Review Article

AN OVERVIEW OF BIOAVAILABILITY AND BIOEQUIVALENCE STUDY IN HUMAN SUBJECTS

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Abstract

Nowadays, the use of generic drug products increases to minimise the healthcare cost. With increased availability and use of generic drug products, healthcare professionals are encountered with a large number of multisource products from which they have to select therapeutically equivalent products. Generic substitution is of concern not only for healthcare professionals but also for pharmaceutical industries. Therefore, the present review described the general aspects of bioavailability and bioequivalence studies conducted in human subjects for developing the generic drug.

Introduction

Life expectancy of patients has increased globally during the last three decades due to the new drug discovery (brand-name drugs) as well as generic drug production. It is well known that most health care interventions occur through medication. The rising cost of medication has been contributing to the total overall cost of health care and thus receives considerable attention globally. A major strategy for lowering the cost of medication and thereby reducing its contribution to total health care costs, has been the introduction of generic equivalents of brand-name drugs (innovator drugs). This strategy has been effective in reducing total prescription cost by 11% without sacrificing.

The increased availability and use of generic drug products, healthcare professionals are encountered with a large number of multisource products from which they have to select therapeutically equivalent products. Generic substitution is of concern not only for healthcare professionals but also for pharmaceutical industries, consumers and government officials. Many research papers have pointed out the concern regarding standards for approval of generic products which may not always ensure therapeutic equivalence. Many guidelines/guidance and regulations covering the licensing of generic products have been introduced to ensure that the medicinal products reaching the market have well-established efficacy and safety profile.

Generic drugs have captured more than 65% of the global market and account for 66% of prescriptions filled in the United States but for less than 13% of the cost. Thus, because of the importance of generic drugs in health care, it is imperative that the pharmaceutical quality, safety, and efficacy of generics should be reliably compared with the corresponding innovator drugs (brand-name drugs). The US Food and Drug Administration (FDA) publishes a list of drug products and equivalents, approved drug products with therapeutic equivalence evaluations, commonly known as the “Orange Book”.

Generic pharmaceutical products need to conform to the same standards of quality, safety and efficacy of the originator's product. In addition, they should be clinically interchangeable with equivalent marketed products. To ensure interchangeability, the generic product must be therapeutically equivalent to the reference product. Therapeutic equivalence can be assured when the generic product is both pharmacologically equivalent/alternative and bioequivalent.

The efficacy and safety of medicinal products should be demonstrated by clinical trials which follow the guidance in 'Good Clinical Practice: Consolidated Guideline' (ICH E6) adopted by the ICH, 1 May 1996.

In BE studies, an applicant compares the systemic exposure profile of a test drug product to that of a reference drug product. For two orally administered drug products to be bioequivalent, the active drug ingredient or active moiety in the test product must exhibit the same rate and extent of absorption as the reference drug product.

Manufacturers seeking regulatory approval of competitive (generic) products (e.g., Abbreviated New Drug Application [ANDA]), must provide detailed bioavailability evidence showing head-to-head comparative performance of their product against the innovator's product. Such trials are fundamentally designed to establish clinical equivalence particularly as it relates to interchangeability or substitutability.
Selected pharmacokinetic parameters and preset acceptance limits allow the final decision on bioequivalence of the tested products. AUC, the area under the concentration time curve, reflects the extent of exposure. Cmax, the maximum plasma concentration or peak exposure, and the time to maximum plasma concentration, tmax, are parameters that are influenced by absorption rate.26

**BIOAVAILABILITY**

According to Food and Drug Administration (FDA) guidance, Bioavailability is defined 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. For drug products that are not intended to be absorbed into the bloodstream, bioavailability may be assessed by measurements intended to reflect the rate and extent to which the active ingredient or active moiety becomes available at the site of action” (FDA, 2003).16

According to World Health Organization (WHO) guidelines, Bioavailability is defined as: “the rate and extent to which the active drug ingredient or therapeutic moiety is absorbed from a drug product and becomes available at the site of drug action” (WHO, 1986).20

**Types of Bioavailability**26,27

1. Comparative bioavailability or Relative bioavailability
2. Absolute bioavailability

**Comparative Bioavailability**

Comparative or relative bioavailability refers to a comparison of two dosage forms in terms of their relative rate and extent of absorption. In some instances, two pharmaceutical alternatives exhibit markedly different bioavailability, for example, a rapidly absorbed elixir and more slowly absorbed capsule. In other cases, two different dosage forms (e.g., a tablet and a capsule) may or may not exhibit very similar bioavailability.

Comparative bioavailability = \( \frac{AUC_{po} \cdot dose_{iv}}{AUC_{iv} \cdot dose_{po}} \)

**Absolute Bioavailability**27

Active pharmaceutical ingredient reaches to the systemic circulation and the range is F= 0 (No drug absorptions) if the drug is completely absorbed in the systemic circulation is F= 1. Since the total amount of drug reaching the systemic circulation is directly proportional to the area under curve (AUC), F is determined by comparing the respective AUCs of the test product and the same dose of drug administered intravenously.

Absolute bioavailability = \( \frac{AUC_{po}}{AUC_{iv}} \)

**Factors Influencing Bioavailability**

The systemic absorption of an orally administered drug can alter the drugs bioavailability and thereby its therapeutic effect. The various factors that can influence the bioavailability of a drug can be broadly classified as dosage form related or patient related.

1. **Disintegration of the drug product**

Disintegration time which measures the rate of breakup of the tablet or the capsule into the drug granules, disintegration time of a tablet is a poor measure of the bioavailability of the contained drug. This is because, in addition to disintegration time and particle size, other factors such as crystalline from (polymorphism), saturation solubility of a drug. The dissolution rate is perhaps a better parameter.

2. **Dissolution of the drug in the fluids at the absorption site**28

The dissolution rate which is the rate at which the drug goes into solution.

3. **Transfer of drug molecule across the membrane lining the gastrointestinal tract into the systemic circulation**29

The availability of drug into the portal system or intestinal mucosa represents an upper limit to the amount of drug that can reach the systemic circulation. While it is difficult to make direct measurements in the GI tract to quantify the rate and extent of drug absorption, important conclusions can be drawn from this point of view that allow a simplification of drug regulatory standards based on this mechanistic approach.

<table>
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<th>Bioavailability Factors Related To Dosage Form27</th>
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The physical and chemical characteristics of a drug as well as its formulation are of prime importance in bioavailability because they can affect not only the absorption of the drug but also its stability. Most important factors that affect the dissolution rate are slowly dissolving substances.

**Drug Interactions Affecting Absorption**

A. Change in gastric or intestinal pH Change in gastrointestinal motility
B. Change in gastrointestinal perfusion
C. Interference with mucosal function (drug-induced mal absorption syndromes)
D. Chelation and absorption
E. Exchange resin binding and solution in poorly absorbable liquid

**Study design and type of studies**30

The pattern of the study is designed in such a way that the effect of formulation can be easily distinguished from other effect. For instance, if the number two formulations were compared, through an open label, balanced, randomized, two-treatment, three-period, three-sequence, single dose, three way cross over design is the design of choice. Generally in practice there are two type of study taken under bioavailability and bioequivalence studies. These are

1. Fasting study
2. Fed study

**Fasting study**

Subjects fast for 10 hours prior to product administration. Normally, the highest safe strength/dose of the test or reference product should be administered on the experimental day with about 8 ounces (240 ml) of water. Further fluid will be withheld for 2 hours standardized meals are to be permitted after four hours after drug administration.

**Fed study**

In studies performed under fed conditions, the composition
of the meal is recommended to be according to the protocol of the originator product. If no specific recommendation is given in the originator protocol, the meal should be a high-fat (approximately 50 percent of total caloric content of the meal). The composition of the meal should be described with regard to protein, carbohydrate and fat.

**BIOEQUIVALENCE**

According to Food and Drug Administration (FDA) Guidance, 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” (FDA, 2003). 16

“Bioequivalence” is a comparison of the bioavailability of two or more drug products. It is the statistical equivalence between the generic and standard formulation of two drug products. When a new formulation of an existing drug is developed, its bioavailability is generally evaluated relative to the standard formulation of the originator. Indeed, a bioequivalence trial against the standard formulation is the key feature of an abbreviated new drug application (ANDA) submitted to the food and drug administration by a manufacturer who wishes to produce a generic drug.

For a generic drug to be considered bioequivalent to a pioneer product, there must be no statistical differences between their plasma concentration-time profiles. Since two products rarely exhibit absolutely identical profiles, some degree of difference must be considered acceptable.

**Reference Product** 22

Reference product is a pharmaceutical product with which the new product is proposed to be identical in clinical practice. The reference product would normally be the innovator product for which efficacy, safety and quality have been well known. When the innovator product is not available in the market leader may be used as a reference product, provided that it has been approved for marketing and its efficacy, safety and quality have been documented.

**Generic products**

Products whose active pharmaceutical ingredients, dosage form strengths and regimen are the same as those of new products. Generic and new products should be similar in the application area of dosage and their physicochemical characteristics should be similar to those of new products.

**Bioequivalence range** 31

Acceptable range of bioequivalence is generally 0.8% - 1.25% for the test or reference ratio of average values, when the parameters are logarithmically transformed. The acceptable range is generally ± 0.2 for the relative difference in vivo parameters between reference and test products, when the raw data are used.

**Equivalence**

Equivalence is a relative term that compares drug products with respect to a specific characteristic or function or to defined set of standards. There are three types of equivalences.

**Types of equivalence** 32

✓ Therapeutic equivalence
✓ Pharmaceutical equivalents

**A. Chemical Equivalence**

Two or more drug products contain the same labelled chemical substance as an active ingredient in the same amount.

**B. Therapeutic equivalence**

Two different medicinal products is said to be therapeutically equivalent when their active constituent is same, meeting same therapeutic moiety and show same clinical efficacy and safety.

**Pharmaceutical equivalents**

Two medicinal products contain the same molar amount of the same therapeutic moiety, pharmaceutical ingredient in the same dosage form, if they meet comparable standards. Pharmaceutical equivalence does not essentially imply therapeutic equivalence, as differences in the excipients or the manufacturing procedure and some other variables can lead to differences in product performance 33.

**PHARMACOKINETIC TERMS** 24

C<sub>max</sub>
This is the maximum drug concentration achieved in systemic circulation following drug administration.

C<sub>min</sub>
This is the minimum drug concentration achieved in systemic circulation following multiple dosing at steady state.

T<sub>max</sub>
It is the time required to achieve maximum drug concentration in systemic circulation.

AUC<sub>0-t</sub>
Areas under the plasma concentration - time curve, from 0 hr to the last quantifiable concentration to be calculated using the trapezoidal rule.

AUC<sub>0-∞</sub>
Area under the plasma concentration - time curve, from zero to infinity to be calculated as the sum of AUC0-t plus the ratio of the last measurable concentration to the elimination rate constant.

AUC<sub>0-t</sub>
Area under the plasma concentration - time curve over one dosing interval following single dose for modified release products.

Kel
Apparent first- order terminal elimination rate constant calculated from a semi-log plot of the plasma concentration versus time curve.

T<sub>1/2</sub>
Elimination half life of a drug is the time necessary to reduce the drug concentration in the blood, plasma or serum to one- half of its initial concentration.

When bioequivalence studies are necessary and types of studies required 35

Bioequivalence can be demonstrated in two types
✓ in vivo
✓ in vitro

**In vivo Bioequivalence Studies**

The following sequence of criteria is useful in assessing the need for in vivo studies:
- Oral immediate-release products with systemic action.
• Indicated for serious conditions requiring assured response.
• Narrow therapeutic margin.
• Pharmacokinetic complicated by absorption < 70% or absorption window, nonlinear kinetics, pre systemic elimination > 70%.
• Unfavourable physiochemical properties, e.g. low solubility, metastable modifications, instability etc.
• Documented evidence for bioavailability problems

**In vitro Bioequivalence Studies**

If none of the above criteria is applicable, comparative in vitro dissolution studies will suffice, in vitro studies, i.e., dissolution studies can be used in lieu of in vivo bioequivalence under certain circumstances called as bio waivers (exemptions)

• The drug product differs only in strength of the active substance it contains, provided all the following conditions hold
• Pharmacokinetics is linear
• The qualitative composition is the same
• The ratio between active substance and the excipients is the same
• Both products are produced by the same manufacturer at the same production site
• A bioavailability or bioequivalence study has been performed with the original product
• Under the same test conditions, the in vitro dissolution rate is the same
• The drug product has been slightly reformulated or the manufacturing method has been slightly modified by the original manufacturer in ways that can convincingly be argued to be irrelevant for the bioavailability

**Design and Conduct of Studies**

The basis of a bioequivalence study is the comparison of the drug product to be tested with an appropriate reference product. In bioequivalence studies an applicant compares the systemic exposure profile of a test drug to that of a reference drug product. Bioequivalence of two products can be assessed using in vitro standards, pharmacokinetic profile, clinical or pharmacodynamic end points.

**Different approaches for determination of bioequivalence of a drug product are:**

1. An in vivo test in humans in which the concentration of the active ingredient and when appropriate, its active metabolites, in blood, plasma, serum or other suitable biological fluid is measured as a function of time.
2. An in vivo test in humans in which the urinary excretion of the active ingredient and when appropriate, its active metabolites are measured as a function of time.
3. An in vitro test that has been correlated with and is predictive of human bioavailability profile or the one acceptable to FDA (e.g. dissolution rate test) that ensures human in vivo bioavailability. An in vivo test in humans in which an appropriate pharmacological effect of the active ingredient and when appropriate, its active metabolites are measured as a function of time if this effect can be measured with adequate accuracy, sensitivity and reproducibility. Well-controlled clinical trials that establish the efficacy and safety of the drug product, for purpose of determining bioavailability, or comparative clinical trials, for purpose of demonstrating bioequivalence. Any other approach considered adequate by the FDA to measure bioavailability or ascertain bioequivalence.

• Bioequivalence for most of oral tablets or capsules is demonstrated in vivo by comparing the rate and extent of absorption that is bioavailability of the generic product with that of the innovator product. This is done by measuring the active ingredient concentration in blood, plasma, serum or other biological fluids over a certain period of time for both the generic and innovator products, also called test and reference drugs respectively. By doing so the bioequivalence studies frequently rely on pharmacokinetic measures such as area under the concentration-time curve (AUC) and peak drug concentration (Cmax).

**Study design**

• Generally single-dose pharmacokinetic studies are recommended for both immediate and extended-release drug products as they are more sensitive in assessing the active ingredient released from drug into circulation. For assessing bioequivalence of two formulations of a drug, two-sequence, two-period, crossover study is conducted after administration of single dose under fasted conditions.

• In crossover design the subjects serve as their own controls and they crossover from one treatment to the other. A large variability in drug clearance often exists among the individuals. However the intra-subject variation is usually smaller relative to inter-subjects variability. Parallel studies are appropriate if the drug has extremely long half life, repeated pharmacokinetic profile is difficult to obtain, or residual pharmacodynamic effects are relevant. Furthermore, if carry over effects from one treatment period to another area of concern or if intra-subject variability is high, then replicate design is used. Non-replicate study designs are usually recommended for bioequivalence studies of most of the orally administered, modified-release and immediate-release dosage forms. Replicate study designs are often recommended for bioequivalence studies of highly variable drug products (intra-subject coefficient of variation ≥ 30%), including those that are modified release, immediate

• Release and other orally administered drug products. Replicate study designs have several scientific advantages compared to non-replicate designs.

**Study subjects**

The subjects should be selected with the objective of minimizing variability and permitting detection of difference between the drug products. Therefore, the study is normally carried out with healthy subjects. The study is performed in accordance with the Declaration of Helsinki for biomedical research involving human subjects35 and the Guideline for Good Clinical Practice.12

The subjects recruited for bioequivalence studies should be 18 years of age or older and capable of giving informed consent. Generally adults between 20-40 years should be selected. According to FDA guidance and Canadian and European guidelines, a minimum of 12 subjects are...
recruited for bioequivalence studies. For logistic reasons the total number normally does not exceed 24 subjects. The subjects should be in good health. The subject’s health is assessed by medical examination including medical history and laboratory tests. They should be screened for the history of use of medications or drugs of abuse, alcohol intake and smoking. The subjects should not take any medication one week before start of study.  

Drug administration and sampling

A bioequivalence study should be a single dose comparison of test drug with appropriate reference drug product carried out in healthy adults. The drug is administered to the subjects in fasting state, unless some other approach is more suitable for valid scientific reasons. Co-administration of food with oral drugs may either enhance or interfere with drug absorption. Thus, feeding increases the inter- and intra-subject variations in rate and extent of absorption. The sponsor should provide the rationale for conducting bioequivalence study under fed or fasting conditions.  

The subjects are randomly selected for each group in the study and the sequence of drug administration is randomly assigned to the individuals. In a typical situation of comparing a test formulation (T) with a reference formulation (R), the two-period, two-sequence crossover design. Subjects are randomly allocated to two treatment sequences; in sequence 1, subjects receive the reference drug and test drug in periods 1 and 2 respectively, on the other hand in sequence 2, subjects receive the drug products in reverse order. The administration of each product is followed by a sufficiently long wash out period of time to ensure complete elimination of drug before next administration. A time period of more than 5 half-lives of the drug is considered adequate washout period.  

In selected cases, it may be necessary for the test and reference products to be compared after multiple-dose administration to determine steady-state levels of the active drug moiety. A multiple-dose study should be crossover in design, unless a parallel or other design is more suitable for valid scientific reasons.  

In fasted state studies an overnight fast of at least 10 hours is recommended. Generally in single dose studies the highest marketed strength is administered. The doses of the test and reference products should be same. The test or reference products are administered with 240 ml of water. Liquids are allowed after one hour and standard meal after 4 hours of drug administration. In all the studies the standardization of study environment, diet, fluid intake and exercise is important.  

In most of the conditions, blood or plasma is collected rather than urine. Blood samples are drawn at appropriate times to assess the absorption, distribution, metabolism and elimination phases of the drug. For most of the drugs 12-18 samples are recommended including pre-dose sample from each subject. Generally sampling for a period equal to at least 3 times the terminal half life of the drug is recommended. Other approach is that the duration of sampling should be sufficient to define at least 80% of the total area under the concentration–time curve (AUC). The exact timings for sampling depend on nature and pharmacokinetic profile of individual drug and its dosage form.  

Experimental Design  

A. Pilot Study

Pilot study in a small number of subjects can be carried out before proceeding with a full BE study. The study can be used to validate analytical methodology, assess variability, optimize sample collection time intervals, and provide other information. A pilot study that documents BE can be appropriate, provided its design and execution are suitable and a sufficient number of subjects [e.g., 12 (maximum 12 subjects)] have completed the study.  

B. Pivotal Study

Those studies that provide the significant evidence that is the basis for the decision as to the risk-benefit assessment for a particular FDC.  

C. Nonreplicate Study

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.  

D. Replicate Study

Replicate study designs are recommended for bioequivalence studies of highly variable drug products (within-subjects coefficient of variation ≥ 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-subjects variance for the test and reference products  
(ii) Indicate whether a test product exhibits higher or lower within-subjects variability in the bioavailability measures when compared to the reference product  
(iii) Suggest whether a subjects-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.  

IND/NDAs

Documentation of bioequivalence may be useful during the investigation of new drug to find links between (I) early and late clinical trial formulations (II) formulations used in clinical trial and constancy studies, if different (III) clinical trial formulations and to be marketed pharmaceutical drug product (IV) other evaluation, as appropriate. In each comparison, the new formulation or new method of manufacture is the pharmaceutical test product and the prior formulation or method of manufacture is the reference product. It is suggested that the determination of re-document bioequivalence during the new drug application period because the pharmaceutical test product produces higher or lower measures of rate and extent of absorption in our body or because the performance of the pharmaceutical...
test or reference is more. In some cases, “BIOINEQUIVALENCE” is observed because of insufficient numbers of subjects entered into the BE study.

ANDAs

Bioequivalence studies are a critical component of ANDA submissions. The purpose of these studies is to demonstrate BE between a pharmaceutically equivalent generic drug product and the corresponding reference listed drug (21 CFR 314.94). Together with the determination of pharmaceutical equivalence, establishing BE allows a regulatory conclusion of therapeutic equivalence. In addition to a BE study under fasting conditions, it is recommended a BE study under fed conditions for administered the immediate-release drug products in orally.

Bioequivalence Studies Needed For Marketing Authorization

Main mechanism of bioequivalence is used to link the multisource (generic) pharmaceutical drug product to the innovator’s original documentation on its safety and efficacy, the following framework is proposed to assist drug regulatory authorities in establishing requirements for proof of interchangeability by describing when bioequivalence testing is required for multisource (generic) pharmaceutical drug products. Further, it also defines the type of testing, in vivo and in vitro, which should be submitted for marketing approval.

Two different medicinal 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:

1. Comparative bioavailability (bioequivalence)
2. Comparative pharmacodynamic studies in humans
3. Comparative clinical trials
4. in vitro Studies

Acceptance of any test procedure in the equivalence documentation between two pharmaceutical products by a drug regulatory authority depends on many factors, including characteristics of the active drug substance and the drug product and the availability of resources to carry out a specific type of study. Wherever a drug produces meaningful concentrations in an accessible biologic fluid, such as plasma, bioequivalence studies are preferred. Wherever a drug does not produce measurable concentrations in an accessible biologic fluid, comparative clinical trials or pharmacodynamic studies may be necessary to document equivalence. In vitro testing, preferably based on a documented in vitro and in vivo correlation or on consideration based on the bio-pharmaceutics classification system, may sometimes provide an 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 urine to indicate the release of the drug substance from the drug product into the systemic circulation. This approach resets 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 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 urine samples are collected and assayed for the concentration of the drug substance and 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 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.

Comparison of BA Measures In BE Studies

An equivalence approach has been and continues to be recommended for BE comparisons. The recommended approach relies on (1) Criterion to allow the comparisons, (2) Confidence interval for the criterion, (3) BE is limit. Log-transformation of exposure measures before statistical analysis is recommended. BE studies are performed as single-dose, crossover studies. To compare measures in these studies, data have been analyzed using an average BE criterion. This guidance recommends continued use of an average BE criterion to compare BA measures for replicate and nonreplicate BE studies of both immediate- and modified-release products.

PHARMACOKINETIC ANALYSIS

Pharmacokinetic data analysis will include data from subjects who complete the study. All concentration values below the limit of quantification (BLQ) will be sent to zero for all pharmacokinetic and statistical calculations. Any missing samples will be reported as M, any non reportable concentration will be reported as NR and will not be included for pharmacokinetic and statistical analysis.

The following pharmacokinetic parameters for paroxetine will be calculated using non compartmental model of win Nonlin version 5.3 or higher version of Pharsight Corporation.

For single dose bioequivalence study the parameters are:

- Area under the plasma concentration-time curve from time zero to time t (AUC<sub>0-t</sub>) calculated by trapezoidal rule, where t is the last measurable time point.
- Area under the plasma / blood concentration-time curve from time zero to time infinity (AUC<sub>0-∞</sub>) where
AUC₀₋∞ = AUCₜ + Ct / λz

- Ct is the last measurable drug concentration and λz is the terminal elimination rate constant calculated according to an appropriate method. The terminal or elimination half life of the drug should also be documented.

- Peak drug concentration (Cmax) and the time to peak drug concentration (Tmax), obtained directly from the data without interpolation.

For multiple-dose studies, the parameters measured are:

- Area under the plasma concentration-time curve from time zero to time t over a dosing interval at steady state (AUC₀ₜ), where t is the dosing interval.
- Peak drug concentration (Cmax) and the time to peak drug concentration (Tmax), obtained directly from the data without interpolation, after the last dose is administered.
- Drug concentrations at the end of each dosing interval during steady state (Cmin).
- Average drug concentration at steady state (Cav), where Cav = AUC₀ₜ / t.
- Degree of fluctuation (DF) at steady state, where DF = 100% × (Cmax — Cmin) / Cav

**Primary pharmacokinetic parameters**

**AUC – ratio and Cmax**

The 90% confidence interval for this measure of relative bioavailability should lie within an acceptance interval of 0.85-1.25 in specific cases of a narrow therapeutic range the acceptance interval may need to be tightened.

Cmax : Maximum measured plasma concentration following each treatment.

AUC₀₋∞: The area under the plasma concentration versus time curve from time 0 to last measurable concentration as calculated by linear trapezoidal method.

AUC₀₋∞: The area under plasma concentration versus time curve from time 0 to infinity.

AUC₀₋∞ = AUCₜ + Ct / λz

**Where**

AUC₀₋∞ = AUC₀ₜ + ct/kel,

ct = last measurable concentration

kel = Terminal elimination rate constant

**Secondary pharmacokinetic parameters**

Tmax: Time of the maximum measured plasma concentration.

T 1/2: The elimination or terminal half life will be calculated as 0.693/kel

kel: The first order rate constant associated with the terminal (long linear) portion of the curve.

This is estimated via linear regression of time vs log concentration.

This parameter will be calculated by linear least squares regression analysis using at least last 3 or more none zero plasma concentration values.

**STATISTICAL ANALYSIS OF THE PHARMACOKINETIC PARAMETERS**

In our study statistical analysis will be performed on PK data of subjects by using SAS statistical software (version 9.2 or higher, SAS inc., USA).

In case of significant violation of inclusion or exclusion criteria affecting or influencing the results of the pharmacokinetic analysis or if they cannot be estimated, the subject will be excluded from the pertaining pharmacokinetic analysis. If necessary an unequal no of subjects per sequence will be used.

**Analysis of Variance**

The various pharmacokinetic parameters (AUC (AUC₀ₜ and AUC₀₋∞), Cmax) derived from the plasma concentration-time curve are subjected to ANOVA in which the variance is partitioned into components due to subjects, periods and treatments. The classical null hypothesis test is the hypothesis of equal means: µT=µR (i.e. products are bioequivalent), where - µT and µR represent the expected mean bioavailabilities of the test and reference formulations, respectively.

The alternate hypothesis therefore is H: µT ≠ µR (i.e. products are bioinequivalent)

An F test will be performed to determine the statistical significance of the effects involved in the model at a significance level of 5% (alpha=0.05).

**Ratio analysis**

Ratio of least squares means for test and reference listed drugs (RLD) formulations will be computed and reported for Ln-transformed pharmacokinetic parameters Cmax, AUC₀ₜ, and AUC₀₋∞.

**Power Analysis**

The power of ANOVA test to detect a 20% mean difference between test and reference listed drug (RLD) formulations will be reported.

**Acceptance Ranges**

**Area under the curve ratio**

The 90% confidence interval for this measure of relative bioavailability should lie within a bioequivalence range of 0.80 to 1.25. If the therapeutic range is particularly narrow, the acceptance range may need to be required based on clinical justification. A larger acceptance range may be acceptable in exceptional cases if justified clinically.

**For highly variable drugs**

In the context of BE, HVDs are considered to be drugs and drug products exhibiting intra-subject variability greater than 30% coefficient of variation in the pharmacokinetic measures, AUC and Cmax. Due to this high variability, large sample size may be needed in BE studies to give adequate statistical power to meet FDA BE limits, and thus designing BE studies for HVDs is challenging. Consequently development of generic products for HVDs is a major concern for the generic drugs industry. Major regulatory agencies also considered different approaches for evaluating BE of highly variable drugs. From 2004 onward the FDA started looking for alternative approaches to resolve this issue and eventually found that replicate crossover design and scaled average BE provides a good approach for evaluating the BE of highly variable drugs and drug products as it would effectively decrease sample size, without increasing patient risk. Recently the FDA has issued Method for Statistical Analysis Using the Reference Scaled Average Bioequivalence Approach for Progesterone Capsules, which clearly states how to perform statistical analysis for HVDs, such as progesterone using the replicate crossover design and reference scaled ABE approach.
Reference

17. Food and Drug Administration (FDA), Bioavailability and bioequivalence requirement 2011.

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