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Review Article

# Bioequivalence and Bioavailability Studies: Regulatory Assessment and Study Design Considerations and Pharmacokinetic Parameters

**Atharva Surendra Bhalerao*, Sandeep Sonawane**

Department of Pharmaceutical Chemistry, MET's Institute of Pharmacy, Adgaon, Nashik, Maharashtra, INDIA.

**ABSTRACT**

Bioequivalence and bioavailability studies are vital components of the pharmaceutical industry, ensuring the safety, efficacy and interchangeability of generic drugs compared to their reference products. This comprehensive research paper dives deeper into the concepts of the principles of bioequivalence and bioavailability. The application of the Biopharmaceutics Classification System (BCS) in evaluating bioequivalence, different study designs used in these assessments. A thorough review of bioequivalence evaluation in line with guidelines from the US Food and Drug Administration (USFDA) and the European Medicines Agency (EMA).

**Keywords:** Bioequivalence, Study Design, EMA, USFDA.

**Correspondence:**
**Mr. Atharva Surendra Bhalerao**
Department of Pharmaceutical Chemistry, MET's Institute of Pharmacy, Adgaon, Nashik-422003, Maharashtra, INDIA.
Email: atharva_bhalerao@yahoo.com
ORCID: 0000-0001-9718-7622

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## INTRODUCTION

In the pharmaceutical field, bioequivalence studies aim to demonstrate that generic drug products have comparable pharmacokinetic characteristics to their corresponding reference products.[1]

Historically, BE regulations developed in the 1970s following the Hatch–Waxman Act (1984) in the United States, which allowed Abbreviated New Drug Applications (ANDAs) based on BE rather than full clinical trials. EMA, established parallel guidelines in the 1990s, while WHO developed frameworks to support entry in developing countries.

These BE studies are crucial for regulatory approval and public health, assuring patients and healthcare professionals that generic drugs can be used interchangeably with branded medications. Bioavailability, the rate and extent of drug absorption, is a fundamental parameter that influences bioequivalence.[1]

The clinical and public health implications of BE are profound. Generic medicines account for over 80% of prescriptions in developed countries, providing significant cost savings while maintaining therapeutic equivalence. However, scientific and regulatory challenges remain, including variability in absorption, design of crossover studies, and harmonization of acceptance criteria.

### Utilization of BCS Classification in Bioequivalence Studies

The Biopharmaceutics Classification System (BCS) is a valuable tool used in bioequivalence studies to categorize drugs based on its solubility and permeability characteristics.[1,2] This system provides a structured frame for evaluating the equivalence of generic drugs to their reference products.[2] Drugs are divided into four classes: Class I drugs have high solubility and permeability. Class II drugs have high solubility but low permeability. Class III drugs have low solubility but high permeability. Class IV drugs have both low solubility and low permeability.

For bioequivalence studies, Class I drugs require less rigorous testing due to their high solubility and permeability, while Class IV drugs demand more extensive assessments due to their challenging properties.[2] Specifically, Class II and Class III drugs often necessitate bioequivalence studies as they possess a combination of high solubility and low permeability.[2] Bioequivalence studies for Class III drugs are important because although they have low solubility, their high permeability allows for efficient absorption.[2] In the case of Class IV drugs, which have both low solubility and low permeability, extensive assessments are necessary to determine bioequivalence due to the challenges in dissolution and absorption.[2]

It is crucial to consider the dosage form of a drug when applying the BCS classification during bioequivalence studies. In certain cases, the dosage form may influence drug solubility and

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absorption. For instance, a drug classified as Class IV due to low solubility and permeability may exhibit improved solubility and absorption when formulated as a solution. In such instances, the drug may be considered as falling into a different BCS class, altering the testing requirements for bioequivalence evaluation.

Understanding the BCS classification of a drug assist in determining the appropriate level of *in vitro* and *in vivo* testing necessary for conducting reliable and robust bioequivalence studies. By considering drug solubility and permeability characteristics, the BCS classification advances in the design and interpretation of bioequivalence studies, facilitating the evaluation of generic drug products against their reference counterparts.[2]

## How to determine required testing

- Class I: Less rigorous testing.
- Class II and III: Bioequivalence studies needed.
- Class IV: Extensive assessments required.

## Different Types of Study Designs

Bioequivalence studies employ various study designs, each tailored to specific drug characteristics and objectives:

- Single-dose, two-period, two-treatment, two-sequence crossover study design is the most common design, where each subject receives both the test and reference product in different sequences.
- Replicate crossover design is used for highly variable drugs to account for intra-subject variability and enhance the precision of the study.
- Partial or fully replicate crossover design is suitable for drugs with a long half-life, ensuring adequate assessment of bioequivalence over extended periods.
- Parallel study design is employed for drugs with narrow therapeutic index, aiming to minimize the risk of unintended differences in pharmacokinetics that could have clinical implications.

## Highly Variable Drugs

Highly variable drugs exhibit substantial intra-subject variability in their pharmacokinetics, leading to challenges in bioequivalence assessment.[1] Conventional single-dose crossover studies may not adequately capture the variability. Therefore, a replicate crossover design is preferred for such drugs, which involves multiple administrations of both the test and reference products to every subject.[1]

## Narrow Therapeutic Index Drugs

Narrow therapeutic index drugs have a small difference between the minimum effective concentration and the minimum toxic concentration. Ensuring bioequivalence for such drugs requires more stringent criteria to minimize the risk of variations that could lead to significant clinical effects. Narrow therapeutic index drugs pose a higher risk if the exposure to the active drug varies between the generic and reference products.[1]

## Bioequivalence Assessment as per USFDA and EMA Regulatory Guidelines

Both the USFDA and EMA have well-defined guidelines for conducting bioequivalence studies. These guidelines outline the acceptable bioequivalence criteria, study design requirements and statistical approaches for data analysis. They also provide clarity on specific considerations for highly variable drugs and narrow therapeutic index drugs. Adhering to these regulatory standards is crucial for obtaining marketing approval for generic drugs in the respective regions.

The below section provides key points from the guidelines on the design, conduct and evaluation of bioequivalence studies, as well as the use of *in vitro* dissolution tests and BCS-based biowaivers.[1,3]

## BCS-Based Biowaivers: Key Criteria for Rapid Dissolution

To be eligible for a BCS-based biowaiver, especially for immediate-release oral formulations, several criteria related to rapid dissolution must be satisfied:

- **High Solubility and Permeability:** The drug must belong to either BCS Class I (high solubility and high permeability) or Class III (high solubility and low permeability).

**Dissolution Requirement:** The drug product should achieve rapid dissolution, defined as:

- A minimum of 85% of the drug must dissolve within 30 min across three different dissolution media (pH 1.2, 4.5 and 6.8).

**Dissolution Profile Similarity:** The dissolution profiles of the test and reference products must be comparable, often evaluated using the f2 similarity factor to meet regulatory standards.

**Excipients:** The formulation's excipients should not significantly impact the drug's absorption.

After meeting these conditions ensures that the drug product will perform similarly in the body, potentially allowing for a waiver of *in vivo* bioequivalence studies in favour of *in vitro* dissolution testing.[1,2]

## Key Points from EMA Guideline for Bioequivalence IR Products

### *Study Design*

- Two-way crossover design is recommended for most bioequivalence studies.
- Alternative designs like parallel design or replicate designs are acceptable in specific cases.
- Multiple-dose studies are allowed if single-dose studies are not feasible.

Highly Variable Drug Products (HVDP) are those with intra-subject variability greater than 30% for a specific parameter. If an applicant believes a drug product to be highly variable, a replicated crossover design study can be conducted. For certain HVDP with clinically justified reasons, the acceptance range for $C_{max}$ can be widened to 69.84-143.19%. This widening can only be applied to replicate studies where the within-subject variability for the $C_{max}$ of the reference compound is >30%. The research/study protocol has to include a specific request for the wider interval.

The extent of widening is determined based on the within-subject variability observed in the bioequivalence study using scaled average bioequivalence. The acceptance range is calculated as [U, L]=exp [±k·sWR], where sWR is the within-subject standard deviation of the log-transformed $C_{max}$ values of the reference product, k is a regulatory constant (0.760) and U is the upper limit and L the lower limit. Examples of different acceptance limits based on variability levels are provided below.

The standard acceptable range of 80.00-125.00% should still be covered by the Geometric Mean Ratio (GMR). But with AUC, the acceptance range stays at 80.00-125.00% regardless of variability, therefore this potential to broaden acceptance criteria based on significant intra-subject variability does not apply.

The study can use either a 3-period or 4-period crossover scheme in the replicate design.

## Selection of Reference and Test Products

- The reference product should be an authorised medicinal product with a complete dossier.
- The test product should match the reference product within 5% in assay content.
- The test product should be from a batch of at least $1/10^{th}$ of the production scale or a full production batch if smaller.

## Subjects

At least 12 evaluable subjects are required in a bioequivalence study.

Subjects should be 18 years of age or older with a BMI between 18.5 and 30 $kg/m^2$.

In some cases, patients may be included instead of healthy volunteers.

## Study Conduct

Standardisation of fasting and administration of test/reference products.

The sampling schedule should allow sufficient data points during the terminal log-linear phase.

Fasting or fed conditions based on product recommendations.

## Pharmacokinetic Parameters

$C_{max}$ and $AUC_{0-t}$ are primary parameters analyzed.

$AUC_{0-\infty}$ and secondary PK parameters are also considered.

$AUC_{(0-72\ hr)}$ can be used for truncated studies.

## Highly Variable Drugs or Drug Products

Replicate crossover studies may be conducted for highly variable drugs.

The acceptance criteria for $C_{max}$ can be widened based on intra-subject variability.

## *In vitro* Dissolution Tests

Dissolution profiles at different pH values and QC media should be reported.

*In vitro* dissolution may support biowaiver studies if similarity criteria are met.

## Narrow Therapeutic Index (NTI) Drugs

For NTIs, acceptance criteria for AUC are tightened to 90.00-111.11%.

## BCS-Based Biowaivers

BCS-based biowaivers apply to immediate release oral products of high solubility and known human absorption.

Specific requirements for rapid dissolution and excipients must be met.

## Fixed Combinations

BCS-based biowaivers are applicable for immediate release of fixed combinations of BCS-class I or III active substances.

In conclusion, these guidelines provide detailed recommendations for conducting bioequivalence studies, including study design, subject selection, *in vitro* dissolution testing and considerations for BCS-based biowaivers and narrow therapeutic index drugs. Adhering to these guidelines ensures a robust and reliable assessment of bioequivalence for generic drug products.

## Key Points from the USFDA Guideline for Bioequivalence

**Draft Guidance 2021:** Bioequivalence Studies with Pharmacokinetic Endpoints for Drugs Filed Under an ANDA.

The provided text contains information from an FDA guideline regarding the establishment of Bioequivalence (BE) for different dosage forms. The key points from the guideline can be summarized as follows:

## General Considerations

BE studies often rely on pharmacokinetic endpoints like $C_{max}$ and AUC, which reflect absorption rate and extent.

A pilot study on a small number of subjects can precede the pivotal study to validate the design and assess variability.

Pivotal BE studies should be conducted under fasting or fed conditions.

High-fat, high-calorie test meals are recommended for fed studies.

Various restrictions apply, such as pre- and post-dose food and alcohol abstinence.

The highest-marketed strength can be used if it is safe, otherwise, lower strengths are considered.

Retain test and reference products for five years.

Plasma or serum is preferred over urine or tissue for analysis.

Subjects with pre-dose plasma drug concentrations greater than 5% of $C_{max}$ should be excluded from BE evaluations.

Data from subjects experiencing vomiting within specified intervals should be excluded.

Outliers can be removed only with valid documentation of protocol violations.

Specific pharmacokinetic information and statistical data are required for submissions.

## Study Designs and Population

The FDA recommends two-way crossover, single-dose parallel, or replicate designs for BE studies.

Healthy subjects, representative of the general population, should be used for BE studies.

A balanced proportion of males and females are recommended for drugs intended for both sexes.

Adequate subject numbers are required for statistical power.

Different study populations may be appropriate for specific drugs.

A replicate crossover study design is suitable for pharmaceuticals, regardless of whether the reference product is highly variable or not. Replicate designs provide the benefit of utilising fewer participants than non-replicate designs; however, each subject in a replicate study will get more treatments.

## Replicate designs are recommended for the following scenarios

For non-Narrow Therapeutic Index (NTI) drugs with high intrasubject variability, a replicate design (either partially or fully) is advantageous. A reference-scaled Bioequivalence (BE) analysis approach should be applied to specific Pharmacokinetic (PK) metrics exhibiting high within-subject variability for the reference product in the pivotal BE study.

For NTI drugs, a fully replicated design is recommended. This allows the computation of within-subject variability for both the reference and test products, enabling a reference-scaled average BE analysis with properly adjusted BE acceptance criteria.

Detailed recommendations and methods of statistical analysis for highly variable drugs and NTI drugs can be found in the respective Product-Specific Guidance (PSGs).

## Different Dosage Forms

Guidelines for establishing BE vary depending on the dosage form (e.g., oral solutions, immediate release, or modified release).

Demonstration of BE for additional strengths can be based on *in vitro* and *in vivo* data.

Post-approval changes require *in vitro* comparisons and may involve *in vivo* BE studies.

## Special Topics

Guidelines for measuring parent drugs versus metabolites and enantiomers versus racemates are provided.

*In vivo* studies for oral immediate-release products with long half-lives may allow crossover or parallel designs.

Sufficient sampling time points before $C_{max}$ are recommended.

Alcohol's effects on modified release drug products should be assessed.

Baseline assessment is recommended for endogenous compounds.

*In vitro* dissolution testing should follow specific methods based on dosage forms.

Enteral feeding tube administration may require comparative *in vitro* testing.

This summary provides a concise overview of the key points from the FDA guideline on establishing bioequivalence for different dosage forms.

In the context of bioequivalence studies, the 90% Confidence Interval (90% CI) is a statistical measure used to assess the variability and comparability between two drug formulations, typically a test product (generic or new formulation) and a reference product (established brand or standard formulation). The 90% CI is calculated to determine whether the test product is bioequivalent to the reference product.

The bioequivalence of two drug formulations implies that they exhibit equivalent pharmacokinetic properties in terms of rate and extent of absorption. In other words, the two formulations are expected to produce similar blood concentration profiles over time, which indicates that they are likely to have similar therapeutic effects and safety profiles.

## Here's how the 90% CI is calculated in the context of bioequivalence

**Pharmacokinetic Parameters:** In a bioequivalence study, pharmacokinetic parameters such as $C_{max}$ (maximum plasma Concentration), AUC (Area under the concentration-time Curve) and $T_{max}$ (time to reach $C_{max}$) are measured for both the test and reference products.

**Geometric Mean Ratios:** The Geometric Mean Ratio (GMR) is calculated for each parameter. The GMR is the ratio of the geometric means of the test product to the reference product. Mathematically:

GMR=(Geometric Mean of Test Product)/(Geometric Mean of Reference Product)

**Standard Deviation (SD):** The standard deviation of the logarithmically transformed pharmacokinetic parameters is calculated for both the test and reference products.

**Calculation of 90% CI:** The 90% confidence interval is calculated using the GMR and the standard deviation. It is typically calculated as follows:

90% CI=exp(ln(GMR)±(t*SE))

**GMR (Geometric Mean Ratio):** The GMR is calculated as the Geometric Mean of the Test Product divided by the Geometric Mean of the Reference Product.

**Standard Deviation (SD):** The standard deviation is calculated for the logarithmically transformed pharmacokinetic parameters for both the test and reference products.

**Calculation of 90% CI:** The 90% Confidence Interval (CI) is calculated using the GMR and the standard deviation. The formula is as follows:

90% CI=exp(ln(GMR)±(t*SE))

Here, t is the *t*-value corresponding to the 90% confidence level and SE is the standard error of the logarithm of the GMR. The t-value is typically derived from the t-distribution and corresponds to the critical value for a 90% confidence level (e.g., t-value for a significance level of 0.05, with 0.025 in each tail).

## Interpretation

If the calculated 90% CI for all the pharmacokinetic parameters ($C_{max}$, AUC and more) falls within the acceptance range of 80% to 125%, it is generally considered that the test product is bioequivalent to the reference product. In other words, the two formulations are considered to be similar enough in terms of pharmacokinetics to be interchangeable from a regulatory perspective.

In summary, the 90% confidence interval is a statistical tool used to determine whether the test product's pharmacokinetic parameters fall within an acceptable range of equivalence compared to the reference product. If the 90% CI falls within the 80% to 125% range, it suggests that the test product is likely to be bioequivalent to the reference product.

The FDA requires companies applying for new drugs to keep backup samples of the drug and any reference products used in testing. These backups are for the FDA to re-run tests if needed.

## Retention of bioavailability samples as per Sec. 320.38 of FDA guidance

As per the guidance the backup samples for new drugs are based on their similarity to current ones.

The organization must have enough backup for the FDA to conduct five tests.

Backups must be clearly marked and preserved for at least 5 years.

The FDA often collects backups during inspections. If not, companies must keep them available for 5 years. This allows the FDA to check the accurateness of the initial testing, if required in future upon submission of the study data.[4]

Bioavailability/Bioequivalence (BA/BE) studies for generic products are typically conducted in accordance with the regulatory guidance of the country of submission. Table 1 below outlines the regulatory landscape, highlighting key parameters and minor variations among the major regulatory agencies.

**Table 1: Regulatory Landscape.**

| Parameter | US FDA | EMA | WHO | ICH |
|---|---|---|---|---|
| CI range (AUC, $C_{max}$) | 80–125% | 80–125% | 80–125% | 80–125% |
| NTI limits | 90–111% | 90–111% | Case-by-case | 90–111% |
| HVD handling | Scaled BE | Scaled BE | Recommend replicate | Harmonized |
| Biowaivers | BCS I, some III | BCS I, some III | BCS I, some III | Under harmonization |

## CONCLUSION

Bioequivalence and bioavailability studies are cornerstones of pharmaceutical research, ensuring patient safety and efficacy when using generic drugs. Understanding the BCS classification, selecting appropriate study designs, addressing highly variable and narrow therapeutic index drugs and adhering to regulatory guidelines are integral aspects of conducting robust and reliable bioequivalence studies. These studies not only facilitate the approval of generic drugs but also contribute to the cost-effective and widespread availability of essential medications, benefiting patients and healthcare systems globally.

**General BE study flow:**

Start: Initiate the bioequivalence study process.

**BCS Classification:**

Classify Drug Based on Solubility and Permeability.

Class I: High solubility, high permeability.

Class II: High solubility, low permeability.

Class III: Low solubility, high permeability.

Class IV: Low solubility, low permeability.

**Determine Required Testing:**

Class I: Less rigorous testing.

Class II and III: Bioequivalence studies needed.

Class IV: Extensive assessments required.

**Study Design Selection:**

Single-Dose, Two-Period, Crossover Study:

Most common design.

**Replicate Crossover Design:**

Generally, for highly variable drugs and for narrow therapeutic index drugs.

**Highly Variable Drugs:**

**Intra-Subject Variability:**

Use replicate crossover design.

Narrow Therapeutic Index Drugs:

**Stringent Criteria:**

Tighten acceptance criteria.

Regulatory Guidelines (USFDA and EMA):

**Study Design:**

Consideration of fasting/fed conditions, sampling schedules.

**Pharmacokinetic Parameters:**

Generally, $C_{max}$, AUC as primary parameters.

90% Confidence Interval (CI) Calculation:

Geometric Mean Ratio (GMR).

Standard Deviation (SD).

**CI Formula:**

$90\%\ CI = \exp(\ln(GMR) \pm (t^{*}SE))$.

Retention of Bioavailability Samples:

**FDA Requirement:**

Preserve backup samples for 5 years.

Available for re-testing.

End: Complete the bioequivalence assessment.

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## ABBREVIATIONS

**BCS:** Biopharmaceutics Classification System, **FDA:** Food and Drug Administration, **EMA:** European Medicines Agency, **$C_{max}$:** Maximum plasma concentration, **AUC:** Area under the concentration-time curve, **$T_{max}$:** Time to reach $C_{max}$, **GMR:** Geometric mean ratio, **SD:** Standard deviation, **CI:** Confidence Interval, **NTI:** Narrow Therapeutic Index, **IR:** Immediate Release.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## REFERENCES


1. Bioequivalence Studies with Pharmacokinetic Endpoints for Drugs Submit. (2021, August 20). Bioequivalence Studies with Pharmacokinetic Endpoints for Drugs Submitted Under an Abbreviated New Drug Application | FDA. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/bioequivalence-studies-pharmacokinetic-endpoints-drugs-submitted-under-abbreviated-new-drug
2. European Medicines Agency. Guideline on the investigation of bioequivalence. Committee for Medicinal Products for Human Use (CHMP). 2010.
3. EMA, Committee for Medicinal Products for Human Use (CHMP). Guideline on the pharmacokinetic and clinical evaluation of modified release dosage forms (EMA/CPMP/EWP/280/96 Corr*). 2014; 1-46.
4. CFR-Code of federal regulations, Title 21, Volume 5, Chapter I, Subchapter D, Part 320.38, Retention of bioavailability Samples. Updated 15 June 2023.


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