

METHODS OF STUDYING BIOAVAILABILITY

AND BIOEQUIVALENCE

INTRODUCTION:

- A *multisource drug product* is a drug product that contains the same active drug substance in the same dosage form and is marketed by more than one pharmaceutical manufacturer.
- *Single-source drug products* are drug products for which the patent has not yet expired or has certain exclusivities so that only one manufacturer can make it. Single-source drug products are usually brand-name (innovator) drug products. After the patent and other exclusivities for the brand-name drug expires, a pharmaceutical firm may manufacture a generic drug product that can be substituted for the branded drug product.
- Since the formulation and method of manufacture of the drug product can affect the bioavailability and stability of the drug, the generic drug manufacturer must demonstrate that the generic drug product is bioequivalent and therapeutically equivalent to the brand-name drug product.
- Drug product selection and generic drug product substitution are major responsibilities for physicians, pharmacists, and others who prescribe, dispense, or purchase drugs. To facilitate such decisions, the U.S. Food and Drug Administration (FDA) publishes annually, in print and on the Internet, *Approved Drug Products with Therapeutic Equivalence Evaluations*, also known as the *Orange Book*.
- The *Orange Book* identifies drug products approved on the basis of safety and effectiveness by the FDA and contains therapeutic equivalence evaluations for approved multisource prescription drug products. These evaluations serve as public information and advice to state health agencies, prescribers, and pharmacists to promote public education in the area of drug product selection and to foster containment of health care costs.

BIOAVAILABILITY(BA):

"Bioavailability means the rate and the extent to which the active drug ingredient of therapeutic moiety is absorbed from a drug product and becomes available at the site of action." (FDA Official Statement in 1977)

- "The rate at which, and the extent to which the drug substance and/or its active metabolites reach(es) the systemic circulation." (International Consensus Statement in 1991)

BIOEQUIVALENCE(BE):

- A relative term which denotes that the drug substance in two or more dosage forms, reaches the systemic circulation at the same relative rate and to the same relative extent i.e., their plasma concentration time profiles will be identical without significant statistical difference.

BIOEQUIVALENT DRUG PRODUCTS:

- This term describes pharmaceutical equivalent or pharmaceutical alternative products that display comparable bioavailability when studied under similar experimental conditions.
- For systemically absorbed drugs, the test (generic) and reference listed drug (brand-name) shall be considered bioequivalent if:
 - (1) the rate and extent of absorption of the test drug do not show a significant difference from the rate and extent of absorption of the reference drug when administered at the same molar dose of the therapeutic ingredient under similar experimental conditions in either a single dose or multiple doses;
 - OR**
 - (2) the extent of absorption of the test drug does not show a significant difference from the extent of absorption of the reference drug when administered at the same molar dose of the therapeutic ingredient under similar experimental conditions in either a single dose or multiple doses and the difference from the reference drug in the rate of absorption of the drug is intentional, is reflected in its proposed labeling, is not essential to the attainment of effective body drug concentrations on chronic use, and is considered medically insignificant for the drug.
- When the above methods are not applicable (eg, for drug products that are not intended to be absorbed into the bloodstream), other *in-vivo* or *in-vitro* test methods to demonstrate bioequivalence may be appropriate.
- Bioequivalence may sometimes be demonstrated using an *in-vitro* bioequivalence standard, especially when such an *in-vitro* test has been correlated with human *in-vivo* bioavailability data. In other situations, bioequivalence may sometimes be demonstrated through comparative clinical trials or pharmacodynamic studies.
- Bioequivalent drug products may contain different inactive ingredients, provided the manufacturer identifies the differences and provides information that the differences do not affect the safety or efficacy of the product.

NEED FOR BIOAVAILABILITY –BIOEQUIVALENCE STUDIES:

BIOAVAILABILITY:

- To evaluate the absolute systemic availability of an oral, topic, intramuscular, or any other dosage form
- To determine if bioavailability parameters are linear over the proposed clinical dose range

- To estimate the inter and intra subject variability
- To study food effects
- To define the effect of changes in the physicochemical properties of the drug substance and the effect of the drug product (dosage form) on the pharmacokinetics of the drug.

BIOEQUIVALENCE:

- Surrogate for therapeutic equivalence to enable “switchability”
- An appropriate measure for the quality control of the product *in vivo*

WHEN SHOULD “BIOEQUIVALENCE STUDIES” CONDUCTED?

- When a generic formulation is tested against an innovator brand
- Where a proposed dosage form is different from that used in a pivotal clinical trial
- When significant changes are made in the manufacture of the marketed formulation

BIOAVAILABILITY:

WHY DO WE CARE ABOUT BA?

- The therapeutic effectiveness of a drug depends upon the ability of the dosage form to deliver the medicament to its site of action at a rate & amount sufficient to produce the desired pharmacologic response.
- For most of the drugs, the pharmacologic response is directly related to the plasma levels. Bioavailability is an absolute term.
- The “true dose” is not the drug swallowed; BUT is the drug available to exert its effect
 - Dissolution
 - Absorption
 - Survive metabolism
- May have a drug with very low bioavailability
 - Dosage form or drug may not dissolve readily
 - Drug may not be readily pass across biological membranes (i.e. be absorbed)
 - Drug may be extensively metabolized during absorption process (first-pass, gut wall, liver)
- Important component of overall variability
 - Variable bioavailability may produce variable exposures

SO,

- The rate of absorption & extent of absorption both are important consideration.
 - A) Rate of absorption: - It may be fast or slow.
 - If fast, we get rapid onset of action, which are desired in treatment of acute conditions like asthma attack, pain etc.
 - Slow rate of absorption is desired when prolong duration of action is needed or to avoid side effects.

B) Extent of absorption:- It is of significance in the treatment of chronic conditions like hypertension, epilepsy etc.

• If the size of the dose administered is same, then the bioavailability of a drug from dosage form depends upon 3 major factors.

1. Pharmaceutical factors
2. Patient related factors
3. Route of administration

➤ The influence of route of administration on drug's bioavailability generally follows this order:

Parenteral > oral > rectal > topical.

- Within parenteral route, i.v. injection of a drug results in 100% bioavailability as the absorption process is bypassed.
- In case of oral route, the dose available to the patient is called Bio available dose which is often less than the administered dose.

Therefore, the bio-available fraction F, refers to the fraction of administered dose that enters the systemic circulation.

$$F = \frac{\text{Bioavailable dose}}{\text{Administered dose}}$$

• **OBJECTIVES OF BA STUDIES:**

Bioavailability studies are important in the...

1. Primary stages of development of a suitable dosage form for a new drug entity.
2. Determination of influence of excipients, patient related factors & possible interaction with other drugs on the efficiency of absorption.
3. Development of new formulations of the existing drugs.
4. Control of quality of a drug product during the early stages of marketing in order to determine the influence of processing factors, storage & stability on drug absorption.

CHARACTERISTICS OF DRUGS WITH GREATEST POTENTIAL

FOR BIOAVAILABILITY PROBLEM:

1. The drug has a narrow therapeutic ratio.
2. The drug has low solubility in water and / or the dissolution rate of the dosage form is slow.
3. The drug product contain high ratio of excipient to active ingredient.
4. The absorption of the drug is limited to a specific region of g.i. tract.
5. The metabolism of the drug is rapid.
6. The drug exhibits dose dependent pharmacokinetics.

CONSIDERATION IN BIOAVAILABILITY STUDY DESIGN:

1. BIOAVAILABILITY ABSOLUTE VS. RELATIVE

Absolute bioavailability

When the systemic availability of a drug administered orally is determined in comparison to its intravenous administration, is called as absolute availability.

$$F = \frac{[AUC]_{\text{oral}} D_{\text{iv.}}}{[AUC]_{\text{iv.}} D_{\text{oral}}}$$

AUC = Area under the Curve

D = Dose of administered drug

- Intravenous dose is selected as a standard due to its 100% bioavailability
- If the drug is poorly water soluble, intramuscular dose can be taken as standard.
- Its determination is used to characterize a drug's inherent absorption properties from extravascular site.

Relative bioavailability

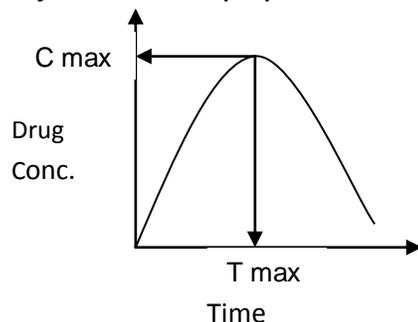
When the systemic availability of a drug after administration is compared with that of standard of the same drug it's referred to as relative bioavailability (Fr).

$$F_r = \frac{[AUC]_{\text{test}} D_{\text{std}}}{[AUC]_{\text{std}} D_{\text{test}}}$$

- The standard is a pure drug evaluated in a crossover study.
 - Its determination is used to characterize absorption of drug from its formulation.
- Both F AND Fr ARE EXPRESSED AS PERCENTAGE.

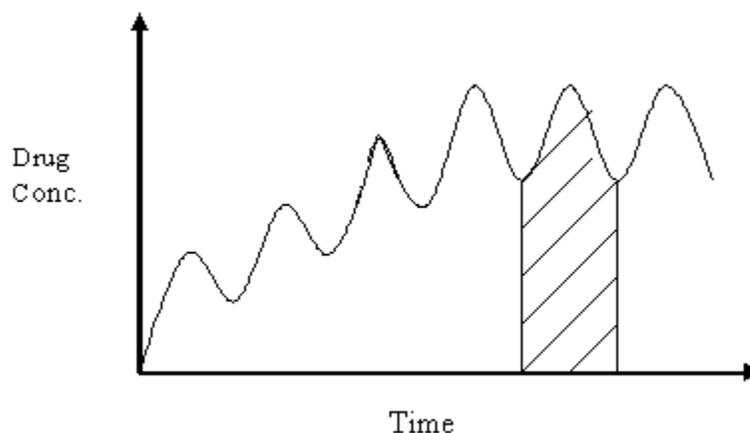
2. SINGLE DOSE VS. MULTIPLE DOSE STUDY

- **Single dose** bioavailability studies are very common, easy, offer less exposure and less tedious. But, it's difficult to predict the steady state characteristics and intersubject variability by this method.



- **Multiple dose** study is difficult to control (poor subject compliance), exposes the subject to more drug, highly tedious and time consuming but has several advantages like:
 1. More accurately reflect the manner in which the drug should be used.
 2. The drug blood levels are higher due to cumulative effect which makes its determination possible using less sensitive analytical method.
 3. Better evaluation of performance of a controlled release formulation is possible.
 4. Nonlinearity in pharmacokinetics, if present, can be easily detected.
 5. Easy to predict the peak & valley characteristic of the drug since the bioavailability is determined at steady – state.
 6. Requires collection of fewer blood samples.
 7. Can be ethically performed in patients because of the therapeutic benefit to the patient.

- In multiple dose study, one must ensure that steady state level has been reached. For this, the drug should be administered for 5-6 elimination half lives before collecting blood sample.



The USFDA requires both single and multiple

dose administration, as well as a determination of the effect of food on the absorption of the drug from the dosage form.

3. HUMAN VOLUNTEERS-HEALTHY SUBJECTS VS. PATIENTS

- **Ideally**, bioavailability studies should be carried out in **patients** for whom the drug is intended to be used because of the apparent advantages___
 1. The patient will be beneficial from the study.
 2. Reflects better therapeutic efficacy of a drug.
 3. Drug absorption pattern in disease states can be evaluated.
 4. Avoids the ethical requirements of administering drugs to the healthy subjects.

- In multiple dose study, they prefer patients rather than healthy humans.

- But, the **drawbacks of using patients as volunteers** are – disease, other drugs the patients may be taking, physiological changes, etc. may modify drug absorption pattern.
- Strict study conditions such fasting state required to be followed by the subject is also difficult. In short, establishing a standard set of conditions necessary for a bioavailability study is difficult with patients as volunteers.
- That's why such studies should be performed in young – 20 – 40 years, **healthy, male, adult volunteers**, body weight within a range $\pm 10\%$, under restricted dietary and fixed activity condition.
- The **consent of volunteers** must be obtained and they must be informed about the conditions to be followed during the course of studies – to abstain from any other medication for at least 2 weeks and to fast overnight prior to and for a minimum of 2-4 hours post dosing as well as possible hazards if any.

METHODS FOR ASSESSING BIOAVAILABILITY:

- Direct and indirect methods may be used to assess drug bioavailability.
- The *in-vivo* bioavailability of a drug product is demonstrated by the rate and extent of drug absorption, as determined by comparison of measured parameters, eg, concentration of the active drug ingredient in the blood, cumulative urinary excretion rates, or pharmacological effects.
- For drug products that are not intended to be absorbed into the bloodstream, bioavailability may be assessed by measurements intended to reflect the rate and extent to which the active ingredient or active moiety becomes available at the site of action.
- The design of the bioavailability study depends on the objectives of the study, the ability to analyze the drug (and metabolites) in biological fluids, the pharmacodynamics of the drug substance, the route of drug administration, and the nature of the drug product.
- Pharmacokinetic and/or pharmacodynamic parameters as well as clinical observations and *in-vitro* studies may be used to determine drug bioavailability from a drug product .

1. Pharmacokinetic methods

- These are indirect methods
- Assumption that –pharmacokinetic profile reflects the therapeutic effectiveness of a drug.
- Advantages: - Accurate, Reliable, Reproducible
 - A. Plasma / blood level time profile.
 - Time for peak plasma (blood) concentration (t_{max})
 - Peak plasma drug concentration (C_{max})
 - Area under the plasma drug concentration–time curve (AUC)
 - B. Urinary excretion studies.
 - Cumulative amount of drug excreted in the urine (D_u)
 - Rate of drug excretion in the urine (dD_u/dt)

Time for maximum urinary excretion (t)
C. Other biological fluids

2. Pharmacodynamic methods

- Involves direct measurement.(measurement of pharmacologic or therapeutic end point)
- **Disadvantages:-** High variability- Difficult to measure- Limited choices- Less reliable- More subjective- Drug response influenced by several physiological & environmental factors
- Maximum pharmacodynamic effect (E_{max})
Time for maximum pharmacodynamic effect
Area under the pharmacodynamic effect–time curve
Onset time for pharmacodynamic effect
- They involve determination of bioavailability from:
 - A. Acute pharmacological response.
 - B. Therapeutic response.

3. In-vitro dissolution studies

- Closed compartment apparatus
- Open compartment apparatus
- Dialysis systems.

4. Clinical observations

- Well-controlled clinical trials

1A. PLASMA DRUG CONCENTRATION

Measurement of drug concentrations in blood, plasma, or serum after drug administration is the most direct and objective way to determine systemic drug bioavailability. By appropriate blood sampling, an accurate description of the plasma drug concentration–time profile of the therapeutically active drug substance(s) can be obtained using a validated drug assay.

t_{max} . The *time of peak plasma concentration*, t_{max} , corresponds to the **time required to reach maximum drug concentration after drug administration**. At t_{max} , peak drug absorption occurs and the rate of drug absorption exactly equals the rate of drug elimination (). Drug absorption still continues after t_{max} is reached, but at a slower rate. When comparing drug products, t_{max} can be used as an approximate indication of drug absorption rate. The value for t_{max} will become smaller (indicating less time required to reach peak plasma concentration) as the absorption rate for the drug becomes more rapid. Units for t_{max} are units of time (eg, hours, minutes).

C_{max} . The *peak plasma drug concentration*, C_{max} , represents **the maximum plasma drug concentration obtained after oral administration of drug**. For many drugs, a

relationship is found between the pharmacodynamic drug effect and the plasma drug concentration. C_{\max} provides indications that the drug is sufficiently systemically absorbed to provide a therapeutic response. In addition, C_{\max} provides warning of possibly toxic levels of drug. The units of C_{\max} are concentration units (eg, mg/mL, ng/mL). Although not a unit for rate, C_{\max} is often used in bioequivalence studies as a surrogate measure for the rate of drug bioavailability.

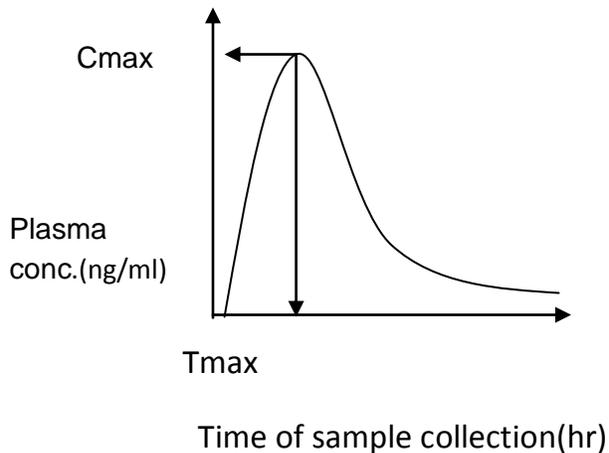
AUC. The area under the plasma level–time curve, AUC, is a measurement of the **extent of drug bioavailability** (). The AUC reflects the total amount of active drug that reaches the systemic circulation. The AUC is the area under the drug plasma level–time curve from $t = 0$ to $t = \infty$, and is equal to the amount of unchanged drug reaching the general circulation divided by the clearance.

$$[AUC]_0^\infty = \int_0^\infty C_p dt$$

$$[AUC]_0^\infty = \frac{FD_0}{\text{CLEARANCE}} = \frac{FD_0}{kV_D}$$

where F = fraction of dose absorbed, D_0 = dose, k = elimination rate constant, and V_D = volume of distribution.

- The AUC is independent of the route of administration and processes of drug elimination as long as the elimination processes do not change. The AUC can be determined by a numerical integration procedure, such as the trapezoidal rule method.
- The units for AUC are concentration time (eg, $\mu\text{g hr/mL}$).
- For many drugs, the AUC is directly proportional to dose. For example, if a single dose of a drug is increased from 250 to 1000 mg, the AUC will also show a fourfold increase (and).
- In some cases, the AUC is not directly proportional to the administered dose for all dosage levels. For example, as the dosage of drug is increased, one of the pathways for drug elimination may become saturated (). Drug elimination includes the processes of metabolism and excretion. Drug metabolism is an enzyme-dependent process.
- For drugs such as salicylate and phenytoin, continued increase of the dose causes saturation of one of the enzyme pathways for drug metabolism and consequent prolongation of the elimination half-life.
- The AUC thus increases disproportionately to the increase in dose, because a smaller amount of drug is being eliminated (ie, more drug is retained). When the AUC is not directly proportional to the dose, bioavailability of the drug is difficult to evaluate because drug kinetics may be dose dependent.



PLASMA DRUG CONCENTRATION TIME CURVE

1B. URINARY DRUG EXCRETION DATA

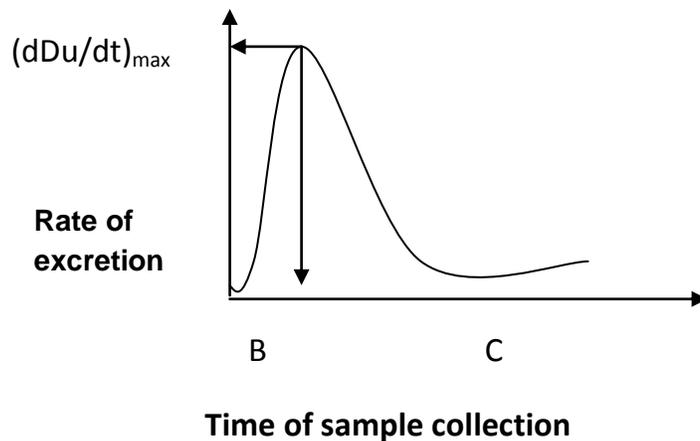
Urinary drug excretion data is an indirect method for estimating bioavailability. The drug must be excreted in significant quantities as unchanged drug in the urine. In addition, timely urine samples must be collected and the total amount of urinary drug excretion must be obtained .

$D_{\infty u}$. The ***cumulative amount of drug excreted in the urine***, $D_{\infty u}$, is related directly to the **total amount of drug absorbed**. Experimentally, urine samples are collected periodically after administration of a drug product. Each urine specimen is analyzed for free drug using a specific assay.

The relationship between the cumulative amount of drug excreted in the urine and the plasma level–time curve shows when the drug is almost completely eliminated , the plasma concentration approaches zero and the maximum amount of drug excreted in the urine, $D_{\infty u}$, is obtained.

dD_u/dt . The ***rate of drug excretion***. Because most drugs are eliminated by a first-order rate process, the rate of drug excretion is dependent on the first-order elimination rate constant k and the concentration of drug in the plasma C_p . In , the ***maximum rate of drug excretion***, $(dD_u/dt)_{max}$, is at point B, whereas the minimum rate of drug excretion is at points A and C. Thus, a graph comparing the rate of drug excretion with respect to time should be similar in shape as the plasma level–time curve for that drug ().

t^{∞} . The ***total time for the drug to be excreted***. In and , the slope of the curve segment A–B is related to the rate of drug absorption, whereas point C is related to the total time required after drug administration for the drug to be absorbed and completely excreted $t = \infty$. The t^{∞} is a useful parameter in bioequivalence studies that compare several drug products.



1C. OTHER BIOLOGICAL FLUIDS

- Bioavailability can also be determined using other biological fluids:
- eg: theophylline → salivary fluid,
Cephalosporin → CSF and bile fluids, etc.

2 A. ACUTE PHARMACOLOGICAL RESPONSE

- In some cases, the quantitative measurement of a drug in plasma or urine lacks an assay with sufficient accuracy and/or reproducibility. For locally acting, nonsystemically absorbed drug products, such as topical corticosteroids, plasma drug concentrations may not reflect the bioavailability of the drug at the site of action.
- An acute pharmacodynamic effect, such as an effect on forced expiratory volume, FEV_1 (inhaled bronchodilators) or skin blanching (topical corticosteroids) can be used as an index of drug bioavailability. In this case, the acute pharmacodynamic effect is measured over a period of time after administration of the drug product.
- Measurements of the pharmacodynamic effect should be made with sufficient frequency to permit a reasonable estimate for a time period at least three times the half-life of the drug ($t_{1/2}$). This approach may be particularly applicable to dosage forms that are not intended to deliver the active moiety to the bloodstream for systemic distribution.
- The use of an acute pharmacodynamic effect to determine bioavailability generally requires demonstration of a dose–response curve .
- Bioavailability is determined by characterization of the dose–response curve. For bioequivalence determination, pharmacodynamic parameters including the total area under the acute pharmacodynamic effect–time curve, peak pharmacodynamic effect, and time for peak pharmacodynamic effect are obtained from the pharmacodynamic effect–time curve. The onset time and duration of the pharmacokinetic effect may also be included in the analysis of the data.
- The use of pharmacodynamic endpoints for the determination of bioavailability and bioequivalence is much more variable than the measurement of plasma or urine drug concentrations.

- Effects such as change in ECG or EEG readings, pupil diameter, etc are related to the time course of a given drugs.
- Bioavailability can be determined by construction of pharmacologic effect time curve as well as dose response graph.
- The drawback of this method is that, the response tends to more variable. Moreover, the observed response may be due to an active metabolite whose concentration is not proportional to concentration of parent drug responsible for the pharmacological effect.

2B. THERAPEUTIC RESPONSE

- Theoretically, this method is most definite among all.
- It's based on observing clinical response to a drug formulation given to a patient suffering from disease for which the drug is intended to be used.
- A major drawback is that quantification of observed response is unreliable for assessment of bioavailability.

3. IN VITRO DISSOLUTION STUDY

- Drug dissolution studies may under certain conditions give an indication of drug bioavailability. Ideally, the *in-vitro* drug dissolution rate should correlate with *in-vivo* drug bioavailability (see and on *in-vivo–in-vitro* correlation, IVIVC). Dissolution studies are often performed on several test formulations of the same drug. The test formulation that demonstrates the most rapid rate of drug dissolution *in vitro* will generally have the most rapid rate of drug bioavailability *in vivo*.
 - The best available tool today which can at least quantitatively assure about the biological availability of a drug from its formulation.
 - The aim of these tests are mimicking the environment offered by the biological system as they must predict *in vivo* behavior to such an extent that *in vivo* bioavailability test need not be performed.
- A. Closed compartment apparatus** : Non sink condition
 - B. Open compartment apparatus** : perfect sink condition
 - C. Dialysis system**
 - This method is useful for very poorly aqueous soluble drugs for which maintenance of sink condition would require large volume of dissolution fluid.

4. CLINICAL OBSERVATIONS

- Well-controlled clinical trials in humans establish the safety and effectiveness of drug products and may be used to determine bioavailability.
- However, the clinical trials approach is the least accurate, least sensitive, and least reproducible of the general approaches for determining *in-vivo* bioavailability.
- The FDA considers this approach only when analytical methods and pharmacodynamic methods are not available to permit use of one of the approaches

described above.

- Comparative clinical studies have been used to establish bioequivalence for topical antifungal drug products (eg, ketoconazole) and for topical acne preparations.
- For dosage forms intended to deliver the active moiety to the bloodstream for systemic distribution, this approach may be considered acceptable only when analytical methods cannot be developed to permit use of one of the other approaches.

Analytical methodology

- The selected analytical method should be
 - Sufficiently sensitive to permit detection of low concentration of drug.
 - Reproducible
 - Must be specific for unmetabolized drug as well as capable of determining concentration of drug in presence of metabolites, constituents of blood/ urine.

Stable Isotope Studies

- This approach involves the simultaneous administration of test product and the reference product, using each subject as his own control.
- The reference contains the drug, which has been synthesized to contain a stable isotope such as such as ²H, ¹⁵N, ¹³C or ¹⁸O in a position in a drug molecule that is not susceptible to metabolism and does not result in kinetic differences due to presence of isotope.
- The sample is collected and the comparisons are made of quantity of labeled and unlabeled drug in each sample, using sophisticated detection systems involving mass spectroscopy.
- **Application of Stable Isotope Method in Study Bioavailability and Bioequivalence of Highly Variable Drugs and Formulations**
 - The stable isotope method has been used successfully in bioavailability studies for highly variable drugs that have extensive first-pass metabolism and exhibit large intra-subject variation in clearance.
 - Recently this technique has been extended to bioequivalence studies where labeled solution is intravenously infused in both occasions while test and reference formulation are administered.
 - This report discusses the advantages and disadvantages of the stable isotope method and its application in bioavailability and bioequivalence studies of highly variable drugs and drug delivery systems.

(REF: Journal of Food and Drug Analysis. 1994.Vol 2(Number3) : Pg155-160)

Bioavailability studies for a Controlled release drug product

- They are tested in a four way cross over with following treatment:
 - ✓ Administration under fasting condition
 - ✓ Administered 1 hr before a high fat content meal

- ✓ Administered immediately after a high fat content meal.
- ✓ Administered 2 hrs after a high fat content meal

➤ **A method for the calculation of bioavailability in slow release formulations in the presence of within-individual variability**

- In the present study they propose a model-independent method based on the combination of the area under the curve of serum drug levels and the mean residence time for evaluating the amount of bioavailability when within-individual variability is present in the serum clearance of the drug, administered as a slow release formulation (SRF), and this follows linear pharmacokinetic behaviour.
- The method assumes that the modifications in the area under the curve of the serum levels induced by the within-individual variability in the kinetic behaviour of the drug lead to a variation of the same proportions in the mean residence time of the serum levels curve and that this parameter can be used as a correction factor in the ratio of the areas under the curve of serum levels in bioavailability studies.
- The method allows one to calculate the fraction of dose absorbed from the SRF without having to measure the disposition clearance of the drug either when using the reference formulation or when the drug is administered as a SRF. The method is easy to apply and has a minimum mathematical complexity. The validity of the method was evaluated using simulated data with either no error or containing a random error of 10%.

(Ref:International journal of p'ceutics
Vol86, issue2-3, 26 oct 1992, pg 123-130)

BIOEQUIVALENCE:

It's commonly observed that there are several formulations of the same drug, in the same dose, in similar dosage form and meant to be given by the same route. In order to ensure clinical performance of such drug products, bioequivalence studies should be performed.

TYPES OF EQUIVALENCE:

Chemical Equivalence:

When 2 or more drug products contain the same labeled chemical substance as an active ingredient in the same amount.

Pharmaceutical Equivalence:

When two or more drug products are identical in strength, quality, purity, content uniformity, disintegration and dissolution characteristics; they may however differ in excipients.

Bioequivalence:

A relative term which denotes that the drug substance in two or more dosage forms, reaches the systemic circulation at the same relative rate and to the same relative

extent i.e., their plasma concentration time profiles will be identical without significant statistical difference.

Therapeutic Equivalence:

When two or more drug products that contain the same therapeutically active ingredient, elicit identical pharmacologic response and can control the disease to the same extent.

- It does not encompass a comparison of different therapeutic agent used in the same condition.
- The FDA considers drug products to be therapeutically equivalent if they meet the following criteria:
 1. Approved as safe and effective.
 2. Pharmaceutically equivalent
 3. Bioequivalent
 4. Adequately labeled
 5. Manufactured in compliance with cGMP.
- Although, they may differ in characteristics like, Shape, release mechanism, excipients, packaging, minor aspects of labeling (like the presence of specific pharmacokinetic information), expiration date/ time, etc.
- The FDA believes that products classified as therapeutically equivalent can be substituted with the same expectation that the substituted product will produce the same clinical effect and safety profile as the prescribed product.

Clinical Equivalence:

When the same drug from 2 or more dosage forms gives identical in vivo effects as measured by pharmacological response or by control over a symptom or a disease.

Drug Products with Possible Bioavailability and Bioequivalence Problems

During the development of a drug product, certain biopharmaceutical properties of the active drug substance or the formulation of the drug product may indicate that the drug may have variable bioavailability and/or a bioequivalence problem. Some of these biopharmaceutic properties include:

- The active drug ingredient has low solubility in water (eg, less than 5 mg/mL).
- The dissolution rate of one or more such products is slow (eg, less than 50% in 30 minutes when tested with a general method specified by the FDA).
- The particle size and/or surface area of the active drug ingredient is critical in determining its bioavailability.
- Certain structural forms of the active drug ingredient (eg, polymorphic forms, solvates, complexes, and crystal modifications) dissolve poorly, thus affecting absorption.
- Drug products that have a high ratio of excipients to active ingredients (eg, greater than 5:1).

- Specific inactive ingredients (eg, hydrophilic or hydrophobic excipients and lubricants) either may be required for absorption of the active drug ingredient or therapeutic moiety or may interfere with such absorption.
- The active drug ingredient, therapeutic moiety, or its precursor is absorbed in large part in a particular segment of the GI tract or is absorbed from a localized site.
- The degree of absorption of the active drug ingredient, therapeutic moiety, or its precursor is poor (eg, less than 50%, ordinarily in comparison to an intravenous dose), even when it is administered in pure form (eg, in solution).
- There is rapid metabolism of the therapeutic moiety in the intestinal wall or liver during the absorption process (first-order metabolism), so that the rate of absorption is unusually important in the therapeutic effect and/or toxicity of the drug product.
- The therapeutic moiety is rapidly metabolized or excreted, so that rapid dissolution and absorption are required for effectiveness.
- The active drug ingredient or therapeutic moiety is unstable in specific portions of the GI tract and requires special coatings or formulations (eg, buffers, enteric coatings, and film coatings) to ensure adequate absorption.
- The drug product is subject to dose-dependent kinetics in or near the therapeutic range, and the rate and extent of absorption are important to bioequivalence.

DIFFERENT METHODS OF STUDYING BIOEQUIVALENCE:

A. IN VIVO BIOEQUIVALENCE STUDY

- a. It requires determination of relative bioavailability after administration of a single dose of test and reference formulations by the same route, in equal doses, but at different times.
- b. The reference product is generally a previously approved product, usually a innovator's product or some suitable reference standard.
- c. The study is performed in fasting, young, healthy, adult male volunteers to assure homogeneity in the population & to spare the patients, elderly or pregnant women from rigors of such a clinical investigation.

TYPES OF DESIGNS:

1. PARALLEL GROUP DESIGN:

- In a parallel group design, subjects are divided randomly into groups, each group receiving one treatment randomly.
- Here number of groups is same as number of treatments to be compared.
- Each subject receives only one treatment.

2. CROSS OVER DESIGN:

- Arrangements in which each subject receives two or more different treatments on successive occasions, are known as cross over designs.
- In this design, the number of treatments is same as the number of periods.
- This design can be used with any number of treatments,

		Period	
		1	2
subjects	1	A	B
	2	B	A
	3	⋮	⋮
	4	⋮	⋮
	⋮	⋮	⋮
	⋮	⋮	⋮

subjects to the restriction that the number of subjects must be a multiple of the number of treatments.

COMPARISON

CROSS OVER DESIGN	PARALLEL GROUP DESIGN
<ul style="list-style-type: none"> • Between subject variability does not enter into the error variability. error. • Number of subjects required are more. are less. Balancing of order takes care of effect sequence or period effects. • Due to long wash out periods, subjects may drop out. • If observations are missing, even design loses efficiency. <p>The trial will be too time consuming. this</p>	<p>Between subject variability is very much and is included in</p> <p>Number of subjects required</p> <p>There is no fear of sequence or period effect.</p> <p>No fear of drop outs.</p> <p>The statistical analysis is simple if some results are missing.</p> <p>If the investigated drug and/or metabolite have long half life, design can be used</p>

CROSS OVER DESIGN(DETAIL):

1.LATIN SQUARE CROSS OVER DESIGN: In which

1. Each formulation is administered just once to each subject & once in each study period, &
2. Unlike parallel design, all the subjects do not receive the same formulation at the same time; in a given study period, they are administered different formulations.

An example of the **Latin square cross- over design** for a bioequivalence study in human volunteers is given in following table:-

- Examples of *Latin-square crossover designs* for a bioequivalence study in human volunteers, comparing three different drug formulations (A, B, C).

- The Latin-square design plans the clinical trial so that each subject receives each drug product only once, with adequate time between medications for the elimination of the drug from the body.
- In this design, each subject is his own control, and subject-to-subject variation is reduced. Moreover, variation due to sequence, period, and treatment (formulation) are reduced, so that all patients do not receive the same drug product on the same day and in the same order.
- Possible carryover effects from any particular drug product are minimized by changing the sequence or order in which the drug products are given to the subject.
- Thus, drug product B may be followed by drug product A, D, or C (). After each subject receives a drug product, blood samples are collected at appropriate time intervals so that a valid blood drug level–time curve is obtained. The time intervals should be spaced so that the peak blood concentration, the total area under the curve, and the absorption and elimination phases of the curve may be well described.

Latin-Square Crossover Design for a Bioequivalence Study of Three Drug Products in Six Human Volunteers

	Drug Product		
Subject	Study Period 1	Study Period 2	Study Period 3
1	A	B	C
2	B	C	A
3	C	A	B
4	A	C	B
5	C	B	A
6	B	A	C

Period refers to the time period in which a study is performed. A two-period study is a study that is performed on two different days (time periods) separated by a *washout period* during which most of the drug is eliminated from the body—generally about 10 elimination half-lives. A *sequence* refers to the number of different orders in the treatment groups in a study. For example, a two-sequence, two-period study would be designed as follows:

	Period 1	Period 2
Sequence 1	T	R
Sequence 2	R	T

where R = reference and T = treatment.

shows a design for three different drug treatment groups given in a three-period study with six different sequences. The order in which the drug treatments are given should not stay the same in order to prevent any bias in the data due to a residual effect from the previous treatment.

2.REPLICATED CROSS OVER DESIGN

Replicated crossover designs are used for the determination of individual bioequivalence, to estimate within-subject variance for both the Test and Reference drug products, and to provide an estimate of the subject-by-formulation interaction variance. Generally, a four-period, two-sequence, two-formulation design is recommended by the FDA.

	Period 1	Period 2	Period 3	Period 4
Sequence 1	T	R	T	R
Sequence 2	R	T	R	T

where R = reference and T = treatment.

The same reference and the same test are each given twice to the same subject. Other sequences are possible. In this design, Reference-to-Reference and Test-to-Test comparisons may also be made.

☒ Advantages of Cross over design:

- ✓ Minimize intersubject variability in plasma drug level.
- ✓ Minimize intrasubject variability → affecting bioavailability of a subsequently administered product.
- ✓ Minimize variation due to time effect.
- ✓ Make it more possible to focus more on formulation variables which is the key to success for any bioequivalence study.

☒ Drawbacks of cross-over design:-

- ⇒ Takes long time since appropriate washout period between 2 administrations is essential.
- ⇒ Time may be longer if the drug has $t_{1/2}$ long.
- ⇒ When the no. of formulations to be tested are more, the study becomes more difficult and subject dropout rate may increase.
This can be overcome by use of a balanced incomplete design in which a subject receives no more than two formulations.

EVALUATION OF THE DATA:

Analytical Method:

The analytical method for measurement of the drug must be validated for accuracy, precision, sensitivity, and specificity. The use of more than one analytical method during a bioequivalence study may not be valid, because different methods may yield different values. Data should be presented in both tabulated and graphic form for evaluation. The plasma drug concentration–time curve for each drug product and each subject should be available.

Pharmacokinetic Evaluation of the Data

For single-dose studies, including a fasting study or a food intervention study, the pharmacokinetic analyses include calculation for each subject of the area under the curve to the last quantifiable concentration (AUC_{0-t}) and to infinity ($AUC_{0-\infty}$), T_{max} , and C_{max} . Additionally, the elimination rate constant, k , the elimination half-life, $t_{1/2}$, and other parameters may be estimated. For multiple-dose studies, pharmacokinetic analysis includes calculation for each subject of the steady-state area under the curve, (AUC_{0-t}), T_{max} , C_{min} , C_{max} , and the percent fluctuation [$100 \times (C_{max} - C_{min})/C_{min}$]. Proper statistical evaluation should be performed on the estimated pharmacokinetic parameters.

Statistical Evaluation of the Data

- Bioequivalence is generally determined using a comparison of population averages of a bioequivalence metric, such as AUC and C_{max} . This approach, termed *average bioequivalence*, involves the calculation of a 90% confidence interval for the ratio of averages (population geometric means) of the bioequivalence metrics for the Test and Reference drug products.
- To establish bioequivalence, the calculated confidence interval should fall within a prescribed bioequivalence limit, usually, 80–125% for the ratio of the product averages. Standard crossover design studies are used to obtain the data.
- Another approach proposed by the FDA and others is termed *individual bioequivalence*. Individual bioequivalence requires a replicate crossover design, and estimates within-subject variability for the Test and Reference drug products, as well as subject-by-formulation interaction.
- Presently, only average bioequivalence estimates are used to establish bioequivalence of generic drug products.
- To prove bioequivalence, there must be no statistical difference between the bioavailability of the Test product and the Reference product. Several statistical approaches are used to compare the bioavailability of drug from the test dosage form to the bioavailability of the drug from the reference dosage form.
- Many statistical approaches (parametric tests) assume that the data are distributed according to a normal distribution or "bell-shaped curve". The

distribution of many biological parameters such as C_{\max} and AUC have a longer right tail than would be observed in a normal distribution ().

- Moreover, the true distribution of these biological parameters may be difficult to ascertain because of the small number of subjects used in a bioequivalence study. The distribution of data that has been transformed to log values resembles more closely a normal distribution compared to the distribution of non-log-transformed data.
- Therefore, log transformation of the bioavailability data (eg, C_{\max} , AUC) is performed before statistical data evaluation for bioequivalence determination.

Analysis of Variance (ANOVA)

- An analysis of variance (ANOVA) is a statistical procedure used to test the data for differences within and between treatment and control groups. A bioequivalent product should produce no significant difference in all pharmacokinetic parameters tested.
- The parameters tested usually include AUC_{0-t} , $AUC_{0-\infty}$, t_{\max} , and C_{\max} obtained for each treatment or dosage form. Other metrics of bioavailability have also been used to compare the bioequivalence of two or more formulations.
- The ANOVA may evaluate variability in subjects, treatment groups, study period, formulation, and other variables, depending on the study design. If the variability in the data is large, the difference in means for each pharmacokinetic parameter, such as AUC, may be masked, and the investigator might erroneously conclude that the two drug products are bioequivalent.
- A statistical difference between the pharmacokinetic parameters obtained from two or more drug products is considered statistically significant if there is a probability of less than 1 in 20 times or 0.05 probability ($p \leq 0.05$) that these results would have happened on the basis of chance alone. The probability, p , is used to indicate the level of statistical significance. If $p < 0.05$, the differences between the two drug products are not considered statistically significant.
- To reduce the possibility of failing to detect small differences between the test products, a *power test* is performed to calculate the probability that the conclusion of the ANOVA is valid. The power of the test will depend on the sample size, variability of the data, and desired level of significance. Usually the power is set at 0.80 with a $\beta = 0.2$ and a level of significance of 0.05. The higher the power, the more sensitive the test and the greater the probability that the conclusion of the ANOVA is valid.

Two One-Sided Tests Procedure

- The two one-sided tests procedure is also referred to as the *confidence interval approach* (). This statistical method is used to demonstrate if the bioavailability of the drug from the Test formulation is too low or high in comparison to that of the

Reference product. The objective of the approach is to determine if there are large differences (ie, greater than 20%) between the mean parameters.

- The 90% confidence limits are estimated for the sample means. The interval estimate is based on a Student's *t* distribution of the data.
- In this test, presently required by the FDA, a 90% confidence interval about the ratio of means of the two drug products must be within $\pm 20\%$ for measurement of the rate and extent of drug bioavailability. For most drugs, up to a 20% difference in AUC or C_{\max} between two formulations would have no clinical significance.
- The lower 90% confidence interval for the ratio of means cannot be less than 0.80, and the upper 90% confidence interval for the ratio of the means cannot be greater than 1.20. When log-transformed data are used, the 90% confidence interval is set at 80–125%. These confidence limits have also been termed the *bioequivalence interval*. The 90% confidence interval is a function of sample size and study variability, including inter- and intrasubject variability.
- For a single-dose, fasting study, an analysis of variance (ANOVA) is usually performed on the log-transformed AUC and C_{\max} values. There should be no statistical differences between the mean AUC and C_{\max} parameters for the Test (generic) and Reference drug products.
- In addition, the 90% confidence intervals about the ratio of the means for AUC and C_{\max} values of the Test drug product should not be less than 0.80 (80%) nor greater than 1.25 (125%) of that of the Reference product based on log-transformed data.

B. IN VITRO BIOEQUIVALENCE STUDY:

In following circumstances equivalence may be assessed by the use of in vitro dissolution testing:

(B.1)Drugs for which the applicant provide data to substantiate all of the following:

1. Highest dose strength is soluble in 250ml of an aqueous media over the pH range of 1-7.5 at 37°C.
2. At least 90% of the administered oral dose is absorbed on mass balance determination or in comparison to an intravenous reference dose.
3. Speed of dissolution as demonstrated by more than 80% dissolution within 15 minutes at 37°C using IP apparatus 1, at 50 rpm or IP apparatus 2, at 100rpm in a volume of 900 ml or less in each of the following media:
 - a. 0.1 N hydrochloric acid or artificial gastric juice (without enzymes)
 - b. A pH 4.5 buffer
 - c. A pH 6.8 buffer or artificial intestinal juice (without enzyme)

(B.2)Different strength of the drug manufactured by the same manufacturer, where all of the following criteria are fulfilled:

1. The qualitative composition between the strengths is essentially the same;

2. The ratio of active ingredients and excipients between the strength is essentially the same or in the case of small strength, the ratio between the excipients is the same;
3. The method of manufacture is essentially the same;
4. An appropriate equivalence study has been performed on at least one of the strength of the formulation (usually the highest strength unless a lower strength is chosen for reasons of safety); and
5. In case of systemic availability-pharmacokinetics have been shown to be linear over the therapeutic dose range.

In vitro dissolution testing may also be suitable to confirm unchanged product quality and performance characteristics with minor formulation or manufacturing changes after approval.

C. PHARMACODYNAMIC STUDIES:

Studies in healthy volunteers of patients using pharmacodynamic parameters may be used for establishing equivalence between two pharmaceutical products. These studies may become necessary

1. If quantitative analysis of the drug and/or metabolite(s) in plasma or urine cannot be made with sufficient accuracy and sensitivity.
2. if measurement of drug concentrations cannot be used as surrogate endpoints for the demonstration of efficacy and safety of the particular pharmaceutical product e.g. topical products without an intended absorption of the drug into the systemic circulation.

D.COMPARATIVE CLINICAL TRIALS

It is carried out when

1. The plasma concentration time-profile data may not be suitable to assess equivalence between two formulations.
2. Pharmacodynamic studies cannot be performed because of lack of meaningful pharmacodynamic parameters, which can be measured.
3. Pharmacodynamic and pharmacokinetic studies are not feasible.

Stereoisomerism in bioequivalence studies

- The use of stereoselective method in bioequivalence studies is an important goal to be realized in future.
- Stereoselective methods should be used in two situation:
 - ✓ When there is high first pass metabolism of the active enantiomer (eutomer) → suggests that both total and eutomer concentrations should be measured.
 - ✓ When there is low first pass metabolism of the eutomer, each enantiomer should be measured separately.
- In all other cases, nonstereoselective methods should suffice.

➤ **The impact of stereoisomerism in a bioequivalence study on two formulations of doxepin**

A bioequivalence study was carried out on two formulations of doxepin containing 15% of the active *cis* isomer and 85% of the less active *trans* isomer. The 90% confidence intervals ($\ln AUC_{last}$, $\ln C_{max}$ and $\ln C_{max}/AUC_{last}$) for *N*-desmethyldoxepin fell entirely within bioequivalence limits whether stereoselective or non-stereoselective data were analyzed. Thus the results of this study did not support arguments in favor of the use of stereoselective methods in bioequivalence studies

(Ref: European journal of pharmaceutical sciences, Vol 4, issue 3, May 1996, pg:133-138)

Bioequivalence studies for controlled release Product

- For controlled release products administered once a day, a three way cross over study comparing the test formulation with an approved controlled release drug product under fasting conditions is conducted to ensure controlled release nature of the test product as well as the absence of dose dumping.

Following are the cases where bioequivalence studies are not necessary:

- a) An aqueous solution for parenteral use
- b) A solution for oral use
- c) A gas
- d) A powder for reconstitution as a solution for oral or parenteral use
- e) An otic or ophthalmic solution
- f) A topical aqueous solution
- g) An inhalation product or nasal spray as an aqueous solution.

Possible Surrogate Markers for Bioequivalence Studies		
Drug Product	Drug	Possible Surrogate Marker for Bioequivalence
Metered-dose inhaler	Albuterol	Forced expiratory volume (FEV ₁)
Topical steroid	Hydrocortisone	Skin blanching
Anion-exchange resin	Cholestyramine	Binding to bile acids
Antacid	Magnesium and aluminum hydroxide gel	Neutralization of acid
Topical antifungal	Ketoconazole	Drug uptake into stratum corneum

Problems in Bioavailability and Bioequivalence

Drugs with high intrasubject variability	Inhalation
Drugs with long elimination half-life	Ophthalmic
Biotransformation of drugs	Intranasal
Stereoselective drug metabolism	Bioavailable drugs that should not produce peak drug levels
Drugs with active metabolites	Potassium supplements
Drugs with polymorphic metabolism	Endogeneous drug levels
Nonbioavailable drugs (drugs intended for local effect)	Hormone replacement therapy
Antacids	Biotechnology-derived drugs
Local anesthetics	Erythropoietin interferon
Anti-infectives	Protease inhibitors
Anti-inflammatory steroids	Complex drug substances
Dosage forms for nonoral administration	Conjugated estrogens
Transdermal	

FDA RECOMMENDATIONS:

- To help avoid complications arising from product substitution, the FDA established a list of generic drugs that can be safely and appropriately substituted for brand products
- The FDA does not recommend substituting drugs that have not been determined to be bioequivalent

FDA POLICY ON DRUG SUBSTITUTION

- The FDA has prepared a list of drugs that are bioequivalent; they can be substituted for each other
- These drugs are listed in a federal publication called *Approved Drug Products With Therapeutic Equivalence Evaluations*, known as the *Orange Book*
- Drugs that are not listed as bioequivalent should not be substituted for each other
- the “orange book” provides therapeutic evaluation codes for drug products:
 - First letter indicates therapeutic equivalence
 - Yes (A) or no (B)
 - Second letter indicates additional information on the basis of FDA evaluation

Coding System :-First Letter.

Drug products that the FDA considers to be therapeutically equivalent to other pharmaceutically equivalent products are coded with the first letter "A". These are drug products for which there are no known or suspected bioequivalence problems.

Drug products that the FDA does not consider to be therapeutically equivalent to other pharmaceutically equivalent products are coded with the first letter "B". These are drug products for which actual or potential bioequivalence problems have not been resolved by adequate in vivo or in vitro evidence.

Often the lack of adequate evidence for bioequivalence is due to specific dosage forms rather than differences in the active ingredients. These are designated BC, BD, BE, BN, BP, BR, BS, BT, BX, or B*.

(I) A-Rated Products.

- A-rated drug products are considered to be therapeutically equivalent to other pharmaceutically equivalent products. A-rated products are those for which actual or potential bioequivalence problems have been resolved with adequate in vivo and/or in vitro evidence supporting bioequivalence. Drug products designated as A-rated fall under 1 of 2 main policies.
- For those active ingredients or dosage forms for which no in vivo bioequivalence issue is known or suspected, bioequivalence is presumed and considered self-evident based on other data in the application for some dosage forms (eg, solutions) or satisfied for solid oral dosage forms by a showing that an acceptable in vitro dissolution standard is met. These are designated AA.
- For drug products that contain active ingredients or dosage forms that have been identified by the FDA as having actual or potential bioequivalence problems, and for post-1962 drug products in a dosage form presenting a potential bioequivalence problem, an evaluation of therapeutic equivalence is assigned if the approved application contains adequate scientific evidence establishing, through in vivo and/or in vitro studies, the bioequivalence of the product. These are designated as AB.

Categories of AB-Rated Products.

- Multisource drug products listed under the same heading (ie, identical active ingredient[s], dosage form, and route[s] of administration) and having the same strength generally will be coded AB if a study is submitted demonstrating bioequivalence.
- In certain instances, a number is added to the end of the AB code to make a 3-character code (eg, AB1, AB2, or AB3). Three-character codes are assigned only in situations when more than 1 reference (innovator) product of the same strength has been designated under the same heading. Two or more reference drugs are generally designated only when there are multiple reference drug products that are not bioequivalent. If a study is submitted that demonstrates a generic

product's bioequivalence to a specific reference drug product, the generic product will be given the same 3-character code as the reference drug it was compared against.

Additional A-Rated Products.

<i>Rating Label</i>	<i>Type of Drug Product</i>	<i>Characteristics</i>
<i>AN</i>	<i>Solutions and powders for aerosolization</i>	<i>Marketed for use in any of several delivery systems and are considered to be PE and TE; bioequivalence standard is based on in vitro methodology</i>
<i>AO</i>	<i>Injectable oil solutions</i>	<i>Considered to be PE and TE only when the active ingredient, its concentration, and the oil type used as a vehicle are identical</i>
<i>AP</i>	<i>Injectable aqueous solutions and, sometimes, intravenous (IV) nonaqueous solutions</i>	<i>Injectable (parenteral) products that are TE, but may have different characteristics (eg, route of administration)</i>
<i>AT</i>	<i>Topical products</i>	<i>Contain the same active ingredient in the same topical dosage form with a bioequivalence waiver</i>

(II) B-Rated Products.

- The BD code denotes products containing active ingredients with known bioequivalence problems and for which adequate studies have not been submitted that demonstrate bioequivalence.
- The FDA's bioequivalence regulations contain criteria and procedures for determining whether a specific active ingredient in a specific dosage form has the potential for causing a bioequivalence problem.¹ It is FDA policy to consider an ingredient meeting these criteria as having a potential bioequivalence problem even in the absence of positive data demonstrating nonequivalence. Pharmaceutically equivalent products containing these ingredients in oral dosage forms are coded BP until adequate in vivo bioequivalence data are submitted.
- The code BX is assigned to specific drug products for which the data are insufficient to determine therapeutic equivalence under the policies stated by the FDA. In these situations, the drug products are presumed to be

therapeutically nonequivalent until the FDA has determined that there is adequate information to make a full evaluation of therapeutic equivalence.

EXAMPLES TAKEN FROM DIFFERENT JOURNALS:

1. Evaluation of the Absolute Oral Bioavailability and Bioequivalence of Erlotinib, an Inhibitor of the Epidermal Growth Factor Receptor Tyrosine Kinase, in a Randomized, Crossover Study in Healthy Subjects.

Both intravenous and oral erlotinib were generally well tolerated with an estimated bioavailability of 59% following oral administration

(Ref: <http://jclinpharm.highwire.org/cgi/content/abstract/46/3/282>)

2. All aciclovir cream formulations are not bioequivalent.

These studies suggest that not all marketed ACV creams are bioequivalent to the clinically proven innovator. Given the magnitude of the differences seen, there is concern over therapeutic inequivalence of generic ACV creams to the innovator cream.

(Ref: International journal of pharmaceutics, Vol.304, issues1-2, 4Nov,2005, pg63-71)

3. Bioequivalence of two oral contraceptive drugs containing norethindrone and ethinyl estradiol

The data support the hypothesis for bioequivalence of the two formulations with respect to total absorption.

(Ref: Contraception, Vol.40, issue 5, nov1989, pg 581-590)

4. Bioequivalence and tolerability study of two brands of clopidogrel tablets, using inhibition of platelet aggregation and pharmacodynamic measures

In this study of healthy male volunteers, the 2 tablet preparations of clopidogrel showed bioequivalence.

(Ref: Current Therapeutic Research, Vol64, issue9, Nov-Dec 2003, pg 685-696)

5. Pharmacokinetics and bioequivalence of Oxybutynin sustained release capsule and conventional tablet in healthy volunteers.

Here sustained release capsules are extendedly released. Test and reference preparations are bioequivalent for AUC_0-V_{Ft} but not bioequivalent for Q_{max} .

(Ref: CA, Vol-151, NO 12, Sept21, 2009, pgn 1525, 272547e)

6. Bioequivalence of simvastatin tablets in Healthy volunteers by randomized crossover study.

Concentration Time curve of two preparations fitted one compartment model. Both preparations are bioequivalent.

(Ref:CA, Vol-151, no13, sept28,2009 ,pg2100, 297392p)

7.Pharmacokinetics and Bioequivalence of Tankejing dripping pills in healthy volunteers by randomized 2cycle crossover study.

Here both formulations were found to be bioequivalent.

(Ref:CA, Vol-151, no13, sept28,2009 ,pg2100, 297393q)

8.Pharmacokinetics of Lofexidine HCl in healthy volunteers.

Single dose,crossover study and a multiple study were conducted. Pharmacokinetic data indicate that Lofexidine has a consistent profile.

(Ref:JPS, Vol98, NO1,Jan 2009, pg319)

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21. JPS, Vol98, NO1,Jan 2009, pg319

QUESTIONS:

1. In a bioavailability study, explain how determination of both rate and extent of absorption are important?
2. Compare single dose vs. multiple dose bioavailability studies.
3. Discuss the merits and demerits of using healthy subjects and patients as volunteers for bioavailability studies.
4. Which is the method of choice in bioavailability determination? On what principle such a study based? (Key: Plasma level time studies)
5. Explain with the significance the parameters used in bioavailability determination using plasma level studies.
6. Determination of metabolites in urine is not used as a measure of bioavailability? Why? (Key: Presystemic metabolism before absorption)
7. Drawbacks of acute pharmacological response and therapeutic response as measures of bioavailability.
8. Explain cross over study for bioequivalence.
9. Layout a Latin square cross over diagram for bioequivalence study on three formulations- X, Y, Z in six volunteers.
10. Generally accepted statistical rules for establishing bioequivalence.
11. Note on Orange Book.

Previous Questions: -

1. Discuss the methods of measuring Bioavailability (2007)
2. Define bioavailability? Why do we care about bioavailability? Mention the objectives of bioavailability studies? Write a note on single dose versus multiple dose studies.
3. Write a note on- Bioavailability –absolute versus relative
4. Explain the coding system for bioequivalent products as per FDA
5. Latin square design: Advantages and drawbacks