Chapter

Bioavailability and Bioequivalence Studies

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Abstract

In vivo bioavailability studies are performed for new drug to establish essential pharmacokinetic parameters including rate of absorption, extent of absorption, rates of excretion and metabolism and elimination half-life after a single and multiple dose administration. These essential pharmacokinetic parameters are useful in establishing dosage regimens. Bioequivalence used to assess the expected *in vivo* biological equivalence of two proprietary preparations of drug products. If two drugs are bioequivalent, it means that they are expected to be same for all intents and purposes. In determining bioequivalence between two drugs such as a reference drug or brand and potential to be test drug or marketed generic drug. Pharmacokinetic studies are conducted whereby each of the drugs is administered in a cross over study to healthy volunteer's subjects. Plasma is obtained at regular intervals and assayed for parent drug or metabolite concentration to compare the two drugs. For comparison purpose of two formulations, the plasma concentration data are used to assess key pharmacokinetic parameters. If 90% confidence interval for the ratio of the geometric least square means of peak plasma concentration, area under curve of test and reference drugs are within 80–125%, then bioequivalence will be established.

Keywords: bioavailability, bioequivalence studies, pharmacokinetic parameters, cross over study, area under curve

1. Introduction

Bioavailability is defined as relative amount of drug from an administered dosage which enters the systemic circulation and the rate at which the drug appears in the systemic circulation. The bioavailability studies are done by measuring the concentration of the drug in the plasma or blood after administration of drug following systemic protocol of studies and documented over time. The systemic protocol is helpful for clinical trials in the early drug development, and the data obtained are used in subsequent bioequivalence studies. Bioequivalence studies were carried out to distinguish between two pharmaceutical products containing the same active substance. One drug formulated into two different formulations if they show to be therapeutically equivalent to one another in order to be considered interchangeable.

Pharmacokinetics of drug deals with the change in drug concentration in plasma and/or its metabolites in the human or animal body with respect to time following administration of the pharmaceutical product. Bioequivalence studies are used to assess the expected *in vivo* biological equivalence of two proprietary preparations of a drug. If two pharmaceutical products are said to be bioequivalent, then they

would be expected to be the same for all intents and purposes. Bioequivalence of a drug product is achieved if its extent and rate of absorption are not statically significantly different from those of reference product when administered at the same molar dose. If the bioavailability of two formulations administered in the same molar dose is similar, then they are said to be bioequivalent [1–10]. Different test methods are available to assess equivalence, including:

1. Comparative bioavailability studies, in which the active drug substance is measured in an accessible biological fluid such as plasma
2. Comparative clinical trials
3. Comparative pharmacodynamic studies in humans

Bioavailability and bioequivalence studies are required to ensure therapeutic equivalence between a pharmaceutically equivalent test drug and a generic drug or reference drug. Ensuring uniformity in standards of quality, efficacy, and safety of pharmaceutical products is the fundamental responsibility of central drugs standard control organization (CDSCO) [11]. Bioequivalence has to be considered for various products containing active ingredients marketed under different licensees are clinically equivalent and interchangeable. Submission of application for new drugs under schedule Y should be required to furnish the bioavailability and bioequivalence data, that is, mainly focus on the drug release from the pharmaceutical dosage form and subsequent absorption into the systemic circulation.

Comparative bioavailability or relative bioavailability refers to a comparison of two pharmaceutical dosage forms in terms of their relative rate and extent of absorption. In some cases, two pharmaceutical formulations exhibit markedly different bioavailability, for example, a rapidly absorbed elixir and more slowly absorbed capsule. In other cases, two different dosage formulations such as tablet and a capsule may or may not exhibit very similar bioavailability [12].

$$\text{Comparative bioavailability} = \frac{AUCpo \times Doseiv}{AUCiv \times Dosepo}. \quad (1)$$

Absolute bioavailability refers to an active pharmaceutical ingredient reaching the systemic circulation and fraction of drug absorbed ranges from 0 to 1. If F is zero, it means no drug absorptions, and the drug is completely absorbed in the systemic circulation if F = 1. The total amount of drug reaching the systemic circulation is directly proportional to the area under curve (AUC), and fraction of drug absorbed is determined by comparing the respective AUCs of the test product and the same dose of the drug administered intravenously [13].

$$\text{Absolute bioavailability} = \frac{AUCpo}{AUCiv} \quad (2)$$

1.1 Types of studies required in bioequivalence studies

For certain drugs, *in vivo* equivalence was done through either a bioequivalence study or a comparative clinical pharmacodynamic study. For oral immediate drug release formulations with systemic action have one or more adverse conditions like narrow therapeutic window, steep dose-response curve, nonlinear pharmacokinetics, presystemic elimination, unfavorable physicochemical properties.

Physicochemical properties such as solubility and instability of the drug, metastable transformation, poor permeability, etc., are bioavailability problems related to the drug or drugs having similar chemical structure or formulations, where a high ratio of excipients to active ingredients exists. Drugs administered other than oral and parenteral formulations design act by systemic absorption, sustained release drug formulations design act by systemic absorption, fixed dose combination products with systemic action, nonsolution pharmaceutical products which are for nonsystemic use and intended act without systemic absorption are also studied.

In these cases, the bioequivalence concept is not suitable, and then comparative clinical or pharmacodynamic studies are required for proving equivalence. Bioequivalence studies are used to establish links between the early and late clinical trial formulations, formulations used in clinical trials and stability studies, clinical trial formulations and to be marketed drug products. In each comparison, the new formulation or new method of manufacture shall be the test drug, and the prior formulation shall be considered as the reference drug.

1.2 When no need of bioequivalence studies

In some formulations, bioequivalence studies are not required if bioequivalence between a test drug and a reference drug may be considered self-evident with no further requirement for documentation such as when a gas is in the form of test drug, when test drugs are to be administered parenterally such as subcutaneous, intramuscular, intravenous, etc. as aqueous solution and contain the same drug in the same concentration and the same excipients in comparable concentrations. Bioequivalence studies are not required for when the test drug is in the form of solution for oral use and contains the drug in the same dose and does not contain an excipient that is known to affect gastro-intestinal absorption of the drug; when the test drug is in the form of an ophthalmic or topical product prepared as aqueous solution and contains the same active ingredients in the same concentrations and essentially the same excipients in comparable concentrations when the test drug is in the form of powder for reconstitution as a solution and the solution meets either above second and third points, when a test drug is in the form of an inhalation or a nasal spray tested by administered with or without the same device used for reference drug.

2. Design and conduct of pharmacokinetic studies

2.1 Study object

The object of the bioavailability study decides the study protocol. A study protocol used for estimating pharmacokinetic parameters is different from a bioequivalence study carried out for comparing the test formulation with standard formulation.

2.2 Study design

The main object of the experimental design is to minimize the experimental variables and to avoid a bias [14]. *In vivo* bioavailability study is determined by taking into consideration of the following points:

1. The nature of the reference drug and the dosage form to be tested

2. Benefit risk ratio considerations in regard to testing in humans

3. The availability of analytical methods

4. What is the scientific questions to be answered

Bioavailability studies are influenced by various factors such as age, sex, disease state, food habits, physical and mental health condition, body weight human volunteer, experimental design, time of administration, time of sampling, analytical method used and compartment model used in estimating pharmacokinetic parameters or bioavailability that contribute to the observed blood concentration time profile. Therefore, it is necessary to consider all these important factors in a study design.

The bioavailability study should be designed in such a way that the formulation effect can be distinguished from other effects. If two formulations are to be compared, a two-period, two-sequence crossover design is the design of choice which should ideally be equal to or more than five half-lives that have to be measured. Alternative study designs include the parallel design for very long half-life substances with highly variable disposition [15].

In the following sections, various factors are discussed keeping the bioequivalence study also in mind. However, they are valid for simple bioavailability studies also.

2.2.1 Parallel design

In a parallel design, two formulations are administered to two groups of volunteers. To avoid a bias, formulations may be administered randomly to the volunteers. The major disadvantage of this design is that the intersubject variation is not being corrected. It has been proved beyond doubt that most of the times intersubject variation is greater than the variation between any formulation. Therefore, a cross over design is preferred in bioavailability or bioequivalence trails to avoid influence of a intersubject variation. This design is used mainly for drug, and its metabolites have long elimination half-life. The carryover effects or dropouts were less in parallel studies compared to crossover studies.

2.2.2 Crossover design

As recommended by the USFDA [7], in most bioequivalence studies, a test drug is compared with the standard reference drug in a group of normal healthy subjects of age 18–55 years, each receives both the treatments alternately, in a crossover fashion (two-period, two-treatment crossover design), with the two phases of treatment separated by a washout period of generally a week's duration and it mainly depends on the half-life of the drug [16]. If elimination half-life of the drug increases, the washout period also increases. The drug formulation either test or reference is given to each human volunteer randomly but an equal number of subjects receives each treatment in each period, as given in **Table 1**. In case of two treatments, groups 1 and 2, one group receives the treatment in the order A and B, and the second group receives in the reverse order B and A. A similar allocation is done in case of a three-treatment crossover design (three-period, three-treatment crossover design). Intersubject variability is observed for several drugs in clearance. The intrasubject coefficient of variation (approximately 15%) is usually substantially smaller than that between subjects (approximately 30%), and therefore crossover designs are generally recommended for bioequivalence studies.

In crossover design, the treatments are compared on the same human subject, and the intersubject variability is reduced. Both the designs depend on the three fundamental statistical concepts of study design, and these are randomization, replication, and error control. Randomization means allocation of treatments to the

Group No.	**Subject in group**	**Treatment for period No.**			
Two-way crossover					
		I		II	
1.	1, 2, 3, 4, 5, 6	A		B	
2.	7, 8, 9, 10, 11, 12	B		A	
Three-way crossover					
		I	II	III	
1.	1, 2, 3, 4, 5, 6	A	C	B	
2.	7, 8, 9, 10, 11, 12	B	A	C	
3.	13, 14, 15, 16, 17, 18	C	B	A	
Four-way crossover					
		I	II	III	IV
1.	1, 2, 3, 4, 5, 6	A	B	C	D
2.	7, 8, 9, 10, 11, 12	B	D	A	C
3.	13, 14, 15, 16, 17, 18	C	A	D	B
4.	19, 20, 21, 22, 23, 24	D	C	B	A

Table 1.
Latin square design.

subjects without bias. Replication involves the application of more than one experimental subject for reliable estimates than a single observation and also provides a more precise measurement of treatment effects. The number of replicates required mainly depends upon the degree of differences to be detected and inherent variability of the data. Commonly used cross over designs in bioavailability trails are Latin square cross over design and balanced incomplete block design.

A standard approach for conducting a comparative bioavailability study to use a randomized, balanced, cross over design called Latin square or complete cross over design is as shown in **Table 1**. Incomplete block design (BIBD) eliminates many of the difficulties encountered with the Latin square design. In this, each subject receives not more than two formulations, each formulation is administered the same number of times and each pair of formulations occurs together in the same number of subjects. **Table 2** shows BIBD four formulations A, B, C, and D. In this design, as discussed above, each subject receives two formulations, each formulation is administered six times and each pair of formulations occurs together in two subjects (the pairs are AB, AC, AD, BC, BD, and CD).

2.3 Washout period

In a Latin square cross over design, each subject receives each formulation, and even in BIBD, each subject receives two formulations at different occasions. The time interval between the two treatments is called "washout period." Washout period is required for the elimination of the administered dose of a drug so as to avoid the carryover. For most of drugs in crossover design, at least 10 half-lives should be allowed between treatments. This should ensure an elimination of 99.9% of the administered dose and a maximum carryover of less than 0.1% from first treatment. The number of washout period is a function of the half-life and the dose of the drug administered. The number of washout periods in a study depends upon the type of crossover design used and the number of formulations to be evaluated.

Subject	Treatment for period No.	
	I	II
1	A	B
2	B	A
3	A	C
4	C	A
5	A	D
6	D	A
7	B	C
8	C	B
9	B	D
10	D	B
11	C	D
12	D	C

Table 2.
Balanced incomplete block design (BIBD) for four formulations.

In case of digitoxin, which has a half-life of 6–9 days, the total study period exceeds 1 year if four formulations have to be evaluated using Latin square design. Because a very large number of drugs have been found to have half-lives between 1 and 10 hours, a washout period of 1 week was usually found suitable in most of the reported studies. It should be noted that the metabolites of the drug should also be eliminated from the body before the commencement of next treatment.

2.4 Drug product and reference standard

Test product may be new drug formulations developed by pharmaceutical technologists or new dosage forms of an existing drug. A test product may be compared to a reference standard recognized by the Food and Drug Administration for getting approval for marketing the drug product. Test product are generally evaluated to select best dosage form of a new drug or existing drug among different dosage forms, to select the best formulation of a new drug or existing drug among different formulations that have shown equal performance *in vitro* tests and to compare biological performance of a test product to that of a recognized standard [17, 18].

A generic product has to compare with some standard dosage form to verify it's *in vivo* performance. In general, Food and Drug Administration (FDA) accepts any innovator's drug product as a reference standard. The innovator is the one who originally received approval from the FDA to market the product in the country. Sometime, several manufactures may hold approval for certain drugs. Therefore, any one of the permitted drug products can be used as a reference standard. In many of these instances, the FDA would request that only of these products be used as a reference product in order to obtain a more easily comparable data.

Most of the times orally administered dosage forms are subjected for bioavailability studies. However, dosage forms administered by other routes such as buccal, transdermal, and intramuscular should also be evaluated for their biological performance. The therapeutic utility of these dosage forms depends on the rate and extent of absorption of the drug from these dosage forms. Orally administered dosage forms show a much variation in their performance because of intersubject and intrasubject variations.

2.5 Single versus multiple dose study design

If the dosage forms are to be evaluated only for bioequivalence purposes, single dose studies are sufficient. This is because the relative bioavailability of most tablets and capsules can be determined on a single dose basis and usually this is predictive of multiple dose levels. Dosage forms determined for a single dose administration for a therapeutic benefit such as analgesic for the relief of head ache needs only single dose studies [19]. However, certain dosage forms designed to achieve special release profiles of drugs may require multiple dose studies like time release products, enteric-coated preparations, and some intramuscular injections. Even the drugs that undergo the first pass metabolism do need a multiple dose study.

2.6 Administration of drug products and sampling

Administration of drug products or formulations to the subjects should be based on randomization. After the administration, blood samples are withdrawn from the subjects at fixed time intervals. Some time is taken to withdraw a sample from each subject, and the total time difference between first subject and the last subject may range from 10 to 20 minutes depending upon the number of subjects and technicians involved in the study. If the sampling schedule is not followed rigorously in the same sequential manner, significant differences can conceivably exist in the actual duration of the drug in the body and the stated sampling time given for each subject. This 10 to 20 min difference in sample withdrawal from each subject during the study would represent a substantial change in the drug concentrations observed in the blood if under these conditions treatments are administered to the subjects in a sequential manner [20, 21].

If the bioavailability of a given dosage form is to be evaluated by a blood level study, some estimate of the area under the serum concentration versus time curve, peak plasma concentration (C_{max}), and time of peak plasma concentration (T_{max}) must be obtained from the study. Therefore, the frequency of sampling and the duration of sampling are very important for study. It will vary with the drug. There must be sufficient sampling points to allow for proper evaluation of the area under the blood level curve. A blood sampling done up to three to five half-lives of the drug, and if the half-life of the drug is not known, blood sampling should proceed until 1/10 or 1/20 of the peak levels are reached.

Urinary excretion studies are used when it is either not possible to measure a given drug in the blood, plasma or serum or when ethical considerations do not allow the collection of samples over a period of time. The advantage of this method is it involves noninvasive method of sampling, concentration of the drug in the urine is often greater than serum and the amount of the drug excreted in urine is obtained directly. But it is not useful in estimating the absorption rate of rapidly absorbed drugs and sometimes metabolites may interfere with the estimation of the unchanged drug in the urine sample.

Sampling must be continued for a sufficient time period to ensure that the area extrapolated from the time of the last measured concentration to infinite time should be less than 20% of the total AUC. AUC calculations are not useful in case of enterohepatic recycling where the terminal elimination rate constant cannot be calculated accurately. In such case, at least three sampling points from absorption phase, three to four points from Tmax and four points during the elimination phase has been taken. Intervals between successive sampling points in terminal elimination phase are used to calculate the elimination rate constant. It should not be longer than the half-life of the study drug.

2.7 Selection of the number of subjects

The number of subjects should be sufficient in the study to allow for possible withdrawals or dropouts. In initial study, it is acceptable to replace a subject withdrawal or dropout once it has provided the substituted subject follows the same protocol originally intended for the withdrawn subject and subject is tested under similar conditions. The number of subjects involved in a study is determined by the following considerations:

1. The level of significant should be 0.05
2. The error variance associated with the primary characteristics to be studied as estimated from a pilot experiment, from previous studies
3. The expected deviation from the reference drug compatible with bioequivalence
4. The required power, normally >80% to detect the maximum allowable difference in primary characteristics to be studied

2.7.1 Selection criteria for subjects

The studies should be performed on healthy adult volunteers with the aim to minimize variability between the study drugs. Subjects may be males or females; however, the choice of gender should be consistent with usage and safety criteria of the drug. To minimize intra and intersubject variation, the study design should be standardized as much as possible and acceptable.

2.7.2 Fasting and fed state considerations

Generally, a single dose study should be conducted after an overnight fast (at least 10 hours) and subsequent fasting of 4 hours after administration dosing. For multiple dose studies, 2 hours of fasting before and after the dose are acceptable. Estimation of C_{max} and T_{max} for the modified release products or drug is given with food in such case fed state studies also been carried out in addition to the normal fasting state bioavailability studies [22]. During fed state studies, the consumption of a high fat breakfast of 950–1000 KCals is required before dosing. The food intake containing at least 50% of these calories must come from fat, 15–20% calories from proteins, and the remaining from carbohydrates. A single standard diet should be followed taking into consideration of all the Indian subcontinent people. The high fat breakfast must be consumed approximately 15 minutes before dosing in fed state condition.

2.8 Study conditions

Study conditions such as study environment, diet, fluid intake, post dosing postures, exercise, sampling schedules, etc. are monitored during studies. These conditions are stated in the protocol, and at the end of the study, these should be complied, to assure that all variability factors involved in the study to minimize the products to be tested. Least 48 hours before commencement, the study subjects abstain from smoking, drinking alcohol, xanthine containing foods, coffee, tea and beverages, and fruit juices.

2.9 Steady state studies

Steady state study is considered in the following conditions:

1. The drug has a long terminal elimination half-life

2. Blood concentrations after a single dose cannot be achieved for a sufficient time.

3. For drugs, which are toxic or have adverse effects that are ethically should not be administered to patients but they are a necessary part of therapy (cytotoxics).

4. For modified release products or sustained release products which assess the fluctuation in plasma drug concentration at steady state.

5. Where the drug is likely to accumulate in the body.

6. For drugs that exhibit nonlinear, that is, dose or time dependent pharmacokinetics.

7. For combination products where the ratio of plasma concentration of the individual drugs is important.

8. For those drug which induce their own metabolism

9. For enteric coated preparations where the coating is innovative.

2.10 Analysis of biological samples

Ideally, the biological samples collected as per the sampling procedure have to be analyzed immediately after the study but most of the times the samples are stored for several days before subjected to analysis. During storage, the drug may undergo a chemical degradation, adsorption on the walls of the container, etc., so storage of plasma samples is an important aspect of bioavailability studies. The analytical method used for the estimation of the active ingredient responsible for the therapeutic efficacy must be selective and sensitive. Drugs, that undergo the first pass effect exhibit different unchanged drug/metabolite ratio depending on the rate of absorption. In the analysis of blood and urine, the major problem is to extract quantitatively and then separate the intact drug from its major metabolites or even to separate a mixture of two or more drugs from their metabolite.

2.11 Methods of assessment of bioavailability

Pharmacokinetic methods are used for the assessment of bioavailability of drug products that exists as a linear relation between the drug level in the biological fluid and therapeutic response. Therefore, these methods are also known indirect methods. Because therapeutically active drug can be accurately measured in biological fluids, plasma and urine data give the most objective information on bioavailability [23].

2.11.1 Indirect methods or pharmacokinetic methods

Plasma data are most widely used and accepted method for the assessment of bioavailability of the drug product. The basic assumption in this method is that drug products that are bioequivalent product super imposable plasma level time curve. The parameters T_{max} and C_{max} are the measures of the rate of absorption of the drug, while the parameters AUC is a measure of the extent of absorption.

Urinary excretion method is based on the general observation that the rate of urinary excretion of a drug is directly proportional to the concentration of the drug in the blood. Therefore, the bioavailability can be calculated as the ratio of the total amount of the unchanged drug recovered in urine following the administration of test and standard formulations. Urinary metabolite excretion data are not used for the estimation of bioavailability since the drug can undergo metabolism at different sites including the gut and liver, and the rates of metabolism may vary because of various reasons.

The relative bioavailability should lie within an acceptance range of 0.80–1.25 if 90% confidence interval is considered. In case of an especially narrow therapeutic range, the acceptance range may need to be tighter. In rare cases such as highly variable drugs, a wider acceptance range may be acceptable if it has right clinical justification. C_{max} ratio is the measure of relative bioavailability that may be more variable than the AUC ratio, and a wider acceptance range may be acceptable. The range used in the protocol should be justified taking into account safety and efficacy consideration. Tmax is a measure of release or action or signs for a relation to adverse effects.

2.11.2 Direct methods or pharmacodynamic methods

The pharmacodynamic methods are used when assessment of bioavailability by pharmacokinetic methods is not possible due to nonavailability of a sensitive analytical method for the measurement of the drug or the analytical methods lacks sufficient accuracy and/or reproducibility. The two pharmacodynamic methods used for the estimation of bioavailability are based on the measurement of acute pharmacological effect and clinical response. In order to estimate the bioavailability of a drug product accurately by measurement of acute pharmacological effect, the following criteria should meet. These are an easily measurable response such as heart rate, ECG, blood pressure, pupil diameter, etc. and an established dose-related response curve.

2.12 Statistical analysis of the data and analysis of variance (ANOVA)

Due to biological and experimental variations, some differences always exist, and it is necessary to ascertain whether these differences are simply chance occurrences or are due to actual differences in treatment administered to the subjects. Statistical methods are used to evaluate the pharmacokinetic data in order to identify the different sources of variation and if possible to measure the contribution of each identified variable and isolate the specific observation of primary interest. The analysis of variance (ANOVA), a statistical procedure that used for a crossover design is widely used method in bioavilability testing [24].

The pharmacokinetic parameters derived from blood drug concentration and time from bioavailability studies are subjected to ANOVA. In ANOVA, the variance is due to subjects, periods, and treatment. The classical null hypothesis test is considered where H0: $\mu T = \mu R$ if the pharmaceutical products are bioequivalent and alternate hypothesis therefore is H1: $\mu T \neq \mu R$ where products are bioinequivalent

where μT and μR are the expected mean bioavailability of the test and reference or standard drug, respectively.

Bioavailability studies are designed in two ways, and these are design 1 and design 2. Design 1 is parallel design in which the subjects divide into two treatment groups and assign one treatment to each group. Design 2 is crossover design in which each subject has one block and applies both the treatments to each subject with washout period in between them. In a parallel design, variability due to the treatment is considered, and in the crossover design, variability due to treatment, subject, and period are considered to minimize variability. Error sum of squares in design 1 (SSE1) and sum of error sum of squares in design 2 (SSE2) are equal. The error mean sum of square for design 1 (MSE1) will be greater than the error mean sum of square for design 2 (MSE2) if the degrees of freedom for SSE are the same in the both designs then error variability is greater in the parallel group design compared to the crossover design (**Tables 3** and **4**).

The mean sum of squares is compared with the mean sum of squares due to error (F = MST/MSE), and if these are comparable, no difference between the levels of a factor is concluded, otherwise a difference is achieved. The treatment mean sum of squares is larger than the error mean sum of squares if difference is achieved between the treatments. Then the chances of getting treatment mean sum of squares being bigger than the error mean sum of squares are more in design 2 compared to design 1. Therefore, chances of showing a statistically significant difference are higher in design 2 compared to design 1. This is equivalent to saying that design 2 is more competent than design 1. Null hypothesis H0 μT = μR provides an assessment amount of drug absorbed from the test product is identical or equal or similar to the amount of drug absorbed from the reference. They may be different or nearly equal but not identical in most of the cases. If the trial is run under tightly controlled conditions and the number of subjects is large enough, no matter how small the difference between the formulations and it will be detected as significant. The difference may give rise to following anomalies due to a large difference between two formulations, sample size not large enough (**Table 5**).

In some cases, simple null hypothesis was inappropriate and alternative approach to ANOVA for bioequivalence studies is considered as Type I and II error. Type I error is a manufacturer's risk that is explained by probability of rejecting a formulation which is in fact bioequivalent. Manufacturer's risk is the probability ($\alpha = 0.05$) of rejecting H0 when H0 is true. Similarly, type II error is the consumer's risk that is explained as the probability (β) of accepting a formulation which is bioinequivalent that is accepting H0 when H0 is false. FDA restricts the power of the test which should be 80% and the consumer's risk β to 20%, but this may not a satisfactory solution for either the consumer or the regulatory agencies. It makes

Sources of variance	Degree of freedom (D F)	Sum of squares (SS)	Mean of squares (MS)	F statistic
Treatment	T-1	SST	MST	MST/MSE
Subjects	N-1	SSS	MSS	MSS/MSE
Period	T-1	SSP	MSP	MSP/MSE
Error	(T-1)(N-2)	SSE	MSE	
Total	Tn-1			

T is the number of treatments, SST-sum of squares due to treatments, SSP-sum of squares due to period, MSS-mean sum of squares due to subjects, MST-mean sum of squares due to treatments, MSP-mean sum of squares due to period, and N is the number of subjects.

Table 3.
Analysis of variance (ANOVA) table for t-period, t-treatment crossover design.

Sources of variations	Sum of squares (SS)	Degree of freedom (DF)	Mean sum of squares (MSS)	F statistic
Between treatments	SST1	1	MST1	MST1/ MSE1
Error	SSE1	N-2	MSE1	
Total		N-1		

Table 4.
Design 1 A comparison of ANOVA for parallel group design and 2-treatment, 2-period crossover design with n subjects.

Sources of variance	Degree of freedom (DF)	Sum of squares (SS)	Mean of squares (MS)	F statistic
Between Treatments	1	SST2	MST2	MST2/MSE2
Subjects	N-1	SSS2	MSS2	
Between Period	1	SSP2	MSP2	
Errors	N-2	SSE2	MSE2	
Total	2N-1			

Table 5.
Design 2A comparison of ANOVA for parallel group design and 2-treatment, 2-period crossover design with n subjects.

sense that the regulatory authorities should control the consumer's risk and let the pharmaceutical company decide how much manufacturer's risk they are willing to accept. According to FDA guidelines for bioavailability studies state that "Products whose rate and extent of absorption differ by 20% or less are generally bioequivalent." The main object of bioequivalence studies is not in testing the null hypothesis of equality but to assess the difference between in two treatments groups and bioequivalence studies of two formulations is concluded that the difference is within 20% of the reference mean.

2.13 Characteristics to be investigated during bioequivalence studies

Evaluation of bioavailability and bioequivalence studies will be based upon the measurement of concentrations of the active drug substances in the plasma with respective of time. In some situations, the measurements of an active or inactive metabolite may be necessary. These situations include where the concentrations of the drugs may be too low to accurately measure in the biological matrix, limitations of the analytical method, unstable drugs, and drugs with a very short half-life. Racemates should be measured by an achiral assay method. Measurement of individual enantiomers in bioequivalence studies is required where they exhibit different primary efficacy, safety activity, pharmacodynamic and pharmacokinetic characteristics with the minor enantiomer. The pharmacokinetic parameters for product are C_{max}, T_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ and for steady state are $AUC_{0-\tau}$, C_{max}, C_{min}, and degree of fluctuation should be calculated from the plasma time concentration profile.

2.14 Bioavailability and bioequivalence testing

Bioavailability and bioequivalence testing are carried out for two formulations such as new and commercially marketed brand drug [25]. These studies are conducted by experimental designs such as parallel and cross over design in healthy

volunteer subjects but occasionally in patients. After administration of formulation under standard study conditions, plasma samples are withdrawn at regular time intervals and assayed for parent drug or occasionally metabolite concentration in plasma or urine. In some cases, concentration of drug in the blood is neither feasible nor possible to compare. Plasma concentration data are used to determine the pharmacokinetic parameters such as AUC, C_{max}, T_{max}, and absorption lag time (T_{lag}). Bioavailability studies should be conducted at different doses, especially when the drug follows nonlinear pharmacokinetics. In addition to a data from bioequivalence studies, other data may need to be submitted for evidence to meet regulatory requirements for bioequivalence includes analytical method validation and *in vitro-in vivo* correlation studies.

2.15 Criteria for bioequivalence

A 90% confidence interval is considered to establish bioequivalence for AUC, T_{max}, and C_{max} which should fall within the range of 80–125%. A 5% level of significance is taken for rejection of one sided t-test with the null hypothesis of bioequivalence. In bioavailability studies, closer limits are considered for drug that have a narrow therapeutic index, serious dose-related toxicity, steep dose, effect curve, and nonlinear pharmacokinetics within the therapeutic dose range. A wider acceptance range may be admissible if it is based on sound clinical justification. In case of suprabioavailability, a reformulation of the drug product is required and again bioequivalence study has to be carried out. Application of new formulation is required to support the clinical trial data especially for dosage recommendations. Such formulations are usually not being accepted as therapeutically equivalent to the existing reference drug.

3. Regulatory definitions

3.1 Australia

The Therapeutics Goods Administration (TGA) considers two formulations to be bioequivalent if the ratios between the two formulations of C_{max} and AUC should lie in the range of 0.80–1.25 and T_{max} should also be similar between the two formulations [25]. There are closer limits for drugs with a narrow therapeutic index and saturable metabolism. Thus, no generic drug formulations exist in for digoxin or phenytoin for instance in the Australian market.

3.2 Europe

European Economic Area considers two formulations to be bioequivalent if they have pharmaceutically equivalency and their bioavailabilities are similar after administration in the same molar dose with respect to both efficacy and safety. For bioequivalence of two dosage forms, 90% confidence intervals are considered as Australia.

3.3 United States

In case of FDA, two formulations are bioequivalent if the 90% confidence interval of the relative mean of C_{max}, $AUC_{0\text{-}t}$, and $AUC_{0\text{-}\infty}$ of the test or generic formulation should be within 80–125% in the fasting state. Sometimes, fed state bioequivalent comparison studies were carried out for test to reference

formulations where required to administer the formulations after an appropriate meal at a specified time before taking the drug to know the food effect. Food effect study requires the same statistical evaluation as the fasting study as described above.

4. Importance of bioavailability and bioequivalence studies

4.1 Universal approach about comparative bioavailability

Most bioavailability studies, whether for a new or generic product, are carried out for the common theme. These studies are conducted to identify the quantitative nature of a specific product comparison. The absolute bioavailability of new drug is used to assess the pharmacokinetic parameters of an oral formulation relative to that of an intravenous dose or performance of a modified release formulation in comparison to a conventional capsule. For a generic product, it is mainly done for comparison of a competitive formulation with a reference or standard drug. Such commonality in comparative bioavailability studies suggests a universal experimental approach.

4.2 Comparative bioavailability studies of new drugs (NDA)

Comparative bioavailability studies for new drug are used to conduct to determine the bioavailability and bioequivalence of the formulation in humans for safety and efficacy. Information about bioavailability of new drug formulation is obtained by comparing the pharmacokinetics parameters of an intravenous and oral administration of new drug formulations having the same dose [26].

4.3 Comparative bioavailability of generic drugs (ANDA)

When a manufacturer wishes to gain therapeutic equivalence for introducing a competitive generic product into the market place, it is not necessary to conduct the full batch of clinical trials needed for the first product. If therapeutic equivalence has been determined, study has to be carried out according to prescribed study requirements, and it should be similar or equivalent to the previous or innovator product. This is regarded as therapeutically equivalent to the innovative drug product [27].

4.4 Testing under fasting conditions or fed conditions

When the particular drug is not showing any expected results under fasting conditions, then the drug can also be tested under fed conditions to meet all conditions as per regulatory norms in bioequivalence studies.

5. Conclusion

The concept of bioavailability and bioequivalence studies has been adopted by the pharmaceutical industry and national regulatory authorities throughout the world over 20 years. It is mainly due to increasing the number of generic drugs and its formulations and marketed after regulatory acceptance. So, the bioavailability and bioequivalence studies carried under stringent protocols and modified according to the needs. Pharmacokinetic parameters are evaluated by the statistical

methods to get accurate results to assure high quality interchangeable and affordable drugs. There is a continuing attempts made by different organizations, authorities, and basic scientists to understand and develop more efficient and scientific valid approaches to evaluate bioavailability and bioequivalence studies of various formulations.

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References

[1] Midhal KK, McKay G. Bioequivalence: Its history, practice, and future. AAPS Journal. 2009;**11**:664-670

[2] Boix-Montanes A. Relevance of equivalence assessment of topical products based on the pharmacokinetics approach. The European Journal of Pharmaceutical Sciences. 2011;**42**:173-179

[3] Skelly JP. A history of bio pharmaceutics in the food and drug administration. AAPS Journal. 2010;**12**:44-50

[4] Midha KK, Rawson MJ, Hubbard JW. The bioequivalence of highly variable drugs and drug products. The International Journal of Clinical Pharmacology and Therapeutics. 2005;**43**:485-498

[5] Chen ML, Lesko LJ. Individual bioequivalence revisited. Clinical Pharmacokinetics. 2001;**40**:701-706

[6] Lamy PP. Generic equivalents: Issues and concerns. The Journal of Clinical Pharmacology. 1986;**26**:309-316

[7] Food and Drug Administration (FDA). Guidance for industry: Statistical Procedures for Bioequivalence Studies Using a Standard Two-treatment Crossover Design. U.S Department of Health and Human Services. Food and Drug Administration, Center for Drug Evaluation and Research (CDER). July 1992. Available from: www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidance/ucm07244.pdf

[8] Food and Drug Administration (FDA). Guidance for Industry, Waiver of *In Vivo* Bioavailability and Bioequivalence Studies for Immediate-Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System. U.S Department of Health and Human Services. Food and Drug Administration, Center for Drug Evaluation and Research (CDER). 2000. Available from: www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidance/ucm07246.pdf

[9] Food and Drug Administration (FDA). Guidance for Industry, Bio-analytical Method Validation. U.S Department of Health and Human Services. Food and Drug Administration, Center for Drug Evaluation and Research (CDER). Center for Veterinary Medicine (CVM). May 2001. Available from: www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidance/ucm070107.pdf

[10] Food and Drug Administration (FDA). Bioavailability and Bioequivalence Requirement. U.S Department of Health and Human Services. Food and Drug Administration, Center for Drug Evaluation and Research (CDER). Center for Veterinary Medicine (CVM). 2011. Available from: www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcf/cfsearch.cfem?Cfrpart=320

[11] Central Drugs Standard Control Organization (CDSCO). Guidelines for Bioavailability and Bioequivalence Studies. Directorate General of Health Service Ministry of Health and Family Welfare, Government of India, New Delhi. 2005. Available from: http://cdsco.nic.in/html/BE%20Guidelines%20Draft%over10%20march2005.pdf

[12] Chereson R. Bioavailability, bioequivalence, and drug selection. Basic Pharmacokinetics. 1997;**4**(8):1-21

[13] Ahmed AN, El Gamal S, Naggar V. Bioavailability: A pharmaceutical review. Journal of Novel Drug Delivery Technology. 2011:77-88

[14] Guidance for Industry Bioavailability and Bioequivalence Studies for Orally Administered Drug Products General Considerations. U.S Department of Health and Human Services. Food and Drug Administration, Center for Drug Evaluation and Research (CDER). 2001. Available from: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidance/uc154838.pdf

[15] Pidgen A. Bioequivalence and generic prescribing: An industrial view. The Journal of Pharmacy and Pharmacology. 1996;**48**:11-16

[16] Food and Drug Administration (FDA). Guidance for Industry, Statistical Approaches to Establishing Bioequivalence. U.S Department of Health and Human Services. Food and Drug Administration, Center for Drug Evaluation and Research (CDER). 2001. Available from: www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidance/ucm07244.pdf

[17] Foood and Drug Administration (FDA). Guidance for Industry, Bioavailability and Bioequivalence Studies for Orally Administered Drug Products—General Considerations. U.S Department of Health and Human Services. Food and Drug Administration, Center for Drug Evaluation and Research (CDER). 2003. Available from: www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidance/ucm072878.pdf

[18] Bioequivalence Requirements Guidelines. Kingdom of Saudi Arabia: Saudi Food and Drug Authority Drug Sector. 2005. pp. 5-8. Available on: http://www.sfda.gov.sa

[19] Asian Guideline for the Conduct Bioavailability & Bioequivalence Studies. 2004. pp. 5-10

[20] Health Canada. Conduct and Analysis of Bioavailability and Bioequivalence Studies: Part A: Oral Dosage Formulations Used for Systemic Effects (1992); Part B: Oral Modified Release Formulations (1996); Report C: Report on Bioavailability of Oral Dosage Formulations, Not in Modified Release Form, or Drugs Used for Systemic Effects, Having Complicated or Variable Pharmacokinetics (1992). Health Products and Food Branch Guidence Document. 1992-1996. Available from: http://www.hc.sc.gc.ca

[21] Satoskar RS, Bhandarakar SD, Ainapure V. Pharmacology and pharmacotherapeutics. Popular Prakashan. 2001;**17**:10-11

[22] Löbenberg R, Amidon GL. Modern bioavailability, bioequivalence and biopharmaceutics classification system. New scientific approaches to international regulatory standards. European Journal of Pharmaceutics and Biopharmaceutics. 2000;**50**:3-12

[23] Qayyum A. Bioequivalence studies. National University of Sciences and Technology Pakistan. 2012;**39**:5-9

[24] Thiessen JJ. Bioavailability and bioequivalence. 2002;**57**:58

[25] Mastan S, Latha TB, Ajay S. The basic regulatory considerations and prospects for conducting bioavailability/bioequivalence (BA/BE) studies—An overview. Comparative Effectiveness Research. 2011;**1**:1-25

[26] Guideline for Bioequivalence Studies of Generic Products for Topical Use. 2003. pp. 6-10

[27] Chen M-L, Shah V, Patnaik R, Adams W, Hussain A, Conner D, et al. Bioavailability and bioequivalence: An FDA regulatory overview. Pharmaceutical Research. 2001;**18**(12):1645-1650