



# BIOEQUIVALENCE REQUIREMENTS GUIDELINES

Draft

May 2005

This Draft is for comments;  
please review and send your comments or suggestions to SFDA  
E-Mail: [Drug-Dept@sfda.gov.sa](mailto:Drug-Dept@sfda.gov.sa) , Fax No. 0096612757195  
P.O. Box 84983 Riyadh 11681  
Saudi Arabia

## Table of Contents

	<b>Page</b>
I. Introduction	1
II. Objectives of the Guidelines	1
III. Definition of Terms	2
IV. Bioequivalence Studies Needed for Marketing Authorization	5
• Bioequivalence Studies in Humans Based on Pharmacokinetic Measures	6
- Ethical Principles	6
- Study Design	7
- Drug Products	9
- Subjects	10
- Drug Dose and Dosing	11
- Moieties to be Measured	12
- Bioanalytical Methodology	13
- Collection of Biological Matrix and Sampling Schedule	13
- Pharmacokinetic Parameters	13
- Pharmacokinetic Measures of Systemic Exposure	15
- Long Half-Life Drugs	16
- Statistical Analysis and Acceptance Criteria	17
- General Aspects	17
- Decision Rules	17
- Data Transformation	18
- Acceptance Ranges	18
- Presentation of Data	19
- Individual and Population Bioequivalence	20
- Waiver of In Vivo Bioequivalence Study Requirements.	21
• Other Approaches to Assess Bioequivalence	23
- Pharmacodynamic Studies	23

- Clinical Studies	26
- In Vitro Dissolution Testing	28
V. Studies Needed to Support New Post-Marketing Manufacturing Conditions.	28
VI. Laboratory Units for Conducting Bioequivalence Studies and Suggested Specifications	29
VII. Format and Content of the Report on Bioequivalence Studies to be Submitted to the Saudi Food and Drug Authority	30
Appendix 1	
General Pharmacokinetic Study Design and Data Handling	34
Appendix 2	
Food-Effect Bioequivalence Studies	37
References	40

## **I. INTRODUCTION**

Multi-source drug products need to conform to the same standards of quality, efficacy and safety required of the originator's product. In addition, reasonable assurance must be provided that they are, as intended, clinically interchangeable with nominally equivalent market products.

With some classes of products, including-most evidently parenteral formulations of highly water-soluble compounds, interchangeability is adequately assured by implementation of Good Manufacturing Practices and evidence of conformity with relevant pharmacopoeial specifications. For other classes of products, including biologicals such as vaccines, animal sera, and products derived from human blood and plasma, and product manufactured by biotechnology, the concept of interchangeability raises complex considerations that are not addressed in this document, and these products are consequently excluded from consideration. However, for most nominally equivalent pharmaceutical products (including most solid oral dosage forms), a demonstration of therapeutic equivalence can and should be carried out, and such assessment should be included in the documentation for marketing authorization.

This guideline refers to the marketing of pharmaceutical products that are intended to be therapeutically equivalent, and thus interchangeable, but produced by different manufacturers.

## **II. OBJECTIVES OF THE GUIDELINES**

The interchangeability of pharmaceutically equivalent drug products is a matter of concern to the Saudi Food & Drug Authority. This can be attributed to the increasing international drug trade, facilitated import and export in Saudi Arabia in addition to the increasing number of locally produced products. Interest in bioavailability and bioequivalence of pharmaceutical products lies within the general frame of concern for safety and efficacy of these products. Over the past 25 years it has become evident that marketed products having the same amounts of the drug chemical entity may exhibit marked differences between their therapeutic responses. In many cases, these differences

were correlated to dissimilar drug blood levels caused mainly by impaired absorption.

In view of the importance of the process of drug absorption as a direct determinant of drug efficacy and safety, and since bioavailability determination has not yet been adopted by official compendia as an efficacy-indicating test. Therefore, it is necessary to define a general scientific framework guideline, including basic methodology, ethical principles as well as regulatory aspects for the conduct of bioavailability studies, so that optimal and relevant data are generated. Such guidelines for planning and evaluating drug bioavailability/bioequivalence studies should facilitate the task of a pharmaceutical company, or others, wishing to carry out bioavailability/bioequivalence studies.

The present guidelines have been prepared taking into consideration the need for worldwide harmonization, and at the same time the specific needs for Saudi Arabia. For example, in many countries of the region laboratory units for conducting bioequivalence studies are of very limited number and not yet fully developed. However, it is anticipated that very soon such advanced units will be emerging. Therefore, one section of the guidelines deals with the specifications of laboratory units for conducting these studies. Their strict adherence to these specifications can be the subject for future inspection, accreditation or certification by drug regulatory agencies. In addition, another section of the guidelines deals with the format and contents of bioequivalence reports. These aspects deem necessary when constructing guidelines for Saudi Arabia.

### **III. DEFINITION OF TERMS**

Explanation of certain pertinent terminology described below will facilitate the discussions on the approaches to the assessment of bioequivalence.

#### **Bioavailability**

Bioavailability means the rate and extent to which the active drug substance or therapeutic moiety is absorbed from a pharmaceutical dosage form and becomes available at the site of action. For drugs intended to exhibit a systemic therapeutic effect, bioavailability can be more simply understood as the rate and extent to which a substance

or its therapeutic moiety is delivered from a pharmaceutical dosage form into the general circulation. Indeed, in the case of such drugs, the substance in the general circulation is in exchange with the substance at the site of action.

### **Bioequivalence**

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”.

### **Generic Drug Product**

The term “generic product” has somewhat different meanings in different jurisdictions. Generic products may be marketed either under the nonproprietary approved name or under a new brand (proprietary) name. They may sometimes be marketed in dosage forms and/or strengths different from those of the innovator products. However, where the term “generic product” had to be used in this document it means a pharmaceutical product, usually intended to be interchangeable with the innovator product, which is usually manufactured without a license from the innovator company and marketed after expiry of patent or other exclusivity rights.

### **Innovator Drug Product**

Generally, the innovator pharmaceutical product is that which was authorized for marketing (normally as a patented drug) on the basis of documentation of efficacy, safety and quality (according to contemporary requirements).

In the case of drugs, which have been available for many years, it may not be possible to identify an innovator pharmaceutical product.

### **Interchangeable Pharmaceutical Product**

An interchangeable pharmaceutical product is one, which is therapeutically equivalent to a reference product.

### **Multi-source Pharmaceutical Products**

Multi-source pharmaceutical products are pharmaceutically equivalent products that may or may not be therapeutically equivalent. Multi-source pharmaceutical products that are therapeutically equivalent are interchangeable.

### **Pharmaceutical Alternatives**

Drug products are considered pharmaceutical alternatives if they contain the same therapeutic moiety, but are different salts, esters, or complexes of that moiety, or are different dosage forms or strengths. Different dosage forms and strengths within a product line by a single manufacturer are thus pharmaceutical alternatives, as are extended-release products when compared with immediate-release or standard-release formulations of the same active ingredients.

### **Pharmaceutical Equivalence**

It refers to drug products, which contain the same active ingredient in the same strength (concentration) and dosage form, and is intended for the same route of administration. In general, it has the same labeling and meets compendial and other standards of strength, quality, purity, and identity.

Pharmaceutical equivalent does not necessarily imply therapeutic equivalence as differences in the excipients and/or the manufacturing process can lead to differences in product performance.

### **Reference Product**

A reference product is a pharmaceutical product with which the new product is intended to be interchangeable in clinical practice. The reference product would normally be the innovator product for which efficacy, safety and quality have been established. When the innovator product is not available the product which is the market leader may be used as a reference product, provided that it has been authorized for marketing and its efficacy, safety and quality have been established and documented.

### **Therapeutic Equivalence**

Two pharmaceutical products are therapeutically equivalent if they are pharmaceutically equivalent and after administration in the same molar dose their effects, with respect to both efficacy and safety, will be essentially the same as can be derived from appropriate studies (bioequivalence, pharmacodynamic, clinical or in-vitro studies). Therapeutically equivalent drug products are interchangeable.

## **IV. BIOEQUIVALENCE STUDIES NEEDED FOR MARKETING AUTHORIZATION**

Pharmaceutically equivalent multi-source pharmaceutical products must be shown to be therapeutically equivalent to one another in order to be considered interchangeable. Several test methods are available to assess equivalence, including:

- (A) Comparative bioavailability (bioequivalence) studies in humans in which the active drug substance or one or more metabolites are measured in an accessible biologic fluid such as plasma, blood or urine.
- (B) Comparative pharmacodynamic studies in humans.
- (C) Comparative clinical trials.
- (D) In-Vitro Studies.

Applicability of each of these four methods is discussed in subsequent sections of this guideline and special guidance is provided to conduct an assessment of bioequivalence studies.

Acceptance of any test procedure in the documentation of equivalence between two pharmaceutical products by the drug regulatory authority depends on many factors, including characteristics of the active drug substance and the drug product. Where a drug produces meaningful concentrations in an accessible biological fluid, such as plasma, comparative bioavailability (bioequivalence) studies are preferred. Where a drug does not produce measurable concentrations in an accessible biological fluid, comparative clinical trials or pharmacodynamic studies may be necessary to document equivalence. In vitro testing, preferably based on a documented in vitro / in vivo correlation, may sometimes provide same indication of equivalence between two pharmaceutical products.

## **BIOEQUIVALENCE STUDIES IN HUMANS BASED ON PHARMACOKINETIC MEASURES**

The definition of bioequivalence expressed in terms of rate and extent of absorption of the active ingredient or moiety to the site of action, emphasize the use of pharmacokinetic measures in an accessible biological matrix such as blood, plasma, or serum and/or urine to indicate the release of the drug substance from the drug product into the systemic circulation. This approach rests on the understanding that measuring the active moiety or ingredient at the site of action is generally not possible and, furthermore, that some relationship exists between the efficacy/safety and concentration of the active moiety and / or its important metabolite(s) in the systemic circulation.

Bioequivalence studies are designed to compare the in vivo performance of a test pharmaceutical product (multi-source) compared to a reference pharmaceutical product. A common design for a bioequivalence study involves administration of the test and reference products on two occasions to volunteer subjects, with each administration separated by a washout period. The washout period is chosen to ensure that drug given in one treatment is entirely eliminated prior to administration of the next treatment. Just prior to administration, and for a suitable period afterwards, blood and/or urine samples are collected and assayed for the concentration of the drug substance and/or one or more metabolites. The rise and fall of these concentrations over time in each subject in the study provide an estimate of how the drug substance is released from the test and reference products and absorbed into the body. To allow comparisons between the two products, these blood (to include plasma or serum) and/ or urine concentration time curves are used to calculate certain pharmacokinetic parameters of interest. These parameters are calculated for each subject in the study and the resulting values are compared statistically. Details of the general approach are provided in the following sections.

### **a. Ethical Principles**

All research involving human subjects should be conducted in accordance with the ethical principles contained in the current version of the Declaration of Helsinki. It is

essential to have a review committee to confirm that the protocol complies with ethical standards for research on human subjects. The voluntary informed written constant of **the** healthy volunteers to participate in the study must be obtained. Information given to each volunteer should include details of the study, risks associated with participation and information regarding the right to withdraw at any time from participation without jeopardy.

**b. Study Design**

**1. Pilot Study**

A pilot study in a small number of subjects can be carried out before proceeding with a full bioequivalence study. The study can be used to validate analytical methodology, assess variability, optimize sample collection time intervals, and provide other information. For example, for conventional immediate-release products, careful timing of initial samples may avoid a subsequent finding in a full-scale study that the first sample collection occurs after the plasma concentration peak. For modified-release products, a pilot study can help determine the sampling schedule to assess lag time and dose dumping. A pilot study that documents bioequivalence may be acceptable, provided that its design and execution are suitable and a sufficient number of subjects (e.g., 12) have completed the study.

**2. Nonreplicate Study Designs**

Nonreplicate study designs are recommended for bioequivalence studies of most orally administered, immediate-release and modified-release dosage forms. The general recommendations for nonreplicate designs are provided in Appendix 1.

**3. Replicate Study Designs**

Replicate study designs are recommended for bioequivalence studies of highly variable drug products (within-subject coefficient of variation  $\geq 30\%$ ), including those

that are immediate release, modified-release, and other orally administered drug products.

Replicate study designs offer several scientific advantages compared to nonreplicate designs. The advantages of replicate study designs are that they:

- (i) Allow comparisons of within-subject variance for the test and reference products.
- (ii) Indicate whether a test product exhibits higher or lower within-subject variability in the bioavailability measures when compared to the reference product.
- (iii) Suggest whether a subject-by-formulation interaction may be present.
- (iv) Provide more information about factors underlying formulation performance.
- (v) Reduce the number of subjects needed in the bioequivalence study.

The recommended method of analysis of nonreplicate and replicate studies to establish bioequivalence is average bioequivalence.

#### **4. Food-Effect Studies**

Food-effect bioequivalence studies focus on demonstrating comparable bioavailability between test and reference products when administered with meals. Usually, a single-dose, two-period, two-treatment, two-sequence crossover study is recommended for food-effect bioequivalence study. Food-effect bioequivalence studies are generally recommended for modified-release products. The general recommendations for food-effect bioequivalence study designs are provided in Appendix 2.

Food-effect bioequivalence studies are also recommended for certain conventional release drug products. Selection of conventional release drug products that require food studies is based upon certain considerations, such as:

- (i) Documented evidence of effect of food on drug absorption (e.g. cefaclor);
- (ii) The drug is recommended to be administered with food; and
- (iii) The drug may produce gastric irritation under fasting conditions, thus may be taken with food (e.g. NSAIDs).

Food-effect bioequivalence studies can be waived under the following conditions:

- (i) When both the test and reference products are rapidly dissolving, have similar dissolution profiles, and contain a drug substance with high solubility and high permeability.
- (ii) When the dosage and administration of the reference drug label states that the product can be taken with or without food.
- (iii) When the reference drug label does not make any statements about the effect of food on absorption or administration.

**c. Drug Products**

**1. Immediate-Release Products**

For immediate-release oral solid dosage forms such as capsules, tablets and also suspension dosage forms, a single-dose, two-treatment, two-way, two-period, two-sequence crossover fasting study design should be performed. The bioequivalence study should be performed between the test product and the reference listed drug using the highest strength available.

**2. Modified-Release Products**

Modified-release products include delayed-release products such as enteric-coated dosage forms and extended (controlled)-release products. Bioequivalence studies for delayed-release drug products are similar to those for extended-release drug products. Extended-release products can be capsules, tablets, granules, pellets and suspensions. For extended-release and delayed-release drug products, the following studies are recommended:

- (i) A single-dose, nonreplicate, fasting study comparing the highest strength of the test and reference listed drug product.
- (ii) A food-effect, nonreplicate study comparing the highest strength of the test and reference product.

Because single-dose studies are considered more sensitive in addressing the primary question of bioequivalence (i.e., the release of the drug substance from the drug product into the systemic circulation), multiple-dose studies are generally not recommended, even in instances where nonlinear kinetics are present.

**d. Subjects**

For a sound bioequivalence study the sponsor should enroll a number of subjects sufficient to ensure adequate statistical results, which is based on the power function of the parametric statistical test procedure applied. In general, the Saudi Food & Drug Authority recommends a number of 24 normal healthy subjects, preferably non smoking, between 18-50 years in age and within 15% of ideal body weight, height and body build (Metropolitan Life Insurance Company Statistical Bulletin, 1983) to be enrolled in a crossover bioequivalence study. A number of subjects of less than 24 may be accepted (with a minimum of 12 subjects) when statistically justifiable. However, in some cases (e.g., for highly variable drugs) more than 24 subjects are required for acceptable bioequivalence study. The number of subjects should be determined using appropriate methods taking into account the error variance associated with the primary parameters to be studied (as estimated for a pilot experiment, from previous studies or from published data), the significance level desired ( $\alpha = 0.05$ ), and the deviation from the reference product compatible with bioequivalence ( $\pm 20\%$ ) and compatible with safety and efficacy. For a parallel design study a greater number of subjects may be required to achieve sufficient study power.

Sponsors should enter a sufficient number of subjects in the study to allow for dropouts. Because replacement of subjects could complicate the statistical model and analysis, dropouts generally should not be replaced.

The major objective of using a selective demographic profile is to minimize the magnitude of inter-subject variability. In some cases, for bioequivalence studies on specific classes of drugs, for example cytotoxic drugs, drugs solely recommended for a very specific population or gender, etc., and for studies using pharmacodynamic or clinical endpoints, a targeted patient population may be enrolled in the study. If females

are included in the study, the effects of gender differences and menstrual cycle (if applicable) are examined statistically.

A physical examination, medical history (administered within 30 days prior to the initiation of the study), routine blood chemistry and the hematology tests and urinalysis should be performed to ensure normal hepatic, hematological and renal functions of the volunteers selected for the study. Subjects should be free of any history of serious gastrointestinal, renal, hepatic, cardiovascular or hematological disorders and should have no history of adverse reactions to the drug (or its class) under study. Exclusion and inclusion criteria should be stated in the study protocol. The subjects are not permitted to take any prescription or over-the-counter drug products within two weeks of the start of the study. Ingestion of alcohol or caffeine or related xanthines containing food or beverages is not allowed within 48 hours. Each subject is enrolled after signing an Informed Consent Form (ICF). Both the study protocol and ICF are approved by an appropriate Institutional Review Board (IRB) prior to the start of the study. A priori provision is made for the replacement of dropout subjects by enrolling additional subjects.

**e. Drug Dose and Dosing**

The test product should be from a production lot or from a lot produced under production conditions. Each drug product should be clearly identified by its lot number, manufacture, and expiration dates. An approved product serves as the reference drug. A generic drug product (Test Drug) is compared to the designated innovator product (Reference Drug). A reference product is a pharmaceutical product with which the new product is intended to be interchangeable in clinical practice. The reference product would normally be the innovator product for which efficacy, safety and quality have been established.

Bioequivalence studies on generic products are usually conducted on the highest approved strength, unless there are safety concerns preventing the use of this strength. The administered dose does not ordinarily exceed the dose recommended in the labeling.

Typically, in a single dose study, the test and reference drug products whose potencies do not vary more than  $\pm 5\%$ , are administered to the subjects according to their

randomization schedule and pre-assigned sequence. The dose is administered with sufficient fluid after at least 10 hours of fasting which is continued for at least 4 hours post-dose. Appropriate restrictions on fluid intake and physical activities are made, and all vital signs and adverse events are monitored post-dose.

**f. Moieties to be Measured**

The moieties to be measured in biological fluid collected in bioequivalence studies are either the active drug ingredient or its active moiety in the administered dosage form (parent drug) and, when appropriate, its active metabolite(s).

This guidance recommends the following approaches for bioequivalence studies:

Measurement of only the parent drug released from the dosage form, rather than the metabolite, is generally recommended. The rationale from this recommendation is that the concentration-time profile of the parent drug is more sensitive to changes in formulation performance than a metabolite, which is more reflective of metabolite formation, distribution, and elimination. The following are exception:

1. Measurement of a metabolite(s) may be preferred when the parent drug levels are too low to allow reliable analytical measurement in blood, plasma or serum for an adequate length of time. The metabolite(s) data obtained from these studies should be subjects to a full statistical evaluation including a confidence interval approach for bioequivalence demonstration.
2. The metabolite(s) may be formed as a result of gut wall or other presystemic metabolism. If the metabolite(s) contribute meaningfully to safety and/or efficacy, the metabolite(s) and the parent drug should be measured. When the relative activity of the metabolite(s) is low and does not contribute meaningfully to safety and /or efficacy, it does not need to be measured. The parent drug measured in these bioequivalence studies should be subject to full statistical evaluation including a confidence interval approach. The metabolite(s) data can be used to provide supportive evidence of comparable therapeutic outcome.

**g. Bioanalytical Methodology**

Bioanalytical methods for bioequivalence studies should be accurate, precise, selective, sensitive and reproducible. The FDA guidance entitled “Guidance for Industry: Bioanalytical Method Validation” (Published in May 2001), should be adapted in validating bioanalytical, methods.

**h. Collection of Biological Matrix and Sampling Schedule**

Several samples of appropriate biological matrix (blood, plasma/serum, urine) are collected at various time intervals post-dose. The sampling schedule depends on the pharmacokinetic characteristics of the drug tested. In most cases, plasma or serum is the matrix of choice. However, if the parent drug is not metabolized and is largely excreted unchanged and can be suitably assayed in the urine, urinary drug levels may be used to assess bioequivalence, if plasma/ serum concentrations of the drug can not be reliably measured.

Sufficient numbers of samples are collected during the absorption phase to adequately define the ascending portion of the plasma drug level versus time curve. Intensive sampling is carried out around the time of the expected peak concentration. Sufficient numbers of samples should also be collected in the log-linear elimination phase of the drug so that the terminal elimination rate constant and half-life of the drug can be accurately determined. A sampling period extending to at least four to five terminal elimination half-lives of the drug or four to five the longest half-life of the pertinent analyte (if more than one analyte) is usually sufficient. The samples are appropriately processed and stored carefully under conditions that preserve the integrity of the analyte(s).

**i. Pharmacokinetic Parameters (Bioavailability Metrics)**

Examination of the plasma analyte concentration versus time profile provides an overview of the comparative absorption and elimination of the test and reference drug products. In addition, several observed and estimated parameters are also evaluated to assess bioequivalence.

In a single dose bioequivalence study, the following pertinent pharmacokinetic parameters are examined:

$AUC_{0 \rightarrow t}$	=	Area under the curve (from time 0 to time of Last Quantifiable Concentration).
$AUC_{0 \rightarrow \infty}$	=	Area under the curve (from time 0 to infinity).
$C_{max}$	=	Maximum concentration.
$T_{max}$	=	Time to maximum concentration.
$\lambda_z$	=	Terminal elimination rate constant.
$T_{1/2}$	=	Terminal elimination half-life.

A sufficient number of blood samples should be taken to cover at least 80% of the area under the curve as extrapolated to infinity in each individual.

If a multiple dose studies (steady-state studies) were performed, the following pharmacokinetic parameters are examined:

$AUC_{0 \rightarrow T_{ss}}$	=	Area under the curve (from time 0 to dosing interval) over a dosing interval at steady-state.
$C_{maxss}$	=	Maximum concentration at steady state.
$C_{minss}$	=	Minimum concentration at steady state.
$C_{avgss}$	=	Average concentration at steady state.
$T_{maxss}$	=	Time to maximum concentration at steady state.
% Swing	=	$100 (C_{maxss} - C_{minss}) / C_{minss}$
% Fluctuation	=	$100 (C_{maxss} - C_{minss}) / C_{avgss}$

$C_{max}$ ,  $C_{maxss}$ ,  $C_{minss}$ ,  $T_{max}$  and  $T_{maxss}$  are determined directly from the observed data. AUCs are estimated by the conventional trapezoidal rule. In the multiple dose study, at least three consecutive  $C_{minss}$  should be measured to assure attainment of steady state.

Bioequivalence of different formulations of the same drug substance comprises equivalence with respect to rate and extent of drug absorption. It is part of “Good Biometrical Practice” to stipulate the primary characteristics for the confirmative bioequivalence analysis of rate and extent of absorption in the study protocol prior to the commencement of the bioequivalence study. The area under the concentration time curve ( $AUC_{0 \rightarrow \infty}$ ) generally serves as characteristic of the extent of absorption, while in case of fast-releasing (conventional-releasing) formulations the maximum concentration ( $C_{max}$ ), and the time of its occurrence ( $T_{max}$ ), may serve as characteristics of the rate of absorption.

In multiple-dose studies (steady-state) the percent peak-trough fluctuation (% Fluctuation) and the AUC over one steady-state dose interval ( $AUC_{0 \rightarrow T_{ss}}$ ) can be used as primary characteristics of rate and extent of absorption, respectively.

If urinary samples are used as the biological matrix, the following pharmacokinetic parameters are determined from the observed data: cumulative excretion, excretion rate at collection intervals, maximum excretion rate and time to maximum excretion rate.

#### **j. Pharmacokinetic Measures of Systemic Exposure**

Systemic exposure means comparable rate and extent of absorption. Exposure measures are defined relative to early, peak, and total portions of the plasma, serum, or blood concentration-time profile, as follows:

##### **1. Early Exposure**

For orally administered immediate-release drug product, bioequivalence may generally be demonstrated by measurements of peak and total exposure. An early exposure measure may be indicated on the basis of appropriate clinical efficacy/safety trials and/or pharmacokinetic/pharmacodynamic studies that call for better control of drug absorption into the systemic circulation (e.g., to ensure rapid onset of an analgesic

effect or to avoid an excessive hypotensive action of an antihypertensive). In this setting, the guidance recommends use of partial AUC as an early exposure measure. The partial area should be truncated at the population median of  $T_{max}$  values for the reference formulation. At least two quantifiable samples should be collected before the expected peak time to allow adequate estimation of the partial area.

## **2. Peak Exposure**

Peak exposure should be assessed by measuring the peak drug concentration ( $C_{max}$ ) obtained directly from the data without interpolation.

## **3. Total Exposure**

For single-dose studies, the measurement of total exposure should be:

- Area under the plasma/serum/blood concentration-time curve from time zero to time  $t$  ( $AUC_{0 \rightarrow t}$ ), where  $t$  is the last time point with measurable concentration for individual formulation.
- Area under the plasma/serum/blood concentration-time curve from time zero to infinity ( $AUC_{0 \rightarrow \infty}$ ), where  $AUC_{0 \rightarrow \infty} = AUC_{0 \rightarrow t} + C_t/\lambda_z$ ,  $C_t$  is the last measurable drug concentration and  $\lambda_z$  is the terminal or elimination rate constant calculated according to an appropriate method.

The terminal half-life ( $T_{1/2}$ ) of the drug should also be reported.

### **k. Long Half-Life Drugs**

In a bioequivalence study involving an oral product with a long half-life drug, a nonreplicate, single-dose, crossover study can be conducted, provided an adequate washout period is used. If the crossover study is problematic, a bioequivalence study with a parallel design can be used. For either a crossover or parallel study, sample collection time should be adequate to ensure completion of gastrointestinal transit (approximately 2 to 3 days) of the drug product and absorption of the drug substance.

## **I. Statistical Analysis and Acceptance Criteria**

### **1. General Aspects**

Parametric (normal theory) general linear model procedures are recommended for the analysis of pharmacokinetic data derived from in-vivo bioequivalence studies. Analysis of variance (ANOVA) should be performed on the pharmacokinetic parameters AUCs,  $T_{max}$  and  $C_{max}$ . Appropriate statistical models pertaining to the design of the bioequivalence study should be employed. For example, for a conventional two-treatment, two-period, two-sequence (2 x 2) randomized crossover study design, the statistical model often includes factors accounting for the following sources of variation:

- (1) Sequence (Sometimes called Group or Order).
- (2) Subjects nested in sequences.
- (3) Period (or Phase)
- (4) Treatment (sometimes called Drug or Formulation).

The sequence effect should be tested using the [subject (sequence)] mean square from the ANOVA as error term. All other main effects should be tested against the residual error (error mean square) from the ANOVA.

Assumption of the design or analysis should be addressed, and the possibility of differing variation in the formulations should be investigated. This covers investigation of period effects, sequence or carry-over effects, and homogeneity of variance. Outlying observations should be reviewed for their impact on the conclusion. Medical or pharmacokinetic explanations for such observations should be sought.

### **2. Decision Rules:**

Testing the null-hypothesis of equality of two formulation means by the F-test for treatments from ANOVA analysis is not acceptable. In addition, the power approach “80/20” rule of the point hypothesis and the “75/75” rule are also not acceptable as decision rules in assessment of bioequivalence. The “80/20” rule can be used as a pre-study power calculation for sample size determination in the planning stage of the study protocol.

As the consumer (patient) risk of erroneously accepting bioequivalence is of primary concern for health authorities, only statistical procedures not exceeding a nominal consumer risk of 5% are acceptable, and among those the one which minimize the producer (pharmaceutical company) risk of erroneously rejecting bioequivalence has to be selected as the decision procedure of choice.

The statistical methods of choice at present are the two one-sided test procedure (Schuirmann 1987) or to derive a parametric or nonparametric  $100(1-2\alpha)$  % confidence interval for the ratio (or difference) between the test and reference product pharmacokinetic variable averages. Alpha is set at 5% leading, in the parametric case, to the shortest (conventional) 90% confidence interval based on an analysis of variance or, in the nonparametric case, to the 90% confidence intervals (Hauschke et al., 1990).

The statistical procedures should be specified before the data collection starts. The procedures should lead to a decision scheme which is symmetrical with respect to the two formulations (i.e., leading to the same decision whether the generic formulation is compared to reference product or reference product to the generic formulation).

### **3. Data Transformation:**

Concentration and concentration-related quantities e.g., AUC and  $C_{\max}$ , should be analyzed after logarithmic transformation.  $T_{\max}$  should be analyzed without such transformation. For  $T_{\max}$  normally descriptive statistics should be given. Parametric 90%-confidence intervals for the  $T_{\max}$  should be performed on untransformed data and the equivalence range should be expressed in absolute differences of the mean test minus reference.

### **4. Acceptance Ranges:**

Regarding AUCs, the 90% confidence interval should generally be within the acceptance range 80% to 125% (when log-transformed data are used). In specific cases of narrow therapeutic range drugs, the AUC acceptance range may need to be tightened e.g. 90% to 111%. On the other hand, in rare cases a wider acceptance range may be acceptable and this should be justified clinically.

For  $C_{\max}$ , the 90% confidence interval should lie within an acceptance range of 80% to 125% (for log-transformed data). In certain cases a wider interval may be acceptable. The interval must be prospectively defined e.g. 75% to 133% and should be justified taking into account safety and efficacy of patients switched between formulations.

Statistical evaluation of  $T_{\max}$  ( $T_{\max}$  differences) only makes sense if there is a clinically relevant claim for rapid release or action or signs for a relation to adverse effects. The parametric 90% confidence interval (untransformed data are used) and the nonparametric 90% confidence interval for this measure of relative bioavailability should lie within a clinically relevant range.

For multiple dose studies (steady-state) the pharmacokinetic parameters:  $AUC_{0 \rightarrow T_{ss}}$ ,  $C_{\max ss}$ ,  $C_{\min ss}$ ,  $C_{avg ss}$ , % swing and % fluctuation should be analyzed statistically after logarithmic transformation and the 90% confidence interval should be within the acceptance range 80% to 125%.

#### **m. PRESENTATION OF DATA**

The drug concentration in the biological fluid at each sampling time point should be furnished on the original scale for all the subjects who participated in the study. The derived pharmacokinetic parameters should also be furnished on the original scale. The mean, standard deviation, and coefficient of variation for each variable should be computed and tabulated in the final report.

To facilitate bioequivalence comparisons, pharmacokinetic parameters for each individual should be displayed in parallel for the formulations tested. In particular for AUC and  $C_{\max}$ , the difference (T-R), ratio (T/R), and log of ratio (log T/R or Ln T/R) between the test and reference values should be tabulated side by side for all the subjects. For each subject the summary tables should indicate in which sequence (test/reference or reference/test) the subject received the product. In addition to the arithmetic mean for the test and reference products, the geometric means, means of the logs, and standard deviations of the logs should be calculated for AUC and  $C_{\max}$ . All means, including arithmetic mean, geometric mean, and means of the logs, standard deviations, and

coefficients of variation are to be included in the report.

It is acceptable to use logarithms to the base 10 rather than natural logarithms. The report must state unambiguously which logarithms were used, and the use must be consistent throughout.

The pharmacokinetic parameters ( $\lambda_z$  and  $T_{1/2}$ ) should be calculated for each subject following administration of the test and the reference formulations. It is important to document all points used in  $\lambda_z$  determination (i.e., time intervals used for estimation of the elimination rate constant). This will facilitate independent verification of results by health authorities.

For statistical evaluation, plasma concentration at each sampling time point should be evaluated statistically using ANOVA.

Complete analysis of variance (ANOVA) after logarithmic transformation of the AUC and  $C_{max}$  data and untransformed  $T_{max}$  data should be presented in the report. In the ANOVA analysis, source of variations (Formulations, Periods, Sequences and Subjects within sequence), degree of freedom, sum of squares, mean square, F-test and Probability of “ F ” ( $\alpha$ ) values should be included.

Parametric and/or nonparametric 90% confidence intervals for the mean pharmacokinetic parameters as well as the point estimates should be calculated and tabulated in the report.

If the two one-sided t-tests (Schuirmann 1987) were used as the decision criterion, values of both the upper and lower limits of the calculated test statistics and the tabulated t-value should be provided in the report.

#### **n. Individual and Population Bioequivalence**

To date, most bioequivalence studies are designed to evaluate average bioequivalence. Experience with population and individual bioequivalence studies is limited. Therefore, no specific recommendation is given on this matter.

**o. Waiver of In-Vivo Bioequivalence Study Requirements**

It is to be emphasized that the requirement for demonstration of bioequivalence is never waived. However, for certain formulations and circumstances, equivalence between two pharmaceutical products may be considered self-evident with no further requirement for documentation. Examples include:

1. When multi-source pharmaceutical products are to be administered parenterally (e.g., intravenous, intramuscular, subcutaneous, intrathecal administration) as aqueous solutions and contain the same active substance(s) in the same concentration and the same excipients in comparable concentrations;
2. When multi-source pharmaceutical products are solutions for oral use, contain the active substance in the same concentration, and do not contain an excipient that is known or suspected to affect gastrointestinal transit or absorption of the active substance;
3. When multi-source pharmaceutical products are gases;
4. When multi-source pharmaceutical products are powders for reconstitution as a solution and the solution meet either criterion (1) or criterion (2) above,
5. When multi-source pharmaceutical products are otic or ophthalmic products prepared as aqueous solutions and contain the same active substance(s) in the same concentration and essentially the same excipients in comparable concentrations;
6. When multi-source pharmaceutical products are topical products prepared as aqueous solutions and contain the same active substance(s) in the same concentration and essentially the same excipients in comparable concentrations;
7. When multi-source pharmaceutical products are inhalation products or nasal sprays, tested to be administered with or without essentially the same device, prepared as aqueous solution and contain the same active substance(s) in the same concentration and essentially the same excipients in comparable concentrations. Special in-vitro testing should be required to document comparable device performance of the multi-source inhalation aqueous product.

For elements (5), (6) and (7) above, it is the duty of the applicant to demonstrate that the excipient in the multi-source products are essentially the same and in comparable concentrations as those in the reference product. In the event this information about the reference product can not be provided by the applicant and the drug regulatory authority does not have access to these data, in vivo studies should be performed.

For conventional (immediate release) solid oral drug products, in vivo bioequivalence studies are conducted on the highest strength. This requirement for the lower strengths can be waived provided: (a) in vivo bioequivalence is demonstrated on the highest strengths; (b) in-vitro dissolution testing is acceptable; and (c) the formulation for the lower strengths are proportionally similar to the strength which has undergone in vivo bioequivalence testing (i.e., the ratio of active ingredients and excipients between the strengths is essentially the same).

Generally, for extended release (controlled release) drug products, and enteric-coated formulations in vivo bioequivalence study requirements are not waived for the lower strengths. Single dose in-vivo bioequivalence studies under fasting conditions are normally conducted on these strengths.

## **Other Approaches to Assess Bioequivalence**

Bioequivalence of systemically absorbed drugs are assessed using pharmacokinetic (bioavailability) endpoints provided the drug concentrations in the biological matrix can be accurately measured. However, for nonabsorbable drug products and those that are intended for topical administration, bioequivalence is assessed by evaluating pharmacodynamic or clinical endpoints or by in vitro test methods. Some examples are : Topical dermatologic corticosteroids are evaluated by a “ Skin Blanching Test ” (vasoconstrictor assay), topical anti-infective drugs by clinical tests comparing efficacy profiles, metered dose inhalers by pulmonary function tests, and cholesterol lowering resin powder by in vitro binding tests.

### **(B) PHARMACODYNAMIC STUDIES**

Studies in healthy volunteers or patients using pharmacodynamic measurements may be used for establishing equivalence between two pharmaceutical products. These studies may become necessary if quantitative analysis of the drug and/or metabolite(s) in plasma or urine cannot be made with sufficient accuracy and sensitivity. Furthermore, pharmacodynamic studies in humans are required if measurements of drug concentrations cannot be used as surrogate endpoints for the demonstration of efficacy and safety of the particular pharmaceutical product e.g., for topical products without intended absorption of the drug into the systemic circulation.

If pharmacodynamic studies are to be used they must be performed as rigorously as bioequivalence studies, and the principles of Good Clinical Practice (GCP) must be followed.

The following requirements must be recognized when planning, conducting and assessing the results of a study intended to demonstrate equivalence by means of measuring pharmacodynamic drug responses.

1. The response, which is measured, should be pharmacological or therapeutic which is relevant to the claims of efficacy and/or safety.

2. The methodology must be validated for precision, accuracy, reproducibility and specificity.
3. Neither the test nor the reference product should produce a maximal response in the course of the study, since it may be impossible to distinguish differences between formulations given in doses, which give maximum or near-maximum effects. Investigation of dose-response relationships may be a necessary part of the design.
4. The response should be measured quantitatively under double blind conditions and be recordable in an instrument-produced or instrument-recorded fashion on a repetitive basis to provide a record of the pharmacodynamic events which are substitutes for plasma concentrations. In those instances, where such measurements are not possible, recordings on visual analog scales may be used. In other instances where the data are limited to qualitative (categorized) measurements appropriate special statistical analysis will be required.
5. Non-responders should be excluded from the study by prior screening. The criteria by which responders versus non-responders are identified must be stated in the protocol.
6. In instances where an important placebo effect can occur, comparison between pharmaceutical products can only be made by a prior consideration of the placebo effect in the study design. This may be achieved by adding a third phase with placebo treatment in the design of the study.
7. The underlying pathology and natural history of the condition must be considered in the study design. There should be knowledge of the reproducibility of base-line conditions.
8. A crossover design can be used. Where that is not appropriate, a parallel group study design should be chosen.

In studies in which continuous variables could be recorded, the time course of the intensity of the drug action can be described in the same way as in a study in which concentrations were measured, and parameters can be derived which describe the area under the effect-time curve, the maximum response and the time when maximum response occurred.

The statistical considerations for the assessment of the outcome of the study are in principle, the same as outlined for the bioequivalence studies. However, a correction for the potential non-linearity of the relationship between the dose and the area under the effect-time curve should be performed on the basis of the outcome of the dose-ranging study as mentioned above. However, it should be noted that the conventional acceptance range as applied for bioequivalence assessment is not appropriate (too large) in most of the cases but should be defined on a case-by-case basis and described in the protocol.

## **(C) CLINICAL STUDIES**

In several instances, plasma concentration time-profile data are not suitable to assess bioequivalence between two formulations. Whereas in some of the cases pharmacodynamic studies can be an appropriate tool for establishing equivalence, in other instances this type of study cannot be performed because of lack of meaningful pharmacodynamic parameters which can be measured and a comparative clinical trial has to be performed in order to demonstrate equivalence between two formulations.

However, if a clinical study is considered as being undertaken to prove equivalence, the same statistical principles apply as for the bioequivalence studies. The number of patients to be included in the study will depend on the variability of the target parameters and the acceptance range, and is usually much higher than the number of subjects in bioequivalence studies.

The methodology issues for establishing equivalence between pharmaceutical products by means of a clinical trial in patients with a therapeutic endpoint have not yet been discussed as extensively as for bioequivalence trials. However, important items can be identified which need to be defined in the protocol as follows:

1. The target parameters which usually represent relevant clinical endpoints from which the intensity and the onset, if applicable and relevant, of the response are to be derived;
2. The size of the acceptance range has to be defined on case by case basis, taking into consideration the specific clinical conditions. These include, among others, the natural course of the disease, the efficacy of available treatments and the chosen target parameter. In contrast to bioequivalence studies (where a conventional acceptance range is applied) the size of the acceptance range in clinical trials cannot be based on a general consensus on all the therapeutic classes and indications;
3. The presently used statistical method is the confidence interval approach. The main concern is to rule out that the test product is inferior to the reference

pharmaceutical product by more than the specified amount. Hence, a one-sided confidence interval (for efficacy and/or safety) may be appropriate. The confidence intervals can be derived from either parametric or nonparametric methods;

4. Where appropriate, a placebo leg should be included in the design;
5. In some cases, it is relevant to include safety endpoints in the final comparative assessments.

## **(D) IN-VITRO DISSOLUTION TESTING**

Under certain circumstances, bioequivalence can be documented using in vitro approaches. For highly soluble, highly permeable, rapidly dissolving, orally administered drug products, documentation of bioequivalence using an in vitro approach (dissolution studies) is appropriate. In addition to the determination of the in-vivo performance, in vitro dissolution testing is an integral part of the assessment of bioequivalence, especially for the generic drug products. The comparative release profiles of the test and reference drug products are examined. The dissolution testing is conducted by a compendial method and the test product must pass the compendial specifications. For extended release products, the dissolution method and specifications are developed for each drug product. The specifications are applied only to that drug product to maintain its quality and manufacturing controls.

### **Studies Needed to Support Post-Marketing Manufacturing Conditions**

With all pharmaceutical products, in case of post-marketing changes extensive in vitro and/or in vivo testing may be required. Such changes include changes in:

1. Formulations;
2. Site of manufacture;
3. Process of manufacture; and
4. Manufacturing equipment.

The types and extent of further testing required depend on the magnitude of the changes made. If a major change is made, the product might become a new pharmaceutical product. Reference should be made to national regulatory authorities in this regard.

## **Laboratory Units for Conducting Bioequivalence Studies and Suggested Specifications**

The Laboratory units involved in conducting bioequivalence studies are usually affiliated with one of the following parties:

- (i) The manufacturer.
- (ii) An independent body possessing the expertise and facilities required to conduct a bioequivalence study.
- (iii) The drug regulatory authorities.

Most bioequivalence studies are performed by contract bioequivalence testing laboratories, which are generally equipped to conduct both the clinical and analytical phases of a study. Most often, the two study phases are conducted by the same laboratory, although (because of scheduling, special analytical expertise, desire of the pharmaceutical manufacturer to conduct the analytical portion of the study in-house) they may be conducted by different laboratories. The sponsor is urged to rigorously evaluate the candidate contract laboratory (ies) before initiating the study.

The clinical studies must be conducted in compliance with Institutional Review Board (IRB) requirements and with informed consent requirements. For acceptance of data from any laboratory, foreign or domestic, it is important that the laboratory meet good laboratory practices and procedures as certified by an authoritative agency. It must have a qualified staff (pharmacokineticist, physician, statistician and trained personnel) and must keep good records of the procedures undertaken and the results obtained. Monitoring by the sponsor of the each phase of the study, including validation of the assay method, protocol design, subject selection, collection and storage of the blood or urine samples, and pharmacokinetic and statistical analyses of the data is recommended.

## **Format and Content of the Report on Bioequivalence Studies to be Submitted to the Saudi Food & Health Authority.**

The report of a bioequivalence study should give the complete documentation of its protocol, conduct and evaluation complying with the declaration of Helsinki and Good Clinical Practice (GCP) rules. Studies must be approved by an independent ethics committee or institutional review board. The responsible investigator(s) should sign for their respective section of the report. Name and affiliations of the responsible investigator(s), site of the study and period of its execution should be stated. The names and batch numbers of the pharmaceutical products used in the study as well as the composition(s) of the test product should be given. The analytical validation report should be attached.

Results of in vitro dissolution tests should be provided. In addition, the applicant should submit a signed statement confirming the identity of the test product with the pharmaceutical product, which is submitted for registration. All results should be presented clearly. The procedure for calculating the parameters used (e.g., AUC) from the raw data should be stated. Deletion of data should be justified. If results are calculated using a pharmacokinetic model (although not preferred), the model and computing procedure used should be justified. Individual plasma concentration/time curves should be drawn on a linear/linear scale. All individual data and results should be given, including those of eventually dropped-out subjects. Drop-out and withdrawal of subjects should be reported and accounted for. Test results of representative samples should be included.

The statistical report should be sufficiently detailed, so as to enable the statistical analyses to be repeated if necessary. If the statistical methods applied deviate from those specified in the trial protocol, the reasons for the deviations should be stated.

The following is a proposed format and contents of an in vivo bioequivalence study submission and accompanying in vitro data.

Title page.

Study title.

Name of sponsor.

Name(s) and address(es) of the sites where the clinical and analytical aspects of the study were carried out.

Name, address of the investigator(s).

Name, address of the clinical investigator(s).

Table of Contents

I. Study resume.

Name, and signature of the investigator(s).

Name, and signature of the clinical investigator(s).

Products' information including names and batch numbers of the reference and test products compared and the source of the reference product.

Summary of bioequivalence study.

Summary of bioequivalence data.

Plasma.

Urinary excretion.

Figure of mean plasma concentration-time profile.

Figure of mean cumulative urinary excretion.

Figure of mean urinary excretion rates.

II. Clinical Study

Introduction.

Summary of the study.

Details of the study.

Demographic characteristics of the subjects.

Subject assignment in the study.

Details of clinical activity.

Details of, and a justification for, any deviations from the protocol, drop-outs, or withdrawal from the study.

Form for theoretical sampling frequency and actual sampling times.

Adverse reactions report.

### III. Assay Methodology and Validation

Assay method description.

Validation procedure.

Summary of validation.

Data on linearity of standard samples.

Data on interday precision and accuracy.

Data on intraday precision and accuracy.

Data on analyte(s) stability.

Figure for standard curve(s) for low/high ranges.

Representative chromatograms demonstrating the specificity and sensitivity of the assay including chromatograms of LLOQ and blanks (Copies of all the chromatograms should not be included but must be available to be supplied upon request).

Sample calculation.

### IV. Pharmacokinetic Parameters

Definition and calculations of the pharmacokinetic parameters.

Individual and average pharmacokinetic parameters.

Drug levels at each sampling time and pharmacokinetic parameters.

Figure of mean plasma concentration-time profile (presented as both linear-linear and log-linear graphs).

Figures of individual subject plasma concentration-time profiles (presented as both linear-linear and log-linear graphs).

Figure of mean accumulative urinary excretion.

Figures of individual subject cumulative urinary excretion.

Figure of mean urinary excretion rates.

Figures of individual subject urinary excretion rates.

Tables of individual subject data arranged by drug, drug/period, drug/sequence.

### V. Statistical Analyses

Details and results of statistical analysis.

Summary of statistical significance.

- Summary of statistical parameters.
- Analysis of variance.
- Parametric and/or nonparametric 90% confidence intervals (lower limit, upper limit and point estimate).
- Two one-sided t-tests (lower limits, upper limits of the calculated test statistics and the tabulated t-value).
- VI. Protocol including the criteria for inclusion, exclusion or removal of subjects.
- VII. Informed consent.
- VIII. Appendices.
  - Randomization schedule.
  - Analytical raw data.
  - Medical record and clinical reports.
- IX. In vitro Testing
  - Dissolution testing.
  - Dissolution assay methodology.
  - Content uniformity testing.
  - Potency determination.
- X. Batch Size and Formulation
  - Batch record.
  - Quantitative formulation.

## APPENDIX 1

### General Pharmacokinetic Study Design and Data Handling

For replicate and nonreplicate, in-vivo pharmacokinetic bioequivalence studies, the following general approaches are recommended, recognizing that the elements may be adjusted for certain drug substances and drug products.

#### 1. Study Design:

For immediate release solid/ suspension dosage forms, usually a nonreplicate, randomized, single-dose, two-treatment, two-period, two-sequence crossover design is performed.

#### 2. Study Conduct:

- A. The test or reference products should be administered with about 8 ounces (240 ml) of water to an appropriate number of subjects under fasting conditions, unless the study is a food-effect bioequivalence study.
- B. Generally, the highest marketed strength should be administered as a single unit. If necessary for analytical reasons, multiple units of the highest strength can be administered, providing the total single-dose remains within the labeled dose range.
- C. An adequate washout period (e.g., more than 5 half lives of the moieties to be measured) should separate each treatment.
- D. The lot numbers of both test and reference listed products and the expiration date for the reference products should be stated. The drug content of the test product should not differ from that of the reference listed product by more than 5 percent at the date of study. The sponsor should include a statement of the composition of the test product and, if possible, a side by-side comparison of the compositions of the test and reference listed products. Samples of the test and reference listed product must be retained for 5 years.

- E. Prior to and during each study phase, subjects should:
- i. Be allowed water as desired except for one hour before and after drug administration.
  - ii. Be provided standard meals no less than 4 hours after drug administration.
  - iii. Abstain from alcohol, tea and coffee for 24 hours prior each study period and until after the last sample from each period is collected.

### **3. Sample Collection and Sampling Times:**

Under normal circumstances, blood, rather than urine should be used. In most cases, drug, or metabolite(s) are measured in serum or plasma. However, in certain cases whole blood may be more appropriate for analysis. Blood samples should be drawn at appropriate times to describe the absorption, distribution, and elimination phases of the drug. For most drugs, 12 to 18 samples, including a pre-dose sample, should be collected per subject per dose. This sampling should continue for at least three or more terminal half lives of the drug. The exact timing for sample collection depends on the nature of the drug and the input from the administered dosage form. The sample collection should be spaced in such a way that the maximum concentration of the drug in the blood ( $C_{max}$ ) and terminal elimination rate constant ( $\lambda_z$ ) from linear regression can be estimated accurately. At least three to four samples should be obtained during the terminal log-linear phase to obtain an accurate estimate of  $\lambda_z$  from linear regression. The actual clock time when samples are drawn as well as the elapsed time related to drug administration should be recorded.

### **4. Subjects with Pre-dose Plasma Concentration:**

If the pre-dose concentration is less than or equal to 5 percent of  $C_{max}$  value in that subject, the subject's data without adjustment can be included in all pharmacokinetic measurements and calculation. If the pre-dose value is greater than 5 percent of  $C_{max}$ , the subject should be dropped from all bioequivalence study evaluation.

## 5. Data Deletion Due to Vomiting:

Data from subjects who experience emesis during the course of a bioequivalence study for immediate-release products should be deleted from statistical analysis if vomiting occurs at or before 2 times median  $T_{max}$ . In the case of modified-release products, the data from subjects who experience emesis any time during the labeled dosing interval should be deleted.

## 6. Pharmacokinetic Information Recommended for Submission:

- Plasma concentration and time points.
- Subject, period, sequence and treatment.
- $AUC_{0 \rightarrow t}$ ,  $AUC_{0 \rightarrow \infty}$ ,  $C_{max}$ ,  $T_{max}$ ,  $\lambda_z$ , and  $T_{1/2}$ .
- Intersubject, intrasubject, and/or total variability, if available.
- Subject-formulation interaction variance component ( $\sigma_D^2$ ) if individual bioequivalence criterion is used.
- $C_{min}$  (concentration at the end of a dosing interval),  $C_{av}$  (average concentration during a dosing interval), degree of fluctuation  $\{(C_{max}-C_{min})/C_{av}\}$ , and swing  $\{(C_{max}-C_{min})/C_{min}\}$  if steady-state studies are employed.

In addition the following statistical information should be provided for

$AUC_{0 \rightarrow t}$ ,  $AUC_{0 \rightarrow \infty}$ , and  $C_{max}$ .

- Geometric mean.
- Arithmetic mean.
- Ratio of means.
- Confidence intervals.

Logarithmic transformation should be provided for measures used for bioequivalence demonstration.

## 7. Rounding off of Confidence Interval Values:

Confidence interval (CI) values should not be rounded off; therefore, to pass a CI limit of 80-125, the value should be at least 80.00 % and not more than 125.00%.

## APPENDIX 2

### Food-Effect Bioequivalence Studies

#### 1. Study Design

A randomized, balanced, single-dose, two-treatment, two-period, two-sequence crossover design is recommended for food-effect bioequivalence studies. The test product and the reference listed drug product should be administered under fed conditions. An adequate washout period should separate the two treatments.

#### 2. Subject Selection

Food-effect bioequivalence studies are usually carried out in healthy human volunteers. An adequate number of subjects should complete the study so as to achieve sufficient power for appropriate statistical assessment, but should not be less than 12.

#### 3. Strength

Generally, the highest strength of a product should be tested in food-effect bioequivalence studies. In some cases, clinical safety concerns could warrants use of lower strengths of the dosage form. The lot and strength tested in the pivotal bioequivalence fasted study should be tested in the food-effect bioequivalence study. When multiple strengths of MR drug products are intended for marketing and the food-effect study is performed on one of these strengths, in-vitro dissolution testing should be conducted for all other strengths in three different pH media. Similarity of dissolution should be established. Lack of similarity of dissolution could indicate that additional food-effect studies should be performed using other strengths.

#### **4. Food Effect Meal**

The primary food-effect bioequivalence study should be performed under conditions expected to provide maximal perturbation due to presence of food in the gastrointestinal tract. A high fat (approximately 50% of total caloric content of the meal), high calorie (approximately 1000 calories) breakfast is therefore recommended as a test meal for food-effect bioequivalence study. A representative example is 2 eggs fried in butter, 2 slices of beef luncheon, 2 slices of toast with butter, 4 ounces of hash brown potato, 8 ounces of whole milk (i.e., approximately 150 protein calories, 250 carbohydrate calories, 500-600 fat calories). Alternative meals with equivalent nutritional content can be used. Details of the meal should be recorded prior to the study and provided in the study protocol.

#### **5. Administration**

Following an overnight fast of at least 10 hours, subject should be served the food effect meal and ingest this meal within 30 minutes. The drug product should be administered with 180 ml of water immediately (within 5 minutes) after completion of the meal. No food should be allowed for at least 4 hours post-dose. Water can be allowed *ad libitum* after 2 hours. Subjects should be served scheduled standardized meals throughout the remaining study period.

#### **6. Sample Collection**

For both treatment periods, timed biological fluid samples should be collected from the subjects to permit characterization of the complete plasma concentration-time profile for the drug and/or metabolite(s). Caution should be used when studying MR dosage forms (e.g., enteric-coated products) where coadministration with food can delay in vivo drug release. In such instances, sampling times should be adjusted to obtain the complete plasma concentration-time profile.

## 7. Data and Statistical Analysis

The following measurements should be obtained from the resulting concentration-time profiles:

- Area under the concentration-time curve ( $AUC_{0 \rightarrow t}$ ,  $AUC_{0 \rightarrow \infty}$ ).
- Peak concentration ( $C_{max}$ ).
- Time to peak concentration ( $T_{max}$ ).
- Lag-time ( $T_{lag}$ ) for delayed release products.

Individual subject parameters, as well as summary statistics (e.g., group averages, standard deviations, coefficients of variation, 90% confidence intervals {CI}) should be reported. The reference product administered under fed conditions should serve as the reference.

An equivalent food effect will be concluded when the 90% CI for the ratio of the means (population geometric means based on log-transformed data) of the test and the reference product fall within 80 – 125% for AUC and  $C_{max}$ . In certain cases a wider interval of 75% to 133% for  $C_{max}$  may be acceptable. If these CI criteria are not satisfied, the test formulation might not be considered equivalent to and interchangeable with the reference formulation. Clinical relevance of any change in  $T_{max}$ , and  $T_{lag}$  should be considered.

## REFERENCES

- Investigation of bioavailability and bioequivalence: The rules governing medicinal products in European Community. Vol. III, addendum 2, 149-169, 1991.
- Food and Drug Administration (FDA), Guidance for Industry: Bioavailability and bioequivalence studies for orally administered drug products general considerations. Informal communication by the Center for Drug Evaluation and Research (CDER), March 2003.
- Food and Drug Administration (FDA), Guidance for Industry: Statistical approaches to establishing bioequivalence. Informal communication by the Center for Drug Evaluation and Research (CDER), January 2001.
- Food and Drug Administration (FDA), Guidance for Industry: Food-Effect bioavailability and bioequivalence studies, Draft Guidance, Informal communication by the Center for Drug Evaluation and Research (CDER), October 2001.
- Food and Drug Administration (FDA), Guidance for Industry: Bioanalytical method validation. Informal communication by the Center for Drug Evaluation and Research (CDER), May 2001.
- Food and Drug Administration (FDA), Report by the bioequivalence task force on recommendation from the bioequivalence hearing conducted on Sept. 29-Oct. 1, 1986, by the FDA, 1988.
- Food and Drug Administration (FDA), Guidance on statistical procedures for bioequivalence studies using a standard two-treatment crossover design, Informal communication by the Division of Bioequivalence, Office of Generic Drugs, Rockville, MD, 1992.
- Committee for Proprietary Medicinal Products (CPMP), Working Party on Efficacy of Medicinal Products. Note for guidance: Investigation of bioavailability and bioequivalence. The European Agency for the Evaluation of Medicinal Products, July 2001.
- Canadian Health Protection Branch (HPB), Report on bioavailability of oral dosage formulations of drugs used for systemic effects, A. Drugs with uncomplicated characteristics, 1990.

- Canadian Health Protection Branch (HPB), Report on bioavailability of oral dosage formulations of drugs used for systemic effects, B. Drugs with uncomplicated characteristics, 1990.
- Canadian Health Protection Branch (HPB), Drugs Directorate Policy: CI Standard for comparative bioavailability, 1991.
- Multi-source Pharmaceutical Products. WHO guideline on registration requirements to establish interchangeability. WHO Technical Report Series, 1995.
- Midha, KK and Blume, H. Proceedings of bio-international 92, Bad Homburg, Germany, FIP-Paperback, Medpharm Scientific Publishers, Stuttgart, 1993.
- Schuirmann, DJ. A comparison of the two one-sided test procedure and the power approach for assessing the equivalence of average bioavailability. *J. Pharmacokinet. Biopharm.*, 15, 657-680, 1987.
- Hauschke, D., Steinijans, VW. and Diletti, E. A distribution-free procedure for the statistical analysis of bioequivalence studies. *Int. J. Clin. Pharmacol. Ther. Toxicol.*, 28, 72-78, 1990.
- Hamrell, MR. Informed guidance on the submissions of data for bioequivalence studies in computer format. Division of Bioequivalence, Food and Drug Administration, Rockville, MD, 1987.
- Westlake, WJ. Bioavailability and bioequivalence pharmaceutical formulations. In : Peace, KE. (Ed) *Biopharmaceutical statistics for drug development*. Marcell Dekker, New York, PP. 329-352, 1988.
- Abdou, HM. *Dissolution, Bioavailability and Bioequivalence*. Mack Publishing Co., Easton, PA, 1989.
- Food and Drug Administration (FDA), Division of Biopharmaceutics, Bioavailability protocol guideline for ANDA and NDA Submission, 1977.