring configuration, release mechanisms, packaging, expiration time, and within certain limits, labeling. In contrast, pharmaceutical alternatives contain identical therapeutic moiety (or its precursor) but not necessarily in the same amount or dosage form or as the same salt or ester. Based on the Drug Price Competition and Patent Term Restoration Act of 1984 (Hatch-Waxman Act), evidence of pharmaceutical equivalence and bioequivalence provides the assurance of therapeutic equivalence, hence interchangeability between a generic product and its innovator counterpart (Orange Book 2013).

## 2.2 Application of Bioavailability and Bioequivalence Studies

BA/BE information is deemed important in the drug development and for regulatory approval of pharmaceutical products (FDA 2003a). BA and/or BE studies are required in support of drug applications, including Investigational New Drug Applications (INDs), New Drug Applications (NDAs), Abbreviated New Drug Applications (ANDAs), and their amendments and supplements.

During the IND and NDA period, appropriately designed BA studies are necessary to assess performance of the drug product(s) used in clinical trials that provides evidence of safety and efficacy. As described earlier, BA studies can furnish pharmacokinetic information related to drug absorption, distribution, and elimination *in vivo*. BA studies can also be used to achieve many other objectives such as estimating fraction of dose absorbed from an orally administered drug product, providing information on dose proportionality and linearity in pharmacokinetics, and investigating the effect of various intrinsic/extrinsic factors on the pharmacokinetics of the drug under examination. For orally administered drug products with an NME, absolute BA is obtained by comparison to an intravenous dose, while relative BA can be accomplished by comparisons to an oral solution, oral suspension, or other formulation.

On the other hand, BE studies are often used as a bridging tool to support evidence for safety and efficacy between two drug products. During the IND and NDA period, BE studies can be utilized to provide links among formulations used in different phases of clinical trials, as well as to establish links between formulations used in stability studies and clinical trials. In addition, BE studies are critical to the approval of ANDAs. Manufacturers seeking approval to market a generic drug product must submit an ANDA, demonstrating that the drug product is both pharmaceutically equivalent and bioequivalent to the Reference Listed Drug (RLD, i.e., innovator product). Documentation of BE is also essential to ensure product quality throughout the shelf life of a drug product whenever changes occur in the manufacturing or formulation, which applies to both new and generic drug products. Depending on the level of changes, BE may be established through comparative in vivo or in vitro studies between products before and after change (FDA 2003a).

## 2.3 Approaches for Establishment of Bioequivalence

Based on the statutory definition of BE, several in vivo and in vitro methods can be employed for BE establishment. Nonetheless, the US FDA requires that drug applicants conduct BE testing using the most accurate, sensitive, and reproducible approach (FDA 2013b). Hence, in descending order of preference, the following methods have been recommended for BE documentation (FDA 2013b):

- (a) Comparative pharmacokinetic studies
- (b) Comparative pharmacodynamic studies
- (c) Comparative clinical trials
- (d) Comparative in vitro tests
- (e) Any other approach deemed adequate by FDA

Experiences thus far have revealed that comparative pharmacokinetic studies are mostly used for BE demonstration of systemically absorbed drug products while pharmacodynamic studies and clinical trials are generally employed for locally acting drug products. Historically, in vitro tests alone are rarely utilized for the purpose of BE establishment. However, with the recent advances in modern science and technology, comparative in vitro studies have started to take on an added importance for BE demonstration of certain drug products (see Sect. 2.3.4).

### 2.3.1 *Comparative Pharmacokinetic Studies*

As indicated earlier, for systemically acting drug products, demonstration of BE between a test (T) and reference (R) product can be achieved by the conduct of comparative pharmacokinetic studies. These studies are generally performed with a

limited number of healthy volunteers, e.g., 24–36 subjects (FDA 2003a). Most studies have a two-sequence, two-period, crossover design where each subject is randomly assigned to either sequence TR or RT with an adequate washout interval between the two treatment periods (FDA 2003a). Derived from the plasma or serum concentration–time profile, the rate of drug absorption is commonly expressed by maximum concentration ( $C_{\max}$ ) and time to maximum concentration ( $T_{\max}$ ) whereas the extent of absorption is expressed by the area-under-the-curve from time zero after drug administration to time infinity ( $AUC_{\infty}$ ) and/or to the last quantifiable drug concentration ( $AUC_t$ ).  $AUC_t$  may be calculated using the simple trapezoidal rule (Gibaldi and Perier 1982) while  $AUC_{\infty}$  can be estimated by summing up  $AUC_t$  and  $C_t/\lambda_z$  where  $C_t$  is the last quantifiable concentration and  $\lambda_z$  is the terminal rate constant.

With the exception of  $T_{\max}$  parameter, both AUCs and  $C_{\max}$  are statistically analyzed using the two one-sided tests procedure to determine if the average values between the T and R products are comparable (Schuirmann 1987). These comparisons require the calculation of a 90 % confidence interval for the geometric mean ratios of the T and R products. BE is generally declared if the 90 % confidence interval is within the BE limit of 80.00–125.00 % (FDA 2003a). However, the BE limits for highly variable drugs and narrow therapeutic index drugs have been scaled to the intrasubject variability of the reference product in the study (Davit et al. 2012; FDA 2011c, 2012b). To obtain geometric means, the data of AUCs and  $C_{\max}$  are log-transformed prior to conducting an analysis of variance (ANOVA), then back-transformed before calculating the T/R ratio (Davit et al. 2009). Currently, statistical comparison is not performed for  $T_{\max}$  values due to the lack of an appropriate method for this discrete variable (Chen et al. 2001; Davit et al. 2009; Nightingale and Morrison 1987). However, if there is any notable difference in a BE study, consultation on the clinical relevance is sought with medical officers in the FDA.

Since systemic exposure of locally acting drug products may entail a risk of systemic adverse reactions, a comparative pharmacokinetic study is globally required for these products to ensure that systemic drug exposure for the T product is similar to the R product (Chen et al. 2011a). The BE limits of 80–125 % (based on 90 % confidence interval) can be applied to these studies.

### 2.3.1.1 Measures of Systemic Exposure

Despite the US regulations that dictate the reliance of rate and extent of drug absorption for BA/BE determination, there have been concerns regarding the use of  $C_{\max}$  for assessment of absorption rate in BA/BE studies (Chen et al. 2001; FDA 2003a). For example,  $C_{\max}$  is insensitive to changes in rate of input as generally expressed by a rate constant ( $k_a$ ).  $C_{\max}$  is not a pure measure of absorption rate since it is confounded with the distribution (and perhaps elimination) of the drug. In addition, determination of  $C_{\max}$  depends substantially on the sampling schedule and thus this parameter may not be accurate. In recent years, recognizing that systemic

exposure is the key to the efficacy/safety of a drug and that there are multiple challenges inherent in identifying an appropriate pharmacokinetic measure to express both rate and exposure, the US FDA has recommended a change in focus from the measures of “absorption rate and extent” to measures of “systemic exposure” for BA and BE studies (FDA 2003a).

Systematic exposure measures can be used for drugs that achieve therapeutic effects after entry into the systemic circulation. In the FDA Guidance (2003a), these measures are defined relative to the total, peak, and early portions of the plasma/serum profile, which encompasses total exposure ( $AUC_{\infty}$  or  $AUC_t$ ), peak exposure ( $C_{max}$ ), and early exposure (partial AUC to the median  $T_{max}$  of the R product), respectively. In most cases, systemic exposure measures include  $AUC_{\infty}$  (or  $AUC_t$ ) and  $C_{max}$ . Nonetheless, early exposure may be needed in some cases where a better control of the drug input rate is essential for achieving therapeutic effects or circumventing adverse reactions. Notably, these recommendations do not propose a statutory change, given that the conventional measures including  $C_{max}$  and AUC are still used for regulatory determination of BA/BE. More importantly, however, is the conceptual change and understanding that systemic exposure measures based on a concentration–time profile relate directly to efficacy and safety outcomes expressed by therapeutic effects or adverse reactions.

### 2.3.1.2 Measures of Partial Exposure

For immediate-release drug products, consideration of early exposure is needed when the control of drug input rate is critical to achieve a rapid onset of action such as analgesic effect, or avoid a toxic side effect such as hypotensive action from an antihypertensive (FDA 2003a). This notion is unequivocally applicable to modified-release drug products where an appropriate input rate of the drug is necessary to warrant the efficacy and safety profile in the patient (Chen et al. 2011b). In addition to the early exposure measure, the concept of “partial exposure” has recently been expanded to include “late exposure” and any segment of AUC with appropriate cutoff points for better PK/PD characterization and BA/BE assessment. This is exemplified by multiphasic, modified-release drug products that combine both immediate- and extended-release components in a formulation to achieve a quick onset of action as well as a sustained response from the drug afterwards (Chen et al. 2011b; Lionberger et al. 2012; Stier et al. 2012).

Methylphenidate HCl extended-release product is an example for the application of partial AUC measures in establishing BE between an innovator product and its generic versions. Currently, there are three distinct innovator products of extended-release methylphenidate on the market, including a tablet form (Concerta<sup>®</sup>) and two capsule forms (Ritalin LA<sup>®</sup> and Metadate CD<sup>®</sup>). Each product has its unique PK/PD relationship and thus the cutoff for partial AUC may be different from product to product. However, the general principles apply to all three products. For example, the drug labeling of Concerta<sup>®</sup> indicates that this is an extended-release

formulation of methylphenidate with a bimodal release profile. Each Concerta<sup>®</sup> tablet comprises an immediate-release component and an extended-release component, thus providing an instant release followed by sustained release of methylphenidate. Therefore, it is a multiphasic modified-release formulation designed to release a bolus of the drug with a slower drug delivery later in the day. The clinical studies showed a statistically significant improvement in behavioral assessment scores throughout the day for Concerta<sup>®</sup> Tablet relative to placebo, following administration of a single morning dose.

In view of the fact that Concerta<sup>®</sup> Tablet is designed to achieve both rapid onset of action and sustained activity throughout the day, the US FDA has proposed two additional partial AUC metrics for BE demonstration (FDA 2011a). The first partial AUC metric provides assurance that a T and R product will be therapeutically equivalent over the early part of the daily dosing interval, corresponding to the onset of response. The second partial AUC metric ensures that the two products in comparison will be therapeutically equivalent over the later part of the daily dosing interval, corresponding to the duration of the sustained response.

The cutoff point for the first partial AUC metric has been determined using the estimate of  $T_{\max}$  for the immediate-release component of Concerta<sup>®</sup> Tablet. Since the  $T_{\max}$  values of this formulation is  $2 \pm 0.5$  h in a fasting study and  $3 \pm 0.5$  h in a fed study and it is believed that 95 % of observations would fall within two standard deviations of the mean, the cutoff of early partial AUC metric for BE determination was set to be 3 h and 4 h for the fasting and fed study, respectively. Based on the cutoff of the first partial AUC metric, the second partial AUC metric was then determined to be  $AUC_{3-t}$  and  $AUC_{4-t}$  for the respective fasting and fed BE study.

### 2.3.2 Comparative Pharmacodynamic Studies

The use of pharmacodynamic or clinical endpoints for BE demonstration is not recommended for a drug product when the drug is absorbed into the systemic circulation and pharmacokinetic approach can be used to assess systemic exposure for BE evaluation (FDA 2003a). However, in those instances where a pharmacokinetic approach is not possible, determination of BE may be achieved using suitably validated pharmacodynamic or clinical endpoints (FDA 2003a). This can occur to most locally acting drug products and some systemically acting drug products for which drug levels are too low to be measured in biological fluid or there is a safety concern for using the pharmacokinetic approach to assess BE. For locally acting drug products, another reason for not using pharmacokinetic approach to demonstrating BE lies in the fact that drug concentrations in the systemic circulation following administration of these products may not reflect the availability of the drug at the site of action although certain locally acting products are designed for systemic absorption (FDA 2003b). In addition, systemic absorption of some locally acting drug products may have an impact on the safety profile of the product.

### 2.3.2.1 Dose–Response Relationship

An essential component of BE studies based on a pharmacodynamic response is the documentation of a dose–response relationship (FDA 1995a; Holford and Sheiner 1981). Pharmacodynamic endpoints selected for BE studies are required to have the capacity of detecting potential differences between the test and reference products. This can be ascertained by a pilot study that demonstrates the existence of a clear dose–response relationship, which should be done before the conduct of pivotal BE studies (FDA 1995a). Depending on the drugs, the dose–response curve may be linear, nonlinear, steep, or shallow. A shallow dose–response curve may not allow for detection of potential formulation differences between products. Linearity may be obtained in some cases when the dose is expressed on logarithmic scale. For many drugs, however, the dose–response relationship based on a pharmacodynamic endpoint is nonlinear and can be fitted to a hyperbolic  $E_{\max}$  model as follows (Holford and Sheiner 1981):

$$E = E_0 + \frac{E_{\max} \times D}{ED_{50} + D},$$

where  $E$  is the estimated (fitted) value of pharmacodynamic response,  $E_0$  is the baseline pharmacodynamic effect,  $E_{\max}$  is the maximum pharmacodynamic effect, and  $ED_{50}$  is the dose where the pharmacodynamic effect is half-maximal.

Statistical analysis of BE studies using pharmacokinetic measures has been performed with the two one-sided tests procedure (Schuirmann 1987). This procedure, however, would not be appropriate for analysis of a pharmacodynamic endpoint if the dose–response relationship is nonlinear. To circumvent this problem, the US FDA has introduced a “dose-scale” approach where BE is determined based on the projected equivalent dose of the test product in lieu of the pharmacodynamic effect on the dose–response curve (Gillespie 1996; FDA 2010a, 2013c). Specifically, pharmacodynamic responses of the test and reference products determined in the BE study may be converted to estimates of delivered dose of the test and reference products by using the “dose-scale” method. The benefits of the “dose-scale” approach to BE assessment arise from the translation of nonlinear pharmacodynamic measurements to linear dose measurements.

### 2.3.2.2 Sensitivity of Pharmacodynamic Measures

The curvilinear dose–response relationship for pharmacodynamic measures may depend on a number of factors, including the mechanism of drug action and potency, pharmacodynamic measure, study population, and severity of the underlying disease. Therefore, conduct of pharmacodynamic studies warrants careful considerations of screening appropriate subjects for the BE study so that

the likelihood of obtaining discernible response is enhanced (FDA 1995a, 2003b). The doses used in the BE study should be situated in the discriminative region of the dose–response curve, so lower doses are usually recommended for the study (FDA 1995a, 2003b). The basic pharmacodynamic study design for BE determination may include two doses of the reference product. Additional doses can be used to enhance precision in the estimated values. In the case of topical drug products, different doses are normally made by varying the duration of application when there is only one dose strength available for the product (FDA 1995a). For nasal/inhalation products, different doses may be given by single actuation from one or more products. However, multiple strengths are usually available for solid oral dosage forms. In general, a pilot study is first conducted using the reference product to determine the most sensitive dose for the pivotal BE study.

### 2.3.2.3 Examples of Pharmacodynamic Endpoints

The choice of pharmacodynamic endpoints for a drug product depends on the mechanism of drug action. For example, topical dermatologic corticosteroid products along with the comparators can be tested for BE using a vasoconstrictor assay to quantify the “topical bioavailability” between formulations (FDA 1995a). This pharmacodynamic approach is based on the property of corticosteroids to produce blanching or vasoconstriction in the microvasculature of the skin, which presumably relates to the amount of the drug entering the skin. The assay is sometimes referred to as the Stoughton–McKenzie test, vasoconstrictor assay, or skin blanching assay (Stoughton 1992). For most topical drug products, however, comparative clinical trials have been employed to determine BE due to the lack of appropriate pharmacodynamic measures.

Inhalation aerosols represent another example for which pharmacodynamic endpoints are used to evaluate BE. A case in point is short-acting beta-agonists (e.g., albuterol) that are indicated for prevention and treatment of bronchospasm in asthmatic patients. Based on the mechanism of action, pharmacodynamic effects of these drug products are measured in terms of bronchodilation or prevention of experimentally induced bronchoconstriction (FDA 2013c). The most commonly used measure of bronchodilation is an increase in forced expiratory volume within one second ( $FEV_1$ ). In this case, bronchoprovocation with methacholine challenge has been employed to compare the protective effects of beta agonists through the estimation of provocative dose ( $PD_{20}$ ) or concentration ( $PC_{20}$ ) that produces a 20 % decrease in  $FEV_1$  (FDA 2013c).

Many inhalation drug products combine a drug(s) and device in the dosage form. Because of the complexity of these dosage forms, establishment of BE by the US FDA has been based on an “aggregate weight of evidence” approach that utilizes (a) pharmacodynamic or clinical endpoint studies to demonstrate equivalence in



local action, (b) pharmacokinetic studies to ensure minimal systemic exposure, and (c) a battery of in vitro studies to support equivalent performance of the device (FDA 2003b).

### 2.3.3 *Comparative Clinical Trials*

Clinical responses are often located near or at the plateau of the dose–response curve, thus insensitive to distinguish the therapeutic difference between a test and reference formulation (FDA 2003b). As a result, conduct of these studies for BE assessment requires a large number of patients to detect formulation differences. Demonstration of dose–response relationships is not required for clinical BE studies since they are intended only to confirm the lack of important clinical differences between products in comparison. Because of all the reasons mentioned above, BE studies using clinical endpoints will be considered only when both pharmacokinetic and pharmacodynamic approaches are impossible for BE determination.

Several FDA guidance documents for industry are available on the application of clinical approaches to document BE for topical drug products (FDA 2010b). Typically, a randomized, double-blind, placebo-controlled, parallel group study is required. However, placebo treatments are not needed for drugs treating infectious diseases. BE is established if the T product is equivalent to the R product and superior to the placebo treatment. In the case of nasal sprays for local action, the US FDA may waive the in vivo BE studies for solution-based products as BA/BE is self-evident for these products. However, such testing is required for suspension-based nasal sprays due to the lack of a suitable method for particle size determination in suspension formulations (FDA 2003b). Moreover, in vivo BE testing cannot be exempted for nasal solutions in metered dose devices because they are drug-device combination products (FDA 2013c). For establishment of equivalence in local delivery of suspension-based nasal sprays, the US FDA has recommended clinical trials in seasonal allergic rhinitis patients. The study design is a randomized, double-blind, placebo-controlled, parallel group of 14-day duration. The clinical endpoints for equivalence and efficacy analyses are patient self-rated mean total nasal symptom scores.

In general, for drug products that BE determination is made on the basis of pharmacodynamic or clinical endpoints, measurement of the active ingredients, or active moieties in an accessible biological fluid (i.e., pharmacokinetic approach) is necessary to ensure comparable systemic exposure (albeit minimal) between the T and R product (FDA 2003b). However, for some locally acting drug products, such pharmacokinetic studies may be limited by the labeled maximum dose, drug bioavailability, and sensitivity of the bioassay used. In such circumstances, pharmacodynamic or clinical studies could be used to document comparable systemic effects of these drug products.

### 2.3.4 *Comparative In Vitro Studies*

Traditionally, in vitro studies are seldom used alone for BE determination except with some special cases where (1) the drug of interest was approved before 1962 and was determined to be a nonbioproblem drug, or (2) scientific evidences have shown that in vitro test data are correlated with in vivo results (FDA 1997a). Over the decades, however, the evolution in pharmaceutical science and technology may have provided opportunities for relying more on in vitro tests to support BE demonstration. Indeed, this can be exemplified by the recent application of a Biopharmaceutics Classification System (BCS) that classifies drugs based on their biopharmaceutical attributes and predicts BA/BE of the drug products in an immediate-release dosage form. In this case, biowaiver can be granted for a BCS Class I (highly soluble and highly permeable) drug formulated in a rapidly dissolving, immediate-release drug product (FDA 2000). Apart from the enhanced role for in vitro dissolution/release testing, the FDA guidance on BCS has indicated certain in vitro approaches (such as in vitro epithelial cell culture methods) that can be used to determine the permeability class of individual drugs (FDA 2000).

#### 2.3.4.1 *In Vitro Dissolution/Release Testing*

Dissolution/release testing is the most commonly used in vitro method for BE assessment. Although in vitro dissolution/release testing has seldom been used alone as a tool for BE demonstration, dissolution/release information along with the in vivo study data is routinely submitted by drug sponsors for BE documentation of orally administered drug products (FDA 2003a). Dissolution/release data have often been employed to substantiate BE when there is a minor change to formulation or manufacturing (FDA 1995b, c, 1997a, b, 2003a). In addition, in vitro dissolution/release data are utilized to support waiver of BA/BE studies for lower strengths of a drug product, provided that an acceptable in vivo study has been conducted for a higher strength and compositions of these strengths are proportionally similar (FDA 2003a). Together with the use of BCS, in vitro dissolution/release testing has played an increasingly important role in the regulatory determination as to whether the waiver of in vivo BE studies can be granted for an immediate-release drug product (FDA 2000).

In the regulatory arena, to serve as an indicator for BE, an in vitro dissolution/release test should be correlated with and predictive of in vivo BA (FDA 1995c, 2003a). In this setting, the in vitro dissolution/release methodology should be optimized to closely mimic the physiological environment in vivo. For a drug product, proper in vitro dissolution/release behavior in the presence of different formulations with defined in vivo absorption characteristics will be useful to facilitate the establishment of an in vitro–in vivo correlation (IVIVC)

(FDA 1995c). The in vitro dissolution/release method developed in such a manner may be utilized as a surrogate for BA/BE studies when a change occurs in manufacturing or formulation.

#### 2.3.4.2 Other In Vitro Methods

To date, with the better understanding of pharmaceutical attributes, formulation characteristics, and mechanism of action, in vitro studies have taken on an added importance for BE evaluations. A case in point is cholestyramine resin that lowers cholesterol by sequestering bile acid in the gastrointestinal tract (FDA 2012a). For these products, the US FDA has recommended the use of both in vitro equilibrium and in vitro kinetic binding studies of bile acid salts for BE evaluation. The application of these in vitro assays takes advantage of the mechanism of action from resin to assess its binding behavior between the innovator and generic formulation of cholestyramine. Similarly, the Agency has recommended the use of in vitro dissolution, phosphate equilibrium binding, and phosphate kinetic binding studies for BE establishment of lanthanum carbonate chewable tablets (FDA 2011b). Lanthanum is a compound used as a phosphate binder to treat hyperphosphatemia in patients with kidney disease. Lanthanum works in the acid environment of the upper gastrointestinal tract by binding dietary phosphate to form an insoluble complex, which is then eliminated via feces. BE determination with a pharmacokinetic approach is inappropriate for lanthanum because it has an extremely low BA (less than 0.002 %) and the site of drug action lies in the gastrointestinal tract. Likewise, in vitro test methods have been widely used to support BE determination of other locally acting drug products. For example, several in vitro test methods are currently used to support BE assessment of nasal and inhalation products (FDA 2003b). For these products, the key parameters that can be assessed through in vitro tests may include (a) delivered or emitted dose, (b) aerodynamic particle size distribution, (c) spray pattern and plume geometry, and (d) impurities and/or microbial contaminants in formulations and devices during storage or use.

As indicated earlier, pharmaceutical equivalence plays an integral part of therapeutic equivalence between a generic and an innovator product (Orange Book 2013). For simple dosage forms or drug products, pharmaceutical equivalence can be made by a qualitative (Q1) and quantitative (Q2) comparison of composition between formulations. However, this approach may not be sufficient for complex dosage forms or drug products. Use of comparative in vitro test methods may furnish additional evidence to support pharmaceutical equivalence of these products. For instance, the US FDA has suggested the use of a higher level of comparison (Q3) that examines the arrangement of matter (or microstructure) in drug products to supplement the traditional approach for evaluating pharmaceutical equivalence of topical drug products (Lionberger 2005). In this case, the in vitro data for Q3 assessment may include comparisons of physicochemical characteristics as well as in vitro drug release pattern to show structural similarity between formulations.

2.4 Design and Conduct of BE Studies

Currently, the US FDA recommends use of (a) a two-period, two-sequence, two-treatment, single-dose, crossover study design, (b) a single-dose, parallel study design, or (c) a replicate study design for BE studies (FDA 2001, 2003a). Several factors may be considered when choosing appropriate designs for a BE study. For instance, the two-way crossover study design is generally conducted with healthy subjects for most drug products that release drug into the systemic circulation. In this design, each subject will receive each treatment (T or R product) in random order as follows:

Sequence	Period	
	<u>1</u>	<u>2</u>
	T	R
	R	T

For crossover designs, an adequate washout interval is required between the two periods so that drug level at the beginning of each period is almost zero or negligible. In contrast, for parallel designs, each treatment will be administered to a separate group of subjects with similar demographics and no washout period is needed. Parallel designs are often used for BE studies conducted in patients or for drugs with a long half-life where crossover studies are difficult or impossible to perform.

Replicated crossover designs allow for estimation of intrasubject variability of the T and/or R products using a partial (three-way) or full (four-way) replication of treatment as shown below.

Sequence	Period		
	<u>1</u>	<u>2</u>	<u>3</u>
	T	R	T
	R	T	R

Sequence	Period			
	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>
	T	R	T	R
	R	T	R	T

For replicate designs, one or both treatments will be administered to the same subjects on two separate occasions. Replicate design has the advantage of using fewer subjects to achieve the same statistical power compared to the regular two-treatment, two-period crossover design. Replicate designs are particularly useful for highly variable drugs and narrow therapeutic index drugs in that the BE of these drugs can be assessed using a scaling approach based on the intrasubject variability of the R product determined from the study (FDA 2011c, 2012b).

### 2.4.1 Crossover Versus Parallel Design

Single-dose, crossover designs with a washout period between treatments may not be employed for BE studies conducted in patients due to ethical concerns. In such circumstances, parallel designs can be used. Additionally, the crossover design of BE studies may not be practical for drugs with a long half-life because of two reasons. First, adequate characterization of the half-life calls for blood sampling over a long period of time. Secondly, pharmacokinetic principles dictate a washout interval of more than 5 half-lives of the moieties to be measured, which may last for several weeks or months for some drugs. In cases where the conduct of a crossover study is problematic, single-dose parallel designs can be an alternative choice since the latter do not need a washout period between treatments (FDA 2003a) although more subjects are necessary to achieve the same statistical power with parallel designs compared to crossover designs.

Monte Carlo simulations with crossover design studies have demonstrated that using truncated area (such as  $AUC_{0-72\text{ h}}$ ) had the power and accuracy equivalent to those obtained using  $AUC_{0-\infty}$  (sampling up to the last quantifiable concentration) for a long half-life drug with low intrasubject variability in distribution and clearance (Kharidia et al. 1999). Similarly, simulations using parallel design studies for drugs with a half-life of 30 h or more revealed that truncation time range between 60 and 96 h was most informative for BE determination, and that sampling beyond 120 h would not affect BE decision (El-tahtawy et al. 2012). It appears that these simulation results are in agreement with the general belief that completion of gastrointestinal transit of a solid, oral, immediate-release drug product, and absorption of its drug substance will occur within approximately 2–3 days after dosing, regardless of the length of half-life for the drug.

The US FDA has recommended that sample collection be truncated at 72 h for long half-life drugs ( $\geq 24$  h) in oral solid dosage forms, using either a crossover or parallel study (FDA 2003a). However, for drugs demonstrating high intrasubject variability in distribution and/or clearance, AUC truncation cannot be used (FDA 2003a).

### **2.4.2 Single Dose Versus Multiple Doses**

Several simulations have been conducted to investigate the sensitivity of single-dose versus multiple-dose studies in detecting formulation differences using a typical crossover design for BE evaluation. Most simulation results revealed that single-dose studies are more sensitive than multiple-dose studies to detect rate differences between a T and R product, which appears to be consistent with the results found in experimental data. In essence, drugs characterized by low accumulation indices showed virtually no change in the 90 % confidence intervals of AUC and  $C_{\max}$  from single-dose to multiple-dose (El-Tahtawy et al. 1994). However, drugs with higher accumulation indices had smaller confidence interval at steady state, and thus the probability of failing a BE test is dramatically decreased upon multiple dosing (El-Tahtawy et al. 1994).

The US FDA has generally recommended single-dose pharmacokinetic studies for BE demonstration of both immediate- and modified-release products (FDA 2003a). However, steady-state studies may be needed for BE demonstration in some cases (FDA 2003a). As an example, safety considerations for healthy volunteers may suggest the use of patients who are already receiving the medication and it is possible to establish BE without disrupting the ongoing treatment of a patient using a steady-state study. This scenario can be illustrated by clozapine, a drug used to treat the symptoms of schizophrenia (FDA 2005). To demonstrate BE of clozapine tablets, applicants are requested to conduct a single-dose (100 mg), two-treatment, two-period crossover study at steady state. In this case, subjects recruited are patients receiving a stable daily dose of clozapine administered in equally divided doses at 12-h intervals. In addition, patients who are receiving multiples of 100 mg every 12 h can participate in the study of the 100 mg strength by continuing their established maintenance dose. The US FDA recommends that these studies not be conducted using healthy subjects because of safety concerns. According to the crossover randomization schedule, an equal number of patients would receive either the generic or reference formulation in the same dose as administered prior to the study every 12 h for 10 days. Patients would then be switched to the other product for a second period of 10 days. No washout period is necessary between the two treatment periods since it is a steady-state study. After the study is completed, patients could be continued on their current dose of clozapine using an approved clozapine product as prescribed by their clinicians. In all cases where a steady-state study is indicated, applicants are required to carry out appropriate dosage administration and sampling to document the attainment of steady state.

### **2.4.3 Healthy Subjects Versus Patients**

A common practice in conducting pharmacokinetic studies for BE evaluation has been to recruit healthy subjects with 18 years of age or older, which reflects the

common interest of having a homogeneous group of individuals to participate in the study and enhance the likelihood of demonstrating BE. However, recent experiences have revealed that in some instances, albeit rare, there is a lack of subject-to-subject similarity in the difference between the T and R product, the so-called *subject-by-formulation interaction* in statistical term (Hauck et al. 2000). Such interactions can arise when the products (or formulations) differ in a subgroup but not in the remaining subjects of the population.

An earlier report on subject-by-formulation interactions may be related to age (Carter et al. 1993). In this study, one of the generic products had AUC and  $C_{\max}$  values 43 and 77 % higher in the elderly than in the young subjects, while the innovator and another generic product had similar values in the elderly and young. The cause of this interaction had been attributed to the age-related differences in pH, gastric emptying, and/or transit time in the gastrointestinal tract between the two populations. Another example of subject-by-formulation interactions was found from FDA data base with a drug (calcium-channel blocking agent) in two modified-release products (Chen 2005). The drug was a substrate of both CYP3A4 and P-gp. The mean ratio of the T over R product was significantly different between males and females from single-dose and multiple-dose studies, suggesting the presence of a sex-based, group-by-formulation interaction. The in vitro dissolution testing using varying pH media also revealed a pronounced difference in the dissolution behavior of the two products. Based on these data, the interaction was postulated to occur because of different pH-dependent in vivo release profiles between the two products, as well as sex differences in intestinal epithelial drug metabolism and/or transport. In a recent FDA contract study, an apparent subject-by-formulation interaction was also found for ranitidine solution in the presence of a large amount of sorbitol as opposed to sucrose (Chen et al. 2007). A relevant factor accounting for such an interaction may relate to the unique osmotic effect of sorbitol on gastrointestinal physiology observed in various subgroups of the general population (Jain et al. 1985, 1987).

The US FDA currently recommends that in vivo BE studies be conducted in individuals representative of the general population, taking into account age, sex, and race (FDA 2003a). The rationale for having healthy volunteers in most BE studies with pharmacokinetic measures relies on the use of crossover designs where each subject can serve as his/her own control, and thus the conclusion drawn from these study results with respect to BE determination is unbiased, regardless of the populations used. Only under certain circumstances will safety considerations preclude the use of healthy subjects. In such situations, applicants are generally advised to enroll targeted patients with stable disease process and treatments for the duration of the BE study. Depending on the drug characteristics, indications, safety and/or efficacy profiles, the studies may be conducted with crossover and/or parallel designs. Using everolimus as an example, 10 mg tablet of this drug may be dosed once daily for oncology use. Patients who are already receiving everolimus with

such dosing regimen can continue on the same dose for both periods of the crossover or parallel study at steady state without disrupting the course of therapy in the patient (FDA 2012c).

#### **2.4.4 Administered Dose**

In the USA, when a drug product is in the same dosage form, but in a different strength and is proportionally similar in its active and inactive ingredients to the higher strength product on which BE testing has been conducted, an in vivo BE demonstration of one or more lower strengths can be waived based on appropriate dissolution data (FDA 2003a). Hence, the recommended dose used in a BE study is generally the dose corresponding to the highest marketed strength administered as a single unit (FDA 2003a). However, at times a lower strength may have to be administered due to toxicity concerns, as exemplified by clozapine (FDA 2005). The RLD product of clozapine tablets has five dose strengths (12.5, 25, 50, 100, and 200 mg) available on the market. Yet, the BE study of clozapine has been recommended to be performed on 100 mg (instead of 200 mg) strength because of safety considerations. The US FDA has allowed biowaivers for the rest of strengths (including 200 mg) of clozapine tablets, providing that (a) linear elimination kinetics has been established over the therapeutic dose range; (b) acceptable in vivo BE studies on the 100 mg strength; (c) proportional similarity of the formulations across all strengths; and (d) acceptable in vitro dissolution testing of all strengths. Similarly, if warranted for analytical reasons, multiple units of the highest strength can be administered, as long as the total single dose remains within the labeled dose range and the total dose is safe for administration to the study subjects.

For an in vivo BE study, the US FDA has recommended that the assayed drug content of the T product batch should not differ from the R product by more than  $\pm 5\%$ . This is to ensure that comparable doses will be given in the BE study so that no dose correction is necessary for subsequent analysis of study data (FDA 2003a).

#### **2.4.5 Sampling**

In a typical BE study, the T and R product are generally administered with 8 oz (i.e., 240 mL) of water to each participating subject under fasting conditions, unless the study is to be conducted under fed conditions where a high-fat meal will be given (FDA 2002, 2003a). For fasting studies, subjects are usually fasted overnight before drug administration in the following day and standardized meals will be provided to subjects no less than 4 h after dosing.



For BE studies with pharmacokinetic measures, under normal circumstances, a series of blood samples (rather than urine or tissue samples) will be collected after dosing and parent drug (and major metabolites) concentrations in serum or plasma will be measured. However, depending on the drug kinetics, whole blood may be more appropriate for analysis of some drugs, e.g., tacrolimus (FDA 2012d). Tacrolimus is extensively bound to red blood cells with a mean blood to plasma ratio of about 15, while albumin and alpha 1-acid glycoprotein appear to primarily bind tacrolimus in plasma (Venkataramanan et al. 1995).

In a single-dose pharmacokinetic study, collection of blood samples should be scheduled at appropriate times in such a manner that the absorption, distribution, and elimination phases of the drug can be well described. This is generally achieved by collecting 12–18 samples (including a pre-dose sample) for each subject after each dose. More frequent sampling should be made around the anticipated peak time ( $T_{\max}$ ) so that  $C_{\max}$  can be determined with accuracy. The sampling schedule should continue for at least three or more terminal elimination half-life of the drug to ensure complete characterization of the entire pharmacokinetic profile. The exact timing for sample collection depends on the kinetics of the drug and the input rate from the drug product. However, at least three to four samples should be obtained during the terminal log-linear phase to allow for an accurate estimate of terminal rate constant ( $\lambda_z$ ) from linear regression so that  $AUC_{\infty}$  can be calculated without difficulty.

#### **2.4.6 Parent Drug Versus Metabolites**

For most drugs, one or more primary metabolites are formed as a result of biotransformation. Primary metabolites often undergo further metabolic transformation to one or more secondary metabolites. The administered substance (parent drug) and/or its primary/secondary metabolites may produce either desired therapeutic effect or undesired adverse effect or both. If the administered substance is inactive (i.e., has neither therapeutic nor adverse effects), it is termed a pro-drug. After oral administration, biotransformation may occur pre-systemically when the gastrointestinal mucosa and/or liver contribute to the overall metabolism of the administered substance.

The debate over measuring the parent drug versus metabolite(s) is similar to the debate over whether blood level measures or clinical outcomes should be used in BE studies. From a regulatory perspective, reliance on measurement of the parent drug as a marker of rate and extent of release is preferred, even when the parent drug has no clinical activity or the metabolite has a significant therapeutic effect. The rationale for this approach is that the concentration–time profile of the parent drug is more sensitive to changes in formulation performance than the metabolite. The parent drug data mirror the absorption process of the active moiety in the formulation whereas the metabolite data are more reflective of the processes of metabolite formation, distribution, and elimination (FDA 2003a). In many cases,

the formation of metabolite(s) is a sequence secondary to the absorption of parent drug, and thus metabolite(s) data are not useful for distinguishing small differences existing, if any, between formulations. From a clinical perspective, measurement of a metabolite may be desirable when the metabolite possesses most of the clinical activity. Nevertheless, consideration of parent drug versus metabolite for BE evaluation should be focused on the accuracy, sensitivity, and reproducibility of the approach used for assessment.

Indeed, the above notion of using parent drug (rather metabolites) data in BE assessment has been supported by the experimental data and extensive simulations conducted over the years (Chen and Jackson 1991, 1995; Jackson 2000; Jackson et al. 2004; Braddy and Jackson 2010). In most cases, it has been found that 90 % confidence intervals for AUC and/or  $C_{\max}$  of the metabolite are smaller than those of the parent drug, regardless of the drug kinetics and level of error contained in the data. Exceptions arise only when a high degree of intrasubject variability exists in the first-pass metabolism compared to the absorption process of the drug (Chen and Jackson 1995). Under such conditions, the metabolite data is needed in addition to the parent drug data for BE assessment.

In general, it has been concluded that concentration–time profile of the parent drug, as compared to its metabolite(s), is more sensitive to changes in formulation performance, and thus pharmacokinetic data from parent drug should be used for BE assessment. However, metabolite data may be important and should be obtained if a primary metabolite(s) is formed substantially through pre-systemic metabolism (e.g., first-pass, gut wall, or gut lumen metabolism) and contributes significantly to the safety and efficacy of the drug product. This approach should be applied to all drug products, including pro-drugs. To determine BE, the US FDA currently only requires statistical analysis using a confidence interval approach for parent drug while metabolite data are used to provide supportive evidence of comparable therapeutic outcome.

#### **2.4.7 Enantiomers Versus Racemates**

In chemistry, stereoisomers have the same molecular formula with the same atoms, connected in the same sequence, but their atoms are positioned differently in space. Enantiomers are two stereoisomers that are related to each other by a reflection and thus they are mirror images of each other, but they are not superimposable. Analytically, one enantiomer will rotate the plane of polarized light to the right (dextrorotatory, *d* or +), while its antipode will rotate it to the left with the same magnitude (levorotatory, *l* or –). The prefixes R- and S- are assigned to the enantiomers on the basis of their absolute configuration. However, there are no relationships between the *d/l* versus R-/S- nomenclatures.

A drug molecule can be obtained either from natural sources or by chemical synthesis. Natural source drugs may have only one enantiomer whereas chemically

synthesized drugs are generally racemates. Many drugs have been developed and marketed as a racemic (50:50) mixture of the R- and S-enantiomers. For example, nonsteroidal anti-inflammatory drugs (NSAIDs) are an important group of racemic drugs with the S-isomer generally associated with clinical efficacy (Evans 1992). The systemic exposure of many NSAID enantiomers such as ketoprofen and flurbiprofen are found comparable in terms of AUC and the S-/R-concentration ratio in plasma remains constant over time (Ariens 1984). However, it has been observed that for some other NSAIDs, such as fenoprofen and ibuprofen, the AUC of S-isomer may exceed that of the R-isomer (Rubin et al. 1985; Cox 1988; Evans et al. 1990). Due to the low solubility of ibuprofen at acidic pH, different formulations may show different in vivo dissolution rates that in turn, translate into different absorption rates. Substantial unidirectional inversion of the R-(–) to S-(+) enantiomer occurs systemically, which may be influenced by the absorption rate of ibuprofen (Jamali et al. 1988; Davies 1998). In a study comparing two formulations of racemic ibuprofen tablets, results from both chiral (enantiospecific) and achiral (non-enantiospecific) assays showed BE of the two products. However, compared to the achiral assay, the chiral assay detected a larger difference in the eutomer (Garcia-Arieta et al. 2005). In another study with two ibuprofen oral suspensions (2 %), achiral method showed BE of two products for both AUC and  $C_{\max}$ . However, the chiral method showed differences in AUC and  $C_{\max}$ , resulting in non-bioequivalence for the individual enantiomers (Torrado et al. 2010).

Measurement of racemates in plasma or serum using an achiral assay is generally sufficient for BE studies if identical BE outcome can be obtained with the use of racemate or enantiomer data. However, depending on the pharmacokinetic and pharmacodynamic characteristics of the drug under study, BE decision may vary with the use of racemate or enantiomers. As a result, the FDA Guidance (2003a) currently recommends analysis of individual enantiomers for a BE study when all of the following conditions have been met:

- The enantiomers exhibit different pharmacokinetic characteristics
- The enantiomers exhibit different pharmacodynamic characteristics
- Primary efficacy and safety activity reside with the minor enantiomer
- Nonlinear absorption is present for at least one of the enantiomers, as expressed by a change in the enantiomer concentration ratio with change in the input rate of the drug

#### 2.4.8 Endogenous Compounds

Some drug substances are endogenous compounds either because they are naturally produced in the body or because they are present in the normal diet. If the endogenous compound is identical to the drug, BE determination may be difficult since the exogenous drug cannot be distinguished from the endogenous compound.

Baseline-corrected data is generally recommended for BE evaluation when the endogenous levels are fairly constant before and during the study. The baseline levels are often determined by averaging the data from multiple samples taken in the time period before administration of the study drug. In addition, baseline levels should be determined at each dosing interval if they are period specific. Provided below are two examples of endogenous compounds with one (estradiol) produced naturally and the other (potassium chloride) derived from diet intake.

Endogenous estrogens are largely responsible for the development and maintenance of the female reproductive system and secondary sexual characteristics. Although circulating estrogens exist in a dynamic equilibrium of metabolic inter-conversions, estradiol is the principal intracellular estrogen with substantially higher potency than its metabolites, estrone and estriol, at the receptor level. The primary source of estrogens in premenopausal women is ovarian follicles. However, after menopause, most endogenous estrogen is produced by conversion of androstenedione to estrone in peripheral tissues. Therefore, estrone and its sulfate-conjugated form are the most abundant circulating estrogens in postmenopausal women.

In the case of estradiol tablets, a single-dose, two-way, crossover design has been recommended for the BE study in healthy, physiologically or surgically postmenopausal women (FDA 2010d). This population is preferred because estradiol is often used to treat symptoms of menopause and the baseline levels in these subjects are fairly constant. The FDA Guidance on estradiol (2010d) has indicated that BE evaluation of estradiol tablets should be based on 90 % confidence interval of baseline-adjusted data of total estrone, with estradiol (unconjugated) and estrone (unconjugated) data as supportive evidence of comparable therapeutic outcome.

Potassium chloride represents an endogenous compound that comes from dietary intake. In this case, it is best to conduct the BE study by strictly controlling the intake before and during the study. The FDA Guidance on potassium chloride (2011d) recommends that subjects be placed on a standardized diet, with known amounts of potassium, sodium, calories, and fluid intake. Strict control and knowledge of the actual intakes of potassium, sodium, calories, and fluid are critical for study success. In addition, subjects should be placed in a climate-controlled environment, remaining in-house as much as possible. Physical activity should be restricted to avoid excessive sweating and thus potassium loss. Meals, snacks, and fluids should be given at standard times, and subjects are strongly encouraged to ingest the recommended amounts while refraining from unnecessary physical activity.

While baseline-correction can be done for pharmacokinetic data of those endogenous compounds that have constant baseline levels in the body, the issue of whether baseline adjustment is appropriate for BE determination may arise when (a) it is not possible to determine baseline concentrations with accuracy; or (b) a feedback mechanism prevails during the study. Presumably, if the interest is to know whether the exogenous compound administered results in the comparable

systemic levels that are within the normal physiological range, baseline-uncorrected data may be sufficient for BA/BE assessment. However, if the contribution of baseline levels to the total levels in the blood/plasma is substantial for the compound, it may be problematic to use baseline-uncorrected data for BE determination.

## 2.5 Conclusions

BE studies have played an important role in the drug development as well as during the post-approval period for both pioneer and generic drugs. The main objectives of these studies may be twofold. First, they serve as bridging studies in the presence of formulation or manufacturing changes to provide supportive evidence for safety and efficacy of a drug product. Second, they can be utilized to assure product quality and performance throughout the life time of a drug product. In the USA, with the passage of the 1984 Hatch-Waxman Act, considerable interest and attention has been added to focus on the use of these studies for approval of generic drugs.

The statutory definition of BE, expressed in rate and extent of absorption of the active moiety or ingredient to the site of action, emphasizes the use of pharmacokinetic measures to indicate release of the drug substance from the drug product with absorption into the systemic circulation. This approach rests on an understanding that measurement of the active moiety or ingredient at the site(s) of action is generally not possible and that there is some relationship between the drug concentrations at the site of action relative to those in the systemic circulation. In cases where pharmacokinetic approach is impossible, BE studies can be conducted using pharmacodynamic measures, clinical endpoints, or *in vitro* tests, with due considerations.

Extraordinary progress has been made in pharmaceutical science and technology since the enactment of 1977 BA/BE regulations in the USA. The contemporary knowledge and methodologies may provide an opportunity to enhance the regulatory approaches for BE demonstration. An ideal paradigm of BE evaluation may take into account the therapeutic index, clinical importance, and pharmaceutical characteristics of the drug substance and drug product under examination. This can be illustrated by the recent changes in the BE approaches for highly variable drugs and narrow therapeutic index drugs. With modern science and technology, an enhanced reliance on *in vitro* methods for BE demonstration may be possible in the future. Further refinement of the BCS approach may expand the horizon of using *in vitro* studies for establishment of BE. Multiple *in vitro* methods may also be developed to substantiate BE demonstration of complex dosage forms or drug products.

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FDA Bioequivalence Standards

Yu, L.X.; Li, B. (Eds.)

2014, XIII, 465 p. 58 illus., 26 illus. in color., Hardcover

ISBN: 978-1-4939-1251-3