

Bioequivalence Study: Concepts, Approaches, Design, Various Regulatory Prospects and Considerations

Akanksha¹, Roopam Tomar², Dr. Tanuja Singh³, Dr. Shalini Sharma⁴

¹Student, Sunder Deep Pharmacy College

²Assistant Professor, Sunder Deep Pharmacy College

^{3,4}Professor, Sunder Deep Pharmacy College

Abstract:

The study of bioequivalence involves comparing various drug brands and their dosage forms. When the rate of dissolution and absorption of two different formulations of the same drug is identical, they are bioequivalent. Comparing the therapeutic effectiveness of two drugs that have the same active ingredient is necessary to assess the possibility of replacing an innovator with similar pharmaceuticals. Conducting a bioequivalence study involves conducting two methods: in-vitro and in-vivo. In-vivo bioequivalence study is usually carried out in human and animal subjects by measuring the rate and extent of drug absorption in the blood stream after a drug has been administered. Highly reliable information can be obtained from in-vivo studies. Furthermore, living organisms have a greater degree of variability. Conducting multiple trials is necessary, and the cost is also important. A dissolution apparatus is used for in-vitro bioequivalence studies. The necessary biological conditions are provided and samples are collected and analysed on a periodic basis. The system can be controlled by conducting in-vitro studies. It also allows for the imitation of biological conditions. By using in-vitro studies, the cost and number of trials can be decreased. The pharmaceutical industry and national regulatory authorities worldwide have embraced the concept of “BE”. Efforts are being made to understand and develop more effective and scientifically valid approaches to assess bioequivalence of various dosage forms. This article provides a brief review of the BE concepts, approaches, designs, and various basic regulatory considerations and prospects for conducting BE studies.

Keywords: Bioequivalence, Active Pharmaceutical Ingredient, In-vivo, In-vitro

INTRODUCTION:

Assessing the possibility of replacing an innovator with essentially identical pharmaceuticals requires comparing the therapeutic efficacy of two drugs that have the same active ingredient. In practical terms, the best way to support therapeutic equivalence between pharmaceuticals is usually to show bioequivalence. Pharmacokinetic data, rather than therapeutic results, may be used to demonstrate bioequivalence as an established surrogate marker for therapeutic equivalence, assuming that similar plasma concentration time courses in the same subject will result in similar concentrations at the site of action and thus in similar effects.

Two pharmaceutical products are considered to be equivalent when their concentration v/s time profiles, from the same molar dose, are so similar that they are unlikely to produce clinically relevant differences in therapeutic and/or adverse effects.

Bioequivalence is the study of different brands of a same drug and its dosage forms. When the rates of dissolution and absorption of two distinct formulations of the same drug are the same, they are considered bioequivalent.

According to the Food and Drug Administration (FDA) 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”.

Over the past forty years, bioequivalence has drawn more and more attention as it became clear that pharmaceutical medications with identical dosages on the market could have very different therapeutic outcomes. These variations were generally well connected with different medication plasma levels, primarily resulting from poor absorption. Currently, a sizable body of research suggests that the amount of drug in the body or the plasma concentration correlates more strongly with drug response than does the dose given. As a result, bioavailability and bioequivalence studies have been established as appropriate stand-ins for costly, complex, and drawn-out clinical trials based on basic pharmacokinetic concepts and parameters. These studies are widely used globally to establish and ensure consistent quality and a reliable, therapeutically effective performance of marketed dosage forms.

When and what kinds of bioequivalence studies are required:

In vivo Studies:

In vivo documentation of equivalence using a comparative clinical trial, bioequivalence study, or comparative clinical pharmacodynamics study is considered to be particularly significant for certain drugs and dosage forms. Among them are:

- A. Oral immediate release drug formulations with systemic action.
- B. Non-oral and non-parenteral drug formulations designed to act by systemic absorption (such as transdermal patches, suppositories, etc.).
- C. Sustained or otherwise modified release drug formulations designed to act by systemic absorption.
- D. Fixed-dose combination products with systemic action.
- E. Non-solution pharmaceutical products which are for non-systemic use.

In vitro studies:

In vitro dissolution tests can be used to evaluate equivalence in the following situations:

- a. Drugs for which the applicant offers evidence to support each of the following:
 1. At 37°C, the maximum dose strength dissolves in 250 ml of an aqueous medium over a pH range of 1-7.5.
 2. When comparing an oral dose to an intravenous reference dose or doing a mass balance analysis, at least 90% of the dose should be absorbed.
- b. Drugs manufactured in various strengths by the same manufacturer, provided where all of the following criteria are fulfilled:

1. There is nearly no difference in the qualitative composition between the strengths.
2. The ratio of active ingredients and excipients is nearly identical between strengths. Even in small strengths, the ratio of excipients remains consistent.

When it is not required to conduct bioequivalence studies:

Bioequivalence between a new drug and the reference product may be self-evident in the following formulations and circumstances with no additional documentation requirements.

1. When newly developed drugs are to be administered parenterally (e.g., intramuscular, subcutaneous, intrathecal, etc.), they should be aqueous solutions containing the same excipients at comparable quantities and the same active ingredient(s) at the same concentration;
2. When the new drug is an oral solution with the same concentration of the active ingredient and no excipient that could alter the active substance's transit through the gastrointestinal tract or absorption.

Assessment of Bioequivalence Study:

The basic premise behind evaluating the BE of various drug products is that two products are equivalent if, under established experimental conditions, the rate and extent of absorption of the test/generic drug does not differ significantly from that of the reference/brand drug. According to various regulatory bodies, BE studies are often grouped as follows:

1. Pharmacokinetic endpoint studies.
2. Pharmacodynamic endpoint studies.
3. Studies on clinical endpoints.
4. In vitro endpoint studies.

These studies are arranged in general descending order of preference and include in vitro, clinical, pharmacokinetic, and pharmacodynamic research.

Pharmacokinetic endpoint studies:

Studies where the drug level can be found in a biological fluid which is easily accessible (plasma, blood, urine), and where the drug level is connected with the clinical effect, are the most commonly used to evaluate BE for pharmacological products. Utilizing pharmacokinetic measurements to show the release of the drug substance from the drug product with absorption into the systemic circulation is emphasized in the statutory definition of BA and BE, which is stated in the rate and extent of absorption of the drug's active component or ingredient to the site of action. Measures of systemic exposure should be utilized in BA and BE studies to account for clinically significant differences between test and reference products, according to regulatory guidance. These metrics include: i) peak exposure (C_{max}); ii) early exposure (partial AUC to peak time of the reference product for an immediate-release pharmaceutical product); iii) total exposure (AUC_{0-t} or $AUC_{0-\infty}$ for single-dose studies and $AUC_{0-\tau}$ for steady-state studies). In order to ensure equivalent treatment outcomes, reliance on systemic exposure measures will reflect comparable rates and extents of absorption. For the purpose of documenting BE, single dose experiments were chosen over multiple dosage studies due to their generally higher sensitivity in determining the in vivo release of the drug ingredient from the drug product. Regarding single-dose, multiple-dose, and urine data, **Table 1** lists the general pharmacokinetic parameters (primary and secondary).

The following situations require steady state pharmacokinetics and multiple-dose studies:

- Pharmacokinetics that is depending on time or dose.
- For modified-release products, where it is necessary to evaluate the variation in plasma concentration across a dosage interval at steady state.
- If problems of sensitivity preclude sufficiently precise plasma concentration measurements after single-dose administration.
- If BE cannot be demonstrated in an adequately large single-dose research because of intra-individual variability in plasma concentration or disposition, and if this variability reduces at steady state.
- When a single-dose trial is not feasible to do on patients or is not feasible to carry out on healthy volunteers because concerns about tolerability.
- If the medication's terminal elimination half-life is lengthy and it is not possible to track blood concentrations after a single dose for a sufficient amount of time.
- For medications that significantly vary within an individual or that cause their own metabolism.
- For combination products where it depends how much of each component's plasma concentration is available.
- If there's a chance the medication may accumulate in the body.
- For enteric coated preparations in which the coating is innovative.

Table 1 Brief description of the pharmacokinetic parameters used for BA/BE studies

Study type	Primary pharmacokinetic parameters	Secondary pharmacokinetic parameters
Single dose	C_{max} , AUC_{0-t} , $AUC_{0-\infty}$	T_{max} , AUC % extrapolation, MRT, K_{el} , and $T_{1/2}$
Steady state	$C_{max(ss)}$, $C_{min(ss)}$, AUC_{0-t}	$T_{min(ss)}$, $T_{max(ss)}$, C_{avg} , % swing, % fluctuation
Urinary based	$Ae_{(0-t)}$, $Ae_{(0-\infty)}$, R_{max}	T_{lag}

Notes: C_{max} , Maximum plasma concentration; C_{min} , Minimum plasma concentration; $C_{max(ss)}$, Maximum plasma concentration at steady-state; $C_{min(ss)}$, Minimum plasma concentration at steady-state; C_{avg} , Average plasma concentration; T_{max} , Time to C_{max} , AUC_{0-t} , Area under the plasma/serum/blood concentration–time curve from time zero to time t where t is the last time point with measurable concentration; $AUC_{0-\infty}$, Area under the plasma/serum/blood concentration–time curve from time zero to time infinity; AUC_{0-t} , AUC during a dosage interval at steady state; MRT, Mean residence time; $Ae_{(0-t)}$, Cumulative urinary excretion from pharmaceutical product administration until time t; $Ae_{(0-\infty)}$, Amount of unchanged API excreted in the urine at infinite time (7–10 half-lives); $T_{1/2}$, Plasma concentration elimination half-life; % fluctuation, $(C_{max(ss)} - C_{min(ss)})/C_{avg} \cdot 100$; % swing, $(C_{max(ss)} - C_{min(ss)})/C_{min} \cdot 100$.

Abbreviation: API, Active Pharmaceutical Ingredient; R_{max} , maximum rate of excretion or release rate; T_{lag} , lag time.

Blood should be the biological fluid collected to measure the concentration of drugs. The majority of drugs may be measured in serum or plasma; however, certain drugs, such tacrolimus, may require examination of whole blood. Urine can be used as the biological fluid to be sampled if the drug is

removed unchanged from the body in significant amounts (40%) and the blood concentrations are too low to be identified (eg, alendronic acid).

Pharmacodynamic end point studies:

Pharmacokinetic studies are often not adequate for documenting BA and BE from local administration; instead, they measure systemic exposure. If an acceptable pharmacodynamic endpoint is available, in such instances, BA can be evaluated and BE can be established based on a pharmacodynamic research. Pharmacodynamic evaluation measures the impact of two separate product administrations on a pathophysiological process, such as a time function, in order to provide a foundation for BE assessment. Regulatory authorities request applicants to provide explanations for the use of pharmacodynamic parameters and effects in the development of BE criteria. These investigations are usually needed in two situations. 1) if quantitative analysis of the drug and/or metabolite(s) in plasma or urine is not possible with sufficient sensitivity and accuracy; 2) if drug concentration measurement cannot be used as surrogate endpoints for the demonstration of efficacy and safety of the particular pharmaceutical product. Additional requirements for pharmacodynamic research include: i) to demonstrate a dose-response relationship; ii) collecting sufficient information to generate a suitable pharmacodynamic response profile; and iii) ensuring that the entire dose-effect curve remains below the maximum physiological response; iv) Specificity, accuracy, and repeatability should be confirmed for every pharmacodynamic measurement and method. Locally acting pharmaceutical items, oral inhalation drug products (such as metered dosage inhalers and dry powder inhalers), and topically applied dermatologic drug products (such as creams and ointments) are a few examples of these pharmacodynamic investigations. Products containing bronchodilators, like albuterol metered dose inhalers, cause the smooth muscle in the airways to relax. For these drug products, a pharmacodynamic endpoint, based either on increase in forced expiratory volume in 1 second (FEV1) or on measurement of PD20 or PC20 (the dose or concentration, respectively, of a challenge agent) is clinically relevant and may be used for BA and BE studies.

Clinical endpoint studies or comparative clinical trials:

If pharmacokinetic and pharmacodynamic methods are not available, appropriate and carefully monitored clinical trials can be performed to determine BA/BE. General information about the conduct of clinical studies to establish BE is provided by several international regulatory authorities.

In-vitro end point studies:

More recently, drug ingredients and drug products were classified as either having high or low solubility and permeability and quick dissolution, respectively, by the Biopharmaceutics Classification System (BCS). Using this method, drug products can be divided into four main categories: 1) highly soluble and highly permeable; 2) highly permeable and poorly soluble; 3) highly soluble and poorly permeable; 4) poorly soluble and poorly permeable. By using this BCS method, it may be possible to establish BE using just in vitro dissolution tests for a highly soluble and permeable therapeutic component that has been manufactured into a rapid dissolving drug product. Furthermore, according to FDA regulations, non biological issue medications approved prior to 1962 are still permitted to employ in vitro methods to document BE. Dissolution tests can also be used in other circumstances to decrease the number of in vivo studies; i) evaluate the batch-to-batch quality and facilitate batch release; ii) offer process control

and quality assurance; and iii) determine whether additional BE studies are necessary in minor post-approval modifications, where they serve as a bioequivalence signal. Each regulatory body gave the wide range of standards for BA/BE in vitro research.

Design and Conduct of In-vivo bioequivalence studies:

Study Object:

Minimising the experimental variables and preventing bias are the primary goals of the design of the experiment. The determination of in vivo bioavailability study is based on the following factors:

1. The type of reference medication and the dosage form that needs to be examined
2. Benefit-risk ratio factors when it comes to human testing
3. The accessibility of analytical techniques
4. Which scientific queries need to be addressed?

Study design:

Age, sex, disease state, dietary habits, physical and mental health, body weight of human volunteer, experimental design, time of administration, time of sampling, analytical method, and compartment model used in estimating pharmacokinetic parameters or bioavailability that contribute to the observed blood concentration time profile are some of the factors that affect bioavailability studies. As a result, in designing a study, all these crucial variables must be taken into account. It is important to plan the bioavailability study so that the formulation effect can be separated from other effects. A two-period, two-sequence crossover design is the preferred design when comparing two formulations; this design should ideally equal or exceed the five half-lives that need to be observed. One of the alternate study designs is the parallel design for drugs with very long half-life and very variable disposition.

Parallel Design:

Two formulations are given to two volunteer groups in a parallel design. The formulations may be given to the volunteers at random to prevent bias. The main drawback of this design is that there is no correction for intersubject variation. Unquestionably, the most of the time, intersubject variation is larger than the variation across any formulation. To prevent the influence of an intersubject variation, a crossover design is preferred in bioavailability or bioequivalency trials. This design is mainly applied to drugs, whose metabolites have long half-lives for elimination. In comparison to crossover trials, there were fewer carryover effects or dropouts in parallel experiments.

Crossover Design:

According to USFDA guidelines, the majority of bioequivalence studies compare a test drug with a standard reference drug in a group of healthy, normal subjects between the age of 18 and 55. Each subject receives both treatments alternately in a crossover fashion (two-period, two-treatment crossover design), with the washout period, which typically lasts a week, standing between the two phases of treatment. The washout duration increases in tandem with an increase in the drug's elimination half-life. Each human volunteer is randomly assigned to receive either the test or reference medication formulation; however, as shown in **Table 2**, an equal number of participants receive each treatment during each session. When two groups, 1 and 2, are given treatments, the first group gets it in the order A and B, while the second group gets it in the opposite order, B and A. If a three-treatment crossover

design is used (a three-period, three-treatment crossover design), an equivalent allocation is made. There is inter-subject variability for a number of medications in clearance. Since the intrasubject coefficient of variation (about 15%) is typically significantly lower than the inter-subject coefficient of variation (roughly 30%), crossover designs are typically advised for bioequivalence studies.

By comparing the treatments on the same human subject, crossover design lowers inter-subject variability. The three core statistical ideas of study design—randomization, replication, and error control—are the foundation of both approaches. Randomization refers to the unbiased distribution of treatments among the participants. Replication allows for more accurate measurement of treatment effects and uses multiple experimental subjects to generate values that are more dependable than those from a single observation. The degree of differences to be found and the inherent variability of the data are the primary determinants of the number of replicates needed. Latin square cross over design and balanced incomplete block design are two cross over designs that are frequently applied in bioavailability trials.

Table 2 Latin Square Design

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 2 illustrates the typical methodology for carrying out a comparative bioavailability study, which involves using a randomized, balanced cross-over design known as a Latin square or complete cross-over design. Many of the challenges associated with the Latin square design are eliminated by using incomplete block design (BIBD). It includes administering no more than two formulations to each subject, administering each formulation the same number of times, and exposing each pair of formulations in front of the same number of subjects. BIBD four formulations A, B, C, and D are displayed in Table 3. As previously mentioned, this strategy involves giving each subject two formulations, administering each formulation six times, and having each pair of formulations occur together in two subjects (the pairs are AB, AC, AD, BC, BD, and CD).

Table 3: Balanced incomplete block design (BIBD) for four formulations

Subject	Treatment for period No.	
	I	II
01	A	B
02	B	A
03	A	C
04	C	A
05	A	D
06	D	A
07	B	C
08	C	B
09	B	D
10	D	B
11	C	D
12	D	C

Selection of the Number of Subjects:

The following factors determine the appropriate number of subjects for a study to enable the result to be statistically significant:

1. The error variance associated with the primary characteristic to be studied as estimated from a pilot experiment, from previous studies or from published data.
2. The significance level desired: usually 0.05
3. The expected deviation from the reference product compatible with bioequivalence

Selection criteria of the subject:

As much as feasible, subjects should be standardized to reduce intra- and inter-individual variation. Normal adult volunteers in good health should participate in the trials in order to reduce variability and enable the identification of differences between the study medications. Male or female subjects are acceptable, but the gender selection should align with usage and safety requirements. Risks to women who may become pregnant should be taken into account individually. It should be mandatory for women to attest that they are neither pregnant or likely to get pregnant until after the study is completed. A pregnancy test should be performed just before the study's first and last dose to confirm this. Usually, studies should not include women who are using contraceptives. Aim to include as many individuals 60 years of age or older as possible if the medicinal product is intended primarily for elderly users. An effort should be made to include comparable numbers of males and females in the research if the medication product is meant for use by both sexes.

Study conditions:

During studies, multiple study variables are observed, including the study environment, diet, fluid consumption, post-dosing postures, activity, and sample schedules. These requirements are outlined in the protocol and must be met at the conclusion of the investigation to ensure that all variables that affect variability are taken into account in order to reduce the number of goods that need to be evaluated. The

research participants refrain from smoking, drinking alcohol, consuming xanthine-containing meals, coffee, tea, and other beverages, as well as fruit juices, at least 48 hours prior to the study's start.

Selection of Blood Sampling Points/Schedules:

Randomization should be the basis for administering medicinal products or formulations to the participants. At predetermined intervals following administration, blood samples are taken from the participants. Depending on the number of subjects and technicians involved in the study, it may take some time to remove a sample from each subject, and the total time difference between the first and last subject may be ranging from 10 to 20 minutes. The real amount of time the drug remains in the body compared to the stated sample period for each individual may fluctuate significantly if the sampling schedule is not adhered to strictly and in the same order.

When treatments are given to the subjects in sequential manner, the 10–20 min difference in sample withdrawal from each subject during the study would indicate a significant change in the drug concentrations observed in the blood. If a blood level study is to be used to evaluate the bioavailability of a particular dosage form, 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; consequently, the frequency of sampling and the duration of sampling are crucial for the investigation. It depends on the medication. For an accurate assessment of the area under the blood level curve, there must be a sufficient number of sampling points. If the medication's half-life is unknown, blood sample should continue until 1/10 or 1/20 of the peak levels are reached. Blood sampling should be done up to three to five half-lives of the drug. Studies on urine excretion are conducted when measuring a drug in blood, plasma, or serum is not feasible or when collecting samples over an extended period of time is prohibited by ethical reasons. The benefits of this approach include the noninvasive sampling technique, the ability to directly measure the amount of drug discharged in urine, and the fact that urine concentrations are frequently higher than serum concentrations. However, it has no value in determining the rate at which rapidly absorbed medications are absorbed, and occasionally metabolites can cause problems when determining the amount of unaltered medication in a urine sample. To ensure that the region extrapolated from the time of the last measured concentration to infinite time is less than 20% of the total AUC, sampling must be carried out for a long enough duration. When it comes to enterohepatic recycling, AUC estimates are useless because it is impossible to determine the terminal elimination rate constant with accuracy.

Washout Period:

Each subject receives each formulation in a Latin square cross over design, and in BIBD, every subject receives two formulations at different times. The "washout period" is the amount of time that passes between the two treatments. To prevent carryover, a washout interval is necessary to remove the drug's supplied dosage. In crossover design, at least 10 half-lives should be permitted between treatments for the majority of medications. This ought to ensure a maximum carryover of less than 0.1% from the initial treatment and the removal of 99.9% of the dose that was given. The drug's half-life and dosage determine how many washout periods are required. The number of formulations to be examined and the type of crossover design employed determine how many washout periods are needed in a study. For digitoxin, which has a half-life of 6–9 days, evaluating four formulations using the Latin square design would require more than a year of research. A week-long washout period was often deemed appropriate

in the majority of the published research due to the fact that a significant number of medicines had half-lives between one and ten hours. Notably, the drug's metabolites must also be removed from the body prior to starting the following round of treatment.

Reference and test product:

In an application for a generic drug, test products are usually compared with the equivalent dosage form of an innovator pharmaceutical product (reference product). The reference product selection should be supported by the applicant and approved by the regulatory body.

In the event that the innovator product is unavailable, a substitute product that has been authorized by the nation's drug regulatory body may be utilized.

The biostudy's test products have to be made in compliance with GMP guidelines. It is necessary to submit the test product's batch control results.

Unless there is a valid reason otherwise, the test product for oral solid forms intended for systemic action should typically come from a batch of at least 1/10 of the manufacturing scale or 100,000 units, whichever is greater. A full production batch will be needed if the production batch is fewer than 100,000 units. The production of batches utilised should offer a high degree of accuracy that the product and method will be possible on an industrial scale. A thorough validation should be conducted before the product is subjected to additional scaling up.

When using appropriate dissolving test conditions, samples of the product from full production batches should be compared with those from the test batch and should exhibit similar in vitro dissolution profiles.

In order to permit re-testing, should the authorities request it, the study sponsor shall maintain an adequate quantity of all investigational product samples in the study for a period of time that does not exceed the approved shelf life, two years following the trial's conclusion, or until approval, whichever is longer.

For each participant in the bioequivalence trial, the reference and test products need to be packaged separately. Every attempt should be taken to enable accurate tracking of the subjects' administration of the reference and test goods, for as by the use of labels that have a tear-off section.

Bioanalytical methodology:

Good Laboratory Practice (GLP) guidelines should be followed when conducting the bioanalytical portion of bioequivalence experiments. But, as human bioanalytical research is not covered by GLP, monitoring of the study sites is not necessary as part of a national GLP compliance plan.

For the bioanalytical methods to produce accurate information that can be successfully understood, they must be thoroughly validated, well-characterized, and recorded. Every analytical run of the investigation should include quality control samples for validation.

Selectivity, the lower limit of quantitation, the response function (calibration curve performance), accuracy, precision, and stability are the key attributes of a bioanalytical method that are necessary to guarantee the acceptance of the performance and accuracy of analytical results. Since pre-dose concentrations should be detected at 5% of C_{max} or less, the lower limit of quantitation should be 1/20 of C_{max} or less. Before the actual analysis of the samples begins, the reanalysis of the study samples should be predefined in the study protocol and/or SOP.

Reanalyzing subject samples for pharmacokinetic reasons is generally not appropriate. This is particularly crucial for bioequivalence research because it could skew the results.

Sample analysis needs to be done in the absence of treatment-related information.

Data Analysis:

Quantifying the bioavailability difference between the reference and test products and proving the likelihood of any clinically significant deviations are the main goals of the bioequivalence assessment process.

The 90% confidence interval for the ratio of the population means (Test/Reference) for the parameters under consideration forms the basis of the statistical procedure for assessing relative bioavailability (such as bioequivalence).

At the 5% significance level, this approach is comparable to the analogous two-sided test procedure with the null hypothesis of bioinequivalence. The causes of variation that can be presumed to have an impact on the response variable should be considered in the statistical analysis (e.g., ANOVA). It is important to handle a statistically significant sequence effect properly.

ANOVA should be used to assess pharmacokinetic parameters that are obtained from concentration measurements, such as AUC and C_{max} . A logarithmic modification should be applied to the data before analysis.

The analytic method for t_{max} should be non-parametric and applied to untransformed data if the evaluation justifies it. In addition to the required 90% confidence intervals for comparing the two formulations, summary statistics like the median, minimum, and maximum should be included for all relevant pharmacokinetic parameters.

Acceptance range for pharmacokinetic parameters:

The protocol should specify in advance the pharmacokinetic parameters to be examined, the testing process, and the acceptable ranges.

The following is an entire review of the acceptance intervals for the primary characteristics in studies conducted to estimate average bioequivalence.

AUC-ratio

This relative bioavailability measure's 90% confidence interval should fall between 0.80 and 1.25 in the acceptable range. The acceptance interval might need to be tightened in certain situations involving a limited therapeutic range.

Rarely, if a wider acceptability range is supported by strong clinical evidence, it might be appropriate.

C_{max} -ratio

This relative bioavailability measure's 90% confidence interval should fall between 0.80 and 1.25 in the acceptable range. It could be necessary to tighten the acceptance interval in certain situations where the therapeutic range is limited.

A larger interval might be appropriate in some circumstances. The interval needs to be justified and prospectively specified, such as 0.75–1.33, specifically taking into account any safety or efficacy concerns for patients who switch between formulations.

Others:

Only when there is a clinically significant claim for quick release or action or when there are indications of potential side effects, does statistical analysis of t_{max} make sense. This relative bioavailability measure's non parametric 90% confidence interval should fall between a range that has been clinically established. Similar concerns to those for AUC, C_{max} , or t_{max} apply for other pharmacokinetic parameters in comparative relative bioavailability (e.g., C_{min} , Fluctuation $t_{1/2}$, etc.), depending on whether log transformed or untransformed data are used.

Bioequivalence limits:

If the 90% confidence interval of the difference between the test and reference products' average values of the logarithmic parameters to be evaluated falls between $\log(0.80)$ and $\log(1.25)$, then the product is considered bioequivalent.

Tests conducted to study In-vitro bioequivalence:**Uniformity of content:**

Finding out if there are differences in the percentage composition of the active components is crucial for conducting bioequivalence studies. It is important to regularly measure the percentage of drug content in tablets to determine if the dosage is appropriate. The presence and stability of the medicine in dosage form are shown by an analysis of the drug potency in tablets. Each dosage form's monographs contain the content uniformity test, and tablet samples are chosen and examined separately. The assay content of the maximum tablets shall not exceed $\pm 25\%$ and must be within $\pm 15\%$ of the specified potency. During compression, uniformity of weight ensures constancy of dose units.

Weight variation:

Tablet weight is influenced by a number of factors, including as the compression machine's tooling, head pressure, speed, and powder flow characteristics. Twenty tablets from each brand are taken in order to calculate the weight variation. The tablets are usually weighed using an analytical weighing balance. Average weights and percentage deviations for each brand were computed based on the mean value. Pharmacopoeia states that individual weights should not depart from the average weight by more than two.

Hardness:

The hardness test is crucial because it determines how resistant the tablet is to breaking, chipping, or abrasion during handling, shipping, and storage before use. The hardness of the tablet is influenced by the distance between the upper and lower punches at the moment of compression, the weight of the material used, and the pressure utilised. The following are some examples of several types of hardness measuring equipment: Heberlain or Schleeniger hardness tester; Pfizer hardness tester; Strong Cob hardness tester; and Monsanto or Stokes hardness tester.

Friability:

Friability is a phenomenon where surface of tablet is damaged or shows a site of damage due to mechanical shock. The purpose of this test is to ensure that the tablet's corners do not break off. The device in use is a Roche friabilator. 20 pills are selected at random, and their initial weight (W_1) is

determined. The final weight (W2) is computed following a 4-minute, 25-rpm friabilator run on the tablets. The formula is used to get the percentage loss.

$$\% \text{ Friability} = ((W1-W2)/W1) * 100$$

Disintegration:

Disintegration studies are crucial for assessing how well drugs release. A disintegration test is conducted to determine how long it takes for the tablets or capsules to totally dissolve. Previously, the disintegration test is used to determine the homogeneity of compression characteristics. These days, we favour this test for compression characteristic optimisation. The tablet may be overly crushed or the capsule shell gelatine may not be of the necessary quality if the disintegration period is excessively long. Batch inconsistency and lack of uniformity arise from uneven disintegration times. Disintegration apparatuses vary depending on the medication, but their basic design and function are always the same. The device is a basket with six tubes that are all the same diameter.

Each of these tubes has a wire mesh attached to it. The basket moves with the help of a reciprocating motor. The test media is contained in a vessel in which the complete assembly is kept submerged.

Dissolution test:

The amount of medicine that dissolves in bodily fluids and is absorbed into the systemic circulation determines how effective a dosage is. As a result, figuring out a dosage form's dissolving rate is crucial. By using a thermostat to help control temperature and provide the right dissolve medium, biological conditions are maintained in a dissolution apparatus. Samples are taken out on a regular basis. An equal amount of media is supplied to maintain sink conditions. As a result, assays are conducted. The choice of dissolve medium, equipment, and agitation rate is crucial for a successful dissolution test. Dissolution testing is done in order to:

- Enhancing therapeutic efficacy in the course of developing new products and evaluating stability.
- Regular evaluation of production quality to guarantee consistency amongst production lots.
- Evaluation of "bioequivalence"
- Forecasting availability in vivo, or bioavailability (if relevant).

Various kinds of equipment are used for this, according to the Pharmacopoeia, for various dose forms.

DISSOLUTION APPARATUS AND DETAIL AS PER USP		
APPARATUS	NAME	DRUG PRODUCT
Apparatus I	Rotating basket	Tablets
Apparatus II	Paddle	Tablets. capsules modified drug products
Apparatus III	Reciprocating cylinder	Extended-release drug products.
Apparatus IV	Flow cell	Drug products containing low-water-soluble drug
Apparatus V	Paddle over disk	Tran dermal drug products
Apparatus VI	Cylinder	Tran dermal drug products
Apparatus VII	Reciprocating disk	Extended-release drug products

CONCLUSION:

After a review, it is concluded that bioavailability and bioequivalence studies are widely used globally to establish and ensure consistent quality and a reliable, therapeutically effective performance of marketed dosage forms. These studies have been shown to be acceptable stand-ins for costly, complex, and lengthy clinical trials. The development of more effective and scientifically sound methods for evaluating bioequivalence is necessary to attain product quality over time for both innovative and generic drugs. This will require ongoing efforts by international health organizations, pharmaceutical companies, researchers, and regulatory bodies. Statistical techniques are used to assess pharmacokinetic parameters in order to provide precise results and guarantee affordable high-quality pharmaceuticals. Important information about the various brands' qualities can be found in the post-marketing in-vitro bioequivalence studies. Bioequivalence studies performed in vitro and in vivo are necessary and can have an effect on the companies that manufacture dosage forms.

REFERENCES:

1. Mastan, Shaik, Thirunagari Bhavya Latha, and Sathe Ajay, The basic regulatory considerations and prospects for conducting bioavailability/bioequivalence (BA/BE) studies—an overview, *Comparative Effectiveness Research*, 2011; 1-25.
2. Gouthami, A., Ramesh M., Nalini M., Deepthi Nancy M., A review on general concepts of design and conduct of bioequivalence studies, *International Journal of Phytopharmacology*, 2013 4(2); 4-84.
3. Nagadurga, Divvela Hema, Bioavailability and bioequivalence studies, *Pharmaceutical Formulation Design-Recent Practices*. London, UK: Intech Open, 2019.
4. Asean Guidelines for the conduct of Bioavailability and Bioequivalence Studies, (Final Draft) 21 July, 2004
5. Venkateswarlu, K., & Thirumaran, J., Review on Bioavailability and Bioequivalence Studies, *International Journal of Pharmaceutical Sciences Review and Research*, ISSN (2013), 56-64.
6. FDA (U.S. Food and Drug Administration), CDER (Center for Drug Evaluation and Research). Guidance for Industry: Bioavailability and bioequivalence for orally administered drug products general considerations (Draft). 2002.
7. FDA (U.S. Food and Drug Administration), CDER (Center for Drug Evaluation and Research). Guidance for Industry: Food effect bioavailability and fed bioequivalence studies. 2002
8. Food and Drug Administration (FDA), Guidance for Industry: Statistical approaches to establishing bioequivalence, 2001.
9. Abbirami, V., Sainithya, P., Shobana, A., Devi, D. R., & Hari, B. V., A review on in-vitro bioequivalence studies and its methodologies, *Int J Chem Tech Res*, 2013, 5(5), 2295-2302
10. Van der Meersch, A., Dechartres, A., & Ravaud, P, Quality of reporting of bioequivalence trials comparing generic to brand name drugs: a methodological systematic review, *PLoS One* (2011), 6(8), e23611.
11. Aruna, T., Yadav, C. V. B., Nagabhushanam, M., Bonthagarala, B., Reddy, D. N., & Ramakrishna. G, Guidelines for bioavailability and bioequivalence studies: A review, *Pharma Innov. J* 7 (2018): 661-666.
12. Mastan S., Bhavya Latha T., Ajay S., The basic regulatory considerations and prospects for conducting bioavailability/bioequivalence (BA/ BE) studies – an overview, *Comparative Effectiveness Research* (2011): 1-25.

13. Chung Chow S., Bioavailability and Bioequivalence in Drug Development, Wiley Interdiscip Rev Comput Stat. 2014; 6(4): 304–312.
14. Aruna, T., Yadav, C. V. B., Nagabhushanam, M., Bonthagarala, B., Reddy, D. N., & Ramakrishna, G., Guidelines for bioavailability and bioequivalence studies: A review Pharma Innov. J 7 (2018): 661-666.
15. Nakai, K., Fujita, M., & Ogata, H.. New bioequivalence studies: individual bioequivalence and population bioequivalence. Yakugaku Zasshi, 2000;120(11), 1201-1208.
16. Andrade, C. Bioequivalence of generic drugs. The Journal of clinical psychiatry,2015;76(9), 1905.
17. Andrade, C. Bioequivalence of generic drugs: a simple explanation for a US Food and Drug Administration requirement. The Journal of clinical psychiatry,2015;76(6), 1907.
18. Krajcar, D., Jereb, R., Legen, I., Opara, J., & Grabnar, I. Predictive Potential of Acido-Basic Properties, Solubility and Food on Bioequivalence Study Outcome: Analysis of 128 Studies. Drugs in R&D, 2023;23(3), 211-220.
19. Usmani, M. T., Shoaib, M. H., Siddiqui, F., Ahmed, F. R., Yousuf, R. I., & Saleem, M. T. Formulation development, in vivo bioequivalence and pediatric PBPK modeling studies of taste-masked ciprofloxacin chewable tablets. Scientific Reports,2023;13(1), 16070.
20. Tamboli, A. M., Todkar, P., Zope, P., & Sayyad, F. J. An overview on bioequivalence: regulatory consideration for generic drug products. J Bioequiv Availab,2010;2(4), 086-92.
21. Rani, S. Bioequivalence: Issues and perspectives. Indian Journal of Pharmacology,2007; 39(5), 218-225.
22. Kubbinga, M., Langguth, P., & Barends, D. Risk analysis in bioequivalence and biowaiver decisions. Biopharmaceutics & drug disposition,2013; 34(5), 254-261.
23. Endrenyi, L., & Tothfalusi, L. Sample sizes for designing bioequivalence studies for highly variable drugs. Journal of Pharmacy & Pharmaceutical Sciences,2012; 15(1), 73-84.
24. Endrenyi, L., Amidon, G. L., Midha, K. K., & Skelly, J. P. Individual bioequivalence: attractive in principle, difficult in practice. Pharmaceutical research,1998; 15(9), 1321.
25. Mallath, M. K., & Chawla, T. Investigators' viewpoint of clinical trials in India: Past, present and future. Perspectives in Clinical Research, 2017; 8(1), 31-36.
26. Al Shoyaib, A., Riedmaier, A. E., Kumar, A., Roy, P., Parrott, N. J., Fang, L., ... & Wu, F. Regulatory utility of physiologically based pharmacokinetic modeling for assessing food impact in bioequivalence studies: a workshop summary report. CPT: Pharmacometrics & Systems Pharmacology,2023;12(5), 610-618.
27. Mishra, V., Gupta, U., & Jain, N. K. Biowaiver: an alternative to in vivo pharmacokinetic bioequivalence studies. Die Pharmazie-An International Journal of Pharmaceutical Sciences,2010; 65(3), 155-161.
28. Tothfalusi, L., & Endrenyi, L. An exact procedure for the evaluation of reference-scaled average bioequivalence. The AAPS journal,2016; 18, 476-489.
29. Yerlikaya, F., Arslan, A., Atik, Ö., Kozan, S., Parlak, A., Karataş, M. Ö., ... & Aytaç, S. P. The Bioequivalence Study of Two Cefdinir 250 mg/5 mL Oral Suspension Formulations in Healthy Males Under Fasting Conditions. Hacettepe University Journal of the Faculty of Pharmacy,2023; 43(1), 43-49.

30. Benziger DP, Kaiko RF, Miotto JB, Fitzmartin RD, Reder RF, Chasin M. Differential effects of Food on the bioavailability of controlled release oxycodone tablets and immediate-release oxycodone solution. *J Pharm Sci*, 1996; 85, 407-410.
31. Oliveira CH, Abib E, Vannuchi YB, Sucupira M, Ilha J, De Nucci G. Comparative bioavailability of 4 amoxicillin formulations in healthy human volunteers after a single dose administration. *Int J Clin Pharmacol Ther* 2001; 39, 167-172.
32. Guidelines given by Indian regulatory department for the conduct of bioavailability / bioequivalence trials.
33. FDA (U.S. Food and Drug Administration), CDER (Center for Drug Evaluation and Research). Guidance for Industry: Bioavailability and bioequivalence for orally administered drug products general considerations (Draft). 2002.
34. FDA (U.S. Food and Drug Administration), CDER (Center for Drug Evaluation and Research). Guidance for Industry: Food effect bioavailability and fed bioequivalence studies 2002.
35. Guidance for industry: 'Bioavailability and Bioequivalence studies for Orally Administered Drug Products-General Considerations', Food and Drug Administration, USA; March 2003.
36. 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 Administrations. Centre for Drug Evaluation and Research (CDRE), October 2000.
37. Yuksel N, Kanik AE, Baykara T, Comparison of in-vitro dissolution profiles by ANOVA-based, model dependent and independent methods, *Int J Pharm*, 2000; 209, 57-67.
38. Dave. Assesment of similarity factor using different weight variation approaches, *Dissolution Technology*, 2005; 22-27.
39. Joshi A., Kadam S.S., Aatmaram. Technical note: The effect of alcoholic beverages on sustained release, *Pharmaceutical Technology*, 2010; 34(12), 47-49.
40. Tsang, Y. C., Pop, R., Gordon, P., Hems, J., & Spino, M. "High variability in drug pharmacokinetics complicates determination of bioequivalence: experience with verapamil." *Pharmaceutical research* 13 (1996): 846-850
41. Chow, S. C., & Liu, J. P Design and analysis of bioavailability and bioequivalence studies. CRC press, 1999.
42. Hyslop, T., Hsuan, F., & Holder, D. J. "A small sample confidence interval approach to assess individual bioequivalence." *Statistics in Medicine* 19.20 (2000): 2885-2897.
43. Midha, K. K., & McKay, G. "Bioequivalence; its history, practice, and future." *The AAPS journal* 11 (2009): 664-670.
44. Rani, S., & Pargal, A. "Bioequivalence: An overview of statistical concepts." *Indian Journal of Pharmacology* 36.4 (2004): 209-216.
45. Chen, M. L., Shah, V., Patnaik, R., Adams, W., Hussain, A., Conner, D., ... & Williams, R. "Bioavailability and bioequivalence: an FDA regulatory overview." *Pharmaceutical research* 18 (2001): 1645-1650.
46. Hauschke, D., Steinijans, V., & Pigeot, I. Bioequivalence studies in drug development: Methods and applications. John Wiley & Sons, 2007.
47. Sauter, R., Steinijans, V. W., Diletti, E., Böhm, A., & Schulz, H. U. "Presentation of results from bioequivalence studies." *International journal of clinical pharmacology, therapy, and toxicology* 30 (1992): S7-30.

48. Chen, M. L. "An alternative approach for assessment of rate of absorption in bioequivalence studies." *Pharmaceutical research* 9 (1992): 1380-1385.
49. Chow, S. C., & Liu, J. P. *Design and analysis of bioavailability and bioequivalence studies*. CRC press, 1999.
50. Anderson, S., & Hauck, W. W. "Consideration of individual bioequivalence." *Journal of Pharmacokinetics and Biopharmaceutics* 18 (1990): 259-273.
51. Shah, V. P., Midha, K. K., Dighe, S., McGilveray, I. J., Skelly, J. P., Yacobi, A., ... & Spector, S. "Analytical methods validation: bioavailability, bioequivalence, and pharmacokinetic studies." *Journal of pharmaceutical sciences* 81.3 (1992): 309-312.
52. Flouvat, B., Leneveu, A., Fitoussi, S., Delhotal-Landes, B., & Gendron, A. "Bioequivalence study comparing a new paracetamol solution for injection and propacetamol after single intravenous infusion in healthy subjects." *International Journal of Clinical Pharmacology and Therapeutics* 42.1 (2004): 50-57.
53. Kalantzi, L., Goumas, K., Kalioras, V., Abrahamsson, B., Dressman, J. B., & Reppas, C. "Characterization of the human upper gastrointestinal contents under conditions simulating bioavailability/bioequivalence studies." *Pharmaceutical research* 23 (2006): 165-176.
54. Nagadurga, D. H. "Bioavailability and bioequivalence studies." *Pharmaceutical Formulation Design-Recent Practices*. London, UK: IntechOpen, 2019.
55. Sheiner, L. B. "Bioequivalence revisited." *Statistics in medicine* 11.13 (1992): 1777-1788.
56. Galgatte, U. C., Jamdade, V. R., Aute, P. P., & Chaudhari, P. D. "Study on requirements of bioequivalence for registration of pharmaceutical products in USA, Europe and Canada." *Saudi Pharmaceutical Journal* 22.5 (2014): 391-402.
57. Pearce, G. A., McLachlan, A. J., & Ramzan, I. "Bioequivalence: how, why, and what does it really mean?." *Journal of Pharmacy Practice and Research* 34.3 (2004): 195-200.
58. Henney, J. E. "Review of generic bioequivalence studies." *JAMA* 282.21 (1999): 1995-1995
59. Borgheini, G. "The bioequivalence and therapeutic efficacy of generic versus brand-name psychoactive drugs." *Clinical therapeutics* 25.6 (2003): 1578-1592.
60. Meredith, P. "Bioequivalence and other unresolved issues in generic drug substitution." *Clinical therapeutics* 25.11 (2003): 2875-2890.