Review

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Bioavailability and Bioequivalence studies: An Overview & Update

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Abstract

Bioavailability and bioequivalence studies (BA/BE) basically assess the relative concentration of a particular drug (in %) entering the systemic circulation from the administered dose and to determine if it is significant or not from the reference product. Bioavailability and bioequivalence data is mandatory to be furnished while putting in application for any new drug, so it is of utmost importance that the testing methods for measuring availability and assessing equivalence of any given drug should have a scientifically tested and standardised method pertaining to the design and conduct of the given method. The studies conducted *in vitro* and *in vivo* can be helpful in generating data regarding the availability and equivalence of any given drug molecule. While, we have all the methods available for such studies, we also have set guidelines towards carrying out these studies. These guidelines are in place for maintaining the uniformity and quality of the study and also to make certain the efficacy and the lack of significant side effects (safety) of a pharmaceutical product under evaluation. These guidelines are laid down and exercised by the Food and Drug Administration (FDA) & Centre for Drug Evaluation and Research (CDER) in USA and by the Central Drugs Standard Control Organization (CDSCO) in India. This review tries to look into the guidelines laid down by the regulatory authorities, the various designs employed, the facilities supposed to be present at the site for conducting these studies and how the generated data is maintained and used for further analysis.

Keywords: Bioavailability, Bioequivalence, CDSCO, Study designs

Introduction

In developing countries, pharmaceuticals contribute to about 40% of the healthcare budget. The major determinant of health outcome is the access to essential medicines. About 70% of the drugs in market are considered duplicative or non-essential. Hence the concept of essential medicines was embraced to cater the health needs of the people. However in developing countries like India, it is not adequate due to the poverty levels in the communities.(1) To overcome these problems, generic medicines were introduced worldwide to provide affordability to the patients. These are the pharmaceutical products which are bioequivalent to the innovator product in terms of strength, dosage form, route of administration, safety, quality, performance and its intended usage.(2) Even with such an effort, the ordinary people are unable to translate the health expenditure on drugs into savings due to the rise of branded generics which are marketed at a price close to innovator brands. Though the unbranded medicines are cheaper and similar in efficacy to the branded medicines, they are not found in prescriptions due to lack of confidence. The World Health Organization (WHO) estimates that about 30% of the world population lacks a proper access to the essential medicines and it is expected that it will rise to more than 50% in some countries of Africa and Asia in the near future.(3)

For a drug to be introduced in the market preclinical studies are carried out initially and approval is obtained taking the safety aspects into consideration which is depicted in figure 1.

Bioavailability versus Bioequivalence:

Bioavailability is defined as the fraction of administered dose in percentage (0-100) that enters the systemic circulation in either an active or pro-drug form.(4) Various factors are found to influence the bioavailability like food, DDI (drug-drug interactions) and other pathophysiological conditions like age, sex, race, hepatic and renal disorders etc. It also depends on the active ingredient of the drug formulation related to the process of its manufacture, its formulation, metabolism and other biological properties associated.(5)

In the recent years, studies on bioequivalence gained significant attention from the pharmaceutical industries and the regulatory health authorities towards the development of new drugs and to gain access to the essential medicines.(6) Drug products are considered to be bioequivalent when the rate and extent of bioavailability of the active ingredient in the two products are not significantly different under suitable test conditions when administered at the same molar dose.(4)

Some of these terms need to be taken into special consideration while conducting the bioavailability and bioequivalence studies.

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Table 1: Four sequence, four period, four treatment Latin square design

	Period 1	Period 2	Period 3	Period 4
Subject A	1	4	3	2
Subject B	3	2	1	4
Subject C	4	1	2	3
Subject D	2	3	4	1

Absolute bioavailability compares the bioavailability of an active drug through extravascular administration versus an intravenous administration while the relative bioavailability compares the same between two different formulations of the same drug.(7) Two drug products that are found to contain the same active ingredient and identical in terms of their strength, dose and route of administration are referred as pharmaceutical equivalents. However these may differ in their shape, packaging, scoring configuration, excipients etc. Therapeutic equivalent is another similar term in close association which is used to specify the drug products that are pharmaceutically equivalent with the same clinical efficacy and safety. Pharmaceutical alternatives are another group of drug products having the same therapeutic moiety but with different esters, complexes or salts of that particular moiety or with different strengths or dosage forms.(8) Cmax determines the peak estimated concentration of drug in the systemic circulation following its administration and Tmax is the time required to attain the same. AUC is the measure of bioavailability from 0 to ∞.(9) It can be calculated by (F x D)/ CL where F- bioavailability, D- dose of the drug and CL- clearance. Bioavailability in turn can be calculated by the formula F= Fa x Fg xFh where Fa – fraction of drug that is absorbed across intestine, Fg - fraction that escapes presystemic gut wall metabolism and Fh - fraction that escapes hepatic first-pass metabolism.(10)

Overview of CDSCO Guidelines, India:
CDSCO guidelines were mainly framed to ensure the therapeutic equivalence & to measure the quality of the product by *in vivo and in vitro* methods. These guidelines discuss regarding the drug products and its bioequivalence studies if required or not. They compare the new drug formulation with a prior formulation or a reference product. The reference product should also have the same active ingredient as that of the new drug and it is denoted as the "Designated Reference Product" by the Licensing Authority.(11)
Firstly, bioequivalence studies are required in the following *in vivo* conditions: a) Oral drug formulations for immediate release with systemic action and fulfilling one of the criteria as in for serious conditions requiring assured therapeutic response, drugs with

Figure 1: Flowchart depicting the approval of drug process

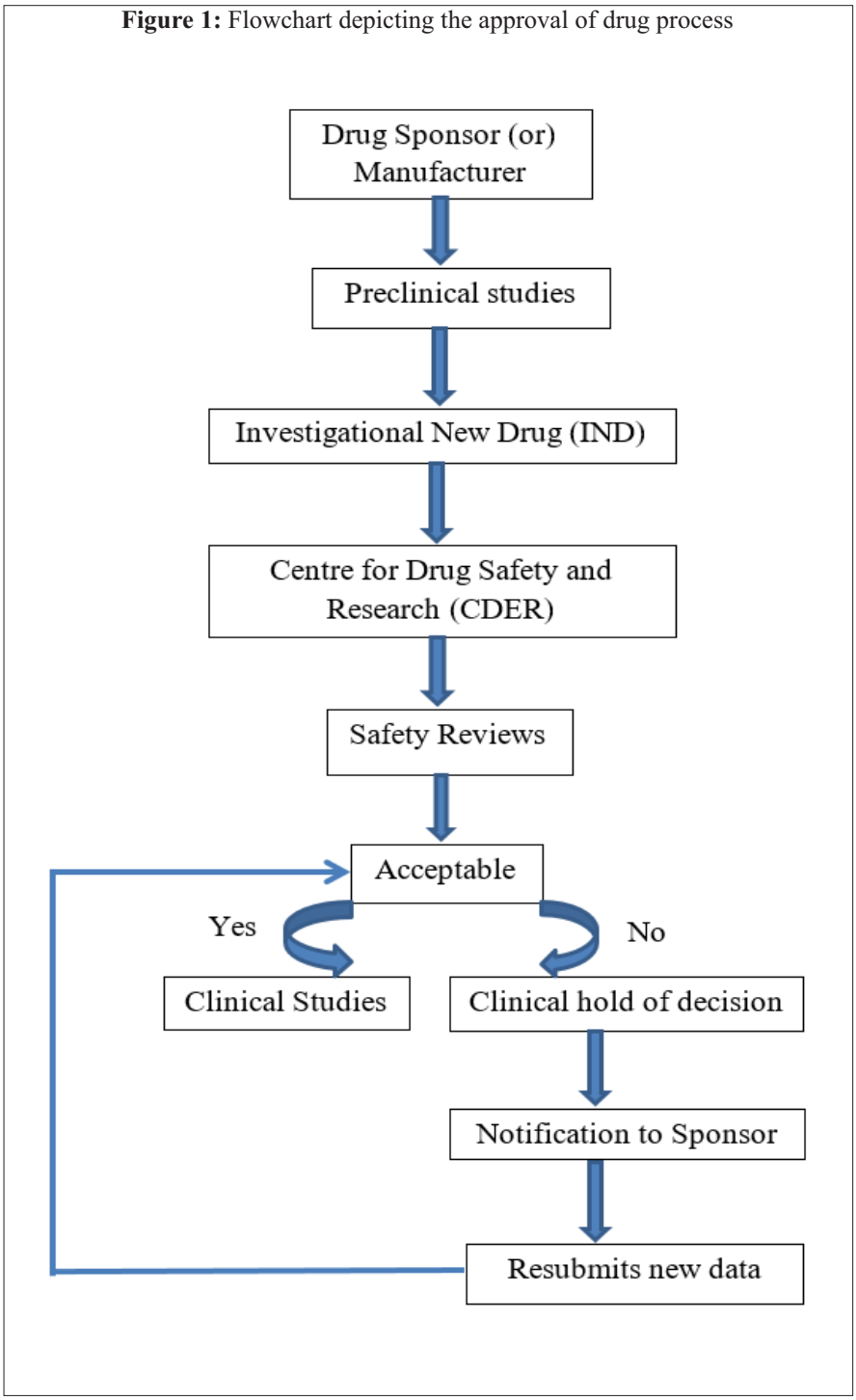


Figure 2: Demonstration of clinical quality according to BCS classification SUPAC- Scale up and post approval changes (13)

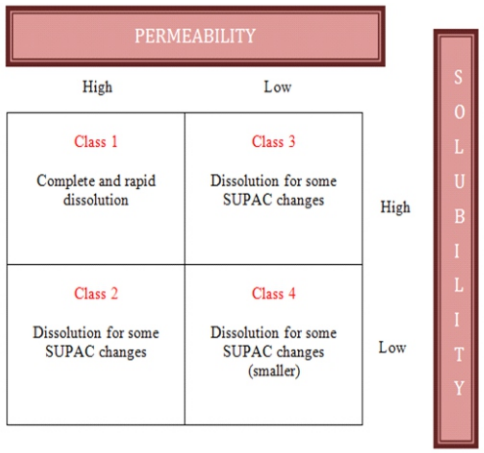
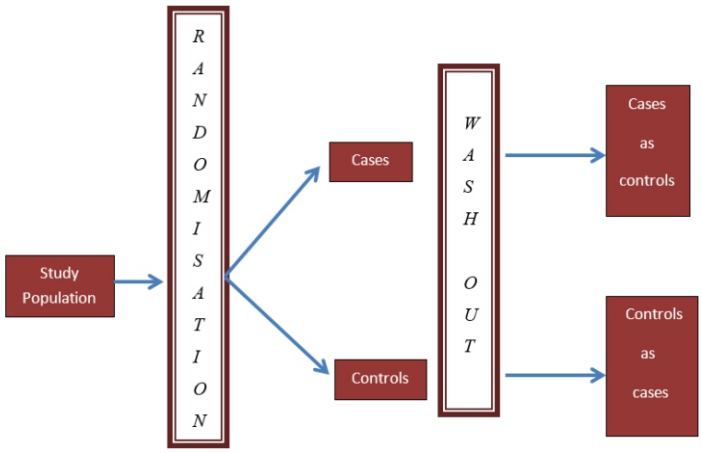


Figure 3: Picture depicting the outline of crossover design



narrow safety margin; steep dose-response curve, high first-pass metabolism >70%, incomplete drug absorption, non-linear pharmacokinetics, unfavourable physicochemical properties (low solubility, instability, poor permeability), high excipients to active ingredients ratio. b) systemically absorbed non-oral and non-parenteral drug formulations (transdermal patches, suppositories etc.,) c) modified release drug formulations with systemic absorption. d) systemically acting fixed-dose combination products. e) Pharmaceutical products which are not in the form of solution (non-systemic use-oral, nasal, ocular, rectal, vaginal) acting without systemic absorption. These studies are also useful in *in vitro* conditions by dissolution testing for the drugs for which the complete data is provided by the applicant to fulfil the following criteria like a) at pH 1-7.5 & 37 ^{0}C, the highest dose strength should be soluble in 250 ml of an aqueous media b) a minimum of 90% absorption of an orally administered dose as determined by a mass balance c) Dissolution speed of >80% at 37 ^{0}C, within 15 minutes at 50 rpm using IP apparatus 1; or at 100 rpm using IP apparatus 2 in a volume of ≤900 ml in some media (0.1 N hydrochloric acid or artificial gastric juice, a pH 4.5 buffer, a pH 6.8 buffer or artificial intestinal juice). Dissolution testing is also useful when the same manufacturer manufactures different strengths of a drug and satisfies the following criteria like a) same qualitative composition between the strengths b) ratio of active constituents and excipients, or that between the excipients of different strengths is essentially the same c) manufacturing method is almost the same d) in case of performing bioequivalence study on at least one of the strengths in an appropriate manner.(11) Bioequivalence studies however are not required in a few conditions to compare a new formulation with the reference product which are referred as bio-waivers. These include a) newer drugs which are given as aqueous solutions administered parenterally or as an otic, ophthalmic or topical preparation and containing the same active ingredient in the same concentration; & with comparable concentrations of the same excipients b) When the newer drug is an oral usage solution containing the active ingredient in the same concentration without an excipient that is found to have an effect on either the absorption of the active ingredient or its transit in the gastrointestinal tract c) newer drug in the form of a powder to be reconstituted as a solution and meets either of the above two criteria d) When the new drug is a gas e) When the new drug is a nasal spray or an inhalational product administered as an aqueous solution containing the active ingredient and excipient in comparable concentrations.(11)The Biopharmaceutics Classification System (BCS) classifies all the pharmaceutical products available into four major classes based on two important

Figure 4: Two group parallel design

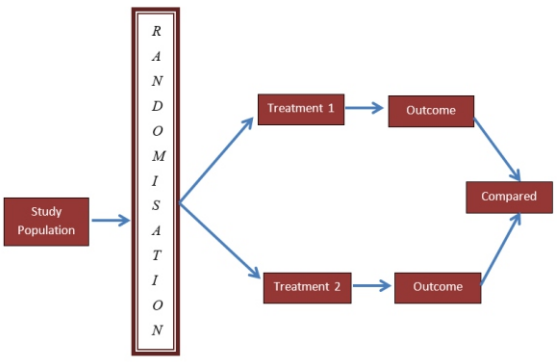
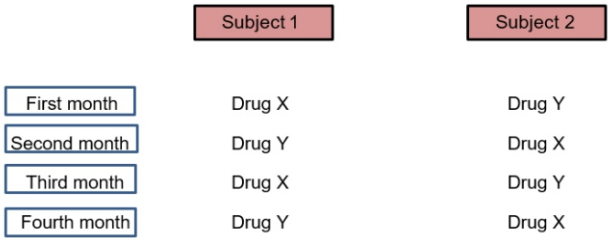


Figure 5: Replicate cross over design



factors which regulate drug absorption i.e. solubility in aqueous media and its permeability through the intestine. Class I: High Solubility, High Permeability, Class II: Low Solubility, High Permeability, Class III: High Solubility, Low Permeability, Class IV: Low Solubility, Low Permeability depicted in figure 2. Presently, the provision for biowaivers is granted for Class I and Class III immediate release solid oral dosage formulations. BCS approach plays a major role towards the evolution of both newer and generic drugs. The present guidelines of US-FDA, EMA (European Medicines Agency) and WHO are shown to have similarities in several aspects like the type of studies to be conducted and documentation to consider it as Class I or Class III. However, the US-FDA maintains some differences with respect to the criteria to be met in terms of solubility, permeability and dissolution. For instance, EMA and WHO consider a requirement volume of 500 ml for rapid dissolution whereas US-FDA specifies 900 ml.(12)

Study Designs

The ideal design of choice to conduct bioavailability and bioequivalence studies comparing two formulations is a crossover design as shown in figure 3.(13) This design is a modification of block randomisation in which at different points of time, each block will be receiving more than one formulation.(14)

Washout period is the period of rest for the two groups under study so that the effect of the formulation administered is not carried over to the next one giving sufficient time for it to be washed out. An adequate time period of minimum 3 half-lives of the components is maintained between the two phases of treatment.

The most common and popularly used crossover design is the two sequence, two period, two treatment design. A crossover deign may be categorised into two types based on the uniformity- uniform within sequences (in each sequence, each treatment occurs the same number of times) and uniform within periods (in each period, each treatment occurs the same number of times). A special type of crossover design is a Latin square design in which within each period and each sequence, each sort of treatment occurs only once.(15) The most common is the four sequence, four period, four treatment type as shown in table 1.

Alternatively, parallel design studies are done for formulations with long half-life which is shown in figure 4.

Replicate study designs are done for formulations with variable disposition depicted in figure 5.

Study Population:

a) Healthy adult volunteers either males or females based on the usage and safety criteria of the particular drug b) Pregnancy & women on contraceptive drugs are excluded from the study; pregnancy test is done before the first & last dose in the study procedure c) For drugs with a potential hazard in one gender, the selection of subjects is narrowed Eg., Studies on teratogenic drugs in males d) For drugs which are intended to be used in a particular sex, participants of that particular gender are included in the study e) Toxicity studies are done in patients with the concerned disease with stable disease condition.

The minimum subjects to be recruited to conduct these studies should be at least 16 unless otherwise justified for ethical reasons.(11)

Pre-requisites for conducting the study:

1. Standardisation of diet, fluid intake, exercise, post-dosing posture 2. Compliance is mentioned in the protocol and is also reported at the end of the study 3. Abstinence from alcohol, smoking, tea, coffee, fruit juices at least 48 hours before the start of the study and during the study process.(11)

Selection of sampling points:

In single-dose trials of a drug with immediate release of the products, the period sampling of blood should be extended to a minimum of 3 elimination half-lives and urine collection to ≥7 half-lives in cases of urinary excretion of the drug. Blood sampling points at different phases after drug administration in: absorption phase – minimum of 3, Tmax – 3 to 4, elimination phase – 4.

Fasting State Studies:

It may be a single dose study which is done with an overnight fasting (at least 10 hours) followed by a fast of 4 hours after the dosing or a multiple dose study ¬ in which 2 hours of fast before and after is acceptable to administer an evening dose of the drug.

Fed State Studies:

The main pre-requisites to perform fed state studies are high fat breakfast at least 15 minutes before dosing i.e. 50% calories from fat, 15-20% from protein, 30-35% of from carbohydrates providing 950-1000 Kcal of energy. It is done in cases of routine clinical practice as the drug is mostly recommended with food, in modified release formulations, and difficulty in the assessment of Cmax and Tmax with fasting studies.(11)

Bioanalytical methodology is used to determine drug and its metabolites in different body fluids like blood, plasma, serum, urine etc. For proper validation, it is categorised into two distinct phases: Pre-study Phase (on human plasma samples) and Study Phase (to confirm stability, accuracy and precision).(11)

The Pre-study Phase ensures evaluation of various characteristics and analyses them for the results to be considered reliable. These include stability data including the influence of at least 3 freezing and thawing cycle representative of the actual sample handling. The data regarding specificity and selectivity should be considered to make sure that the assay does not show any interference by endogenous compounds and other drugs or metabolites in the study samples, or by degradation products. Sensitivity refers to the capacity of the test procedure to record even a small variation in drug concentration. The lowest limit of quantification is usually not >20% and usually the limit of detection is always lower than the quantification limit. The values which lie between limit of quantification and limit of detection are referred to as "Below Quantification Limits". Precision is the degree of reproducibility of the individual assays and is documented at low, medium and high (Cmax) concentrations. It may be an intra-assay precision (within days) where the coefficient of variation is no >15%, although no >20% is also considered sometimes or an inter-assay precision (between days) where it may be higher than 15% but not >20%. Accuracy refers to the extent to which the true value of the drug concentration is estimated by the drug assay and ±15% accuracy is to be attained in general. Recovery is usually done at high, medium & low concentrations; along with documentation of any internal standard if used. In concentration vs. response curve, for the entire range of expected sample concentrations, a quantitative relationship is done. Standard curve for linear relationships is defined by at least five concentrations and for non-linear relationships, additional

points are considered. In any of the conditions, beyond the standard curve, extrapolation is not acceptable. Analytical standards are run at the start and at the end of the assay to ensure stability with monitoring of the standard curve.(11)
The study phase normally involves single determination analysis for validated data. Duplicate or replicate analysis is done for case-by-case basis. In unknown samples, the concentration of the analyte is done by plotting a standard curve for the entire range of concentrations and for each analytical run and each analyte. If the estimation of unknown falls below or above the standard concentration, then redetermination of standard curve (or) re-assay of sample is done after dilution.(11)

Pharmacodynamic Studies:
The requirements of a pharmacodynamics study include the following: a) Response should be related to the efficacy & safety of the drug b) Methodology is to be validated for precision, accuracy, specificity etc., c) During the study, neither the test nor the reference product should produce a maximal response d) Quantitative measurement of the response is done in a double-blind way e) By prior screening, non-responders are excluded & criteria for responders vs. non-responders is mentioned f) Crossover or parallel study design is considered appropriate g) Natural history & underlying pathology is to be mentioned in the study design h) In a nonlinear relationship between dose & AUC, based on the outcome of dose ranging study, correction is done.(11)

Clinical Studies:
This is done in cases where there are no proper pharmacodynamics parameters to be measured and in non-feasibility of pharmacokinetic & pharmacodynamics parameters.(11)
Sample size calculation for BABE studies:
To calculate sample size for any study design, three components are mainly considered to be essential- the variance in response variable which is sought to be analysed, to frame an alternative hypothesis and to have a desired power. The variance is assessed from the data of similar studies in the past. The alternative hypothesis is denoted by delta which is the deviation from the null hypothesis. Power (1-β) is normally taken as 80% or higher.
The principles to design a bioequivalence study are the same as that used for a clinical trial. The only difference is the testing a set of two null hypotheses: a) Ho1: $\mu T/\mu R \leq 0.8$; b) H02: $\mu T/\mu R \geq 1.25$, where μT & μR are expected bioavailabilities for test and reference formulations. This type of study is normally done by a crossover study. For the given two formulations to be considered bioequivalent it should reject the null hypotheses (two one-sided tests) at the 5% level and a power of 1 - β is required. These null hypotheses can also be tested by a 90% two-sided confidence interval. Berlex chose 25% as the variance level after examining the similar studies conducted previously. (16)
The calculated 90% confidence interval for AUC & Cmax should fall within 80-125% (bioequivalence range) to establish bioequivalence. In supra-bioavailability studies, reformulation followed by a fresh bioequivalence study is done.(16)
In a two-period two-treatment crossover bioequivalence pharmacokinetic study, the number of subjects is typically small, mostly less than 60. Among all the available statistical tests, the two one-sided test is the most common approach to test bioequivalence. However, in the two one-sided test method, there is no suitable mathematical formula to calculate the power function in the literature.(17)
Documentation:
The documents to be maintained in a bioequivalence study include clinical data (for compliance with GCP guidelines), the details of validation of analytical method (linearity range, QC sample analysis etc.,), analytical data of the volunteer samples (chromatograms, inter-day & intra-day variation of assay results etc.,), raw data, comments of the chief investigator and copy of the final report.(11)

Sampling Techniques:
The sponsor of the study or the drug manufacturer sends the test product and the reference standard of different batches to the testing facility in a packaged form. The testing facility performs bioequivalence testing by selecting them at random and the samples are maintained as reserve samples. These reserve samples represent the batches that are given by the study sponsor or the drug manufacturer and are in fact retained in the native container. As the sponsor or the manufacturer has a chance of providing the testing facility with a variation in the size of the containers and the way of packaging, FDA has implemented regulations regarding this aspect. If each of the test and reference products is given in single containers to the testing facility, then a sufficient amount of each should be taken from the containers and the remaining is stored in the same containers. If each of them is given in multiple containers, then the testing facility selects some samples randomly and the others are preserved as reserve samples. Each reserve sample should contain a sample of sufficient quantity that helps FDA in performing all the release tests that are mentioned in the application for five times.(18) Drug products of the same batch need to be matched for the bioequivalence study. Reserve sample is to be preserved in a place separated from the area where it is tested with access limited to authorised personnel.(11)
Facilities for conducting BA/BE studies:
The key facilities to conduct bioavailability and bioequivalence studies include: a) Organisation should have a legal identity b) It should be impartial, need to maintain confidentiality, independence & integrity c) organisation with qualified and trained investigators d) It should have a Clinical Pharmacological Unit (CPU) management and analytical laboratory management e) It should have Standard Operating Procedures (SOP) and compliance with aspects of GCP (Good Clinical Practice) guideline and GLP (Good Laboratory Practice) guidelines.(11)
The records concerned with bioavailability and bioequivalence studies are to be maintained by the sponsor minimum for 2 years after the expiry of the marketed drug for which *in vivo & in vitro* studies are conducted and are submitted to the CDSCO on request. The samples are preserved for 3 years after the performance of study or 1 year after the drug expiry by the organisation whichever is earlier.(11)

Generics – A boon:
Introduction of generic medicines has brought a significant development in the process of health care. These drugs are known to have the same chemical composition as that of the brand drug and act at the same site as that of the native drug with similarities in terms of dose, efficacy, potency etc. Generics in the field of

transplantation with regard to immunosuppressants have brought a tremendous development, particularly for drugs like cyclosporine which have become cost effective.(19) They have a widespread use in different fields of medicine contributing to about half of the total volume and 18% of the market value of the pharmaceuticals. Though the increased access to generic medicines all over the world has gained a significant improvement in the health care unit, there are still some hurdles which are yet to be sorted out. Stringent guidelines are to be framed regarding the standards of the generic medicines which helps to reduce the supply of counterfeit drugs and to obtain a proper approval before reaching the market.(20)

Conclusion:

Over the past 20 years, the pharmaceutical industries & the national regulatory authorities all over the world has adopted the concept of bioavailability and bioequivalence studies. A continuous attempt is being made to test the bioequivalence of drugs with varying dosage by the use of efficient techniques and validated approaches in a scientific manner. It is mainly aimed to study the pharmacokinetic and pharmacodynamics parameters after the administration of tested drugs and to bring a breakthrough for developing newer drugs by the pharmaceutical industry. In spite of the existence of pharmaceutical industries concerned with studies on bioavailability and bioequivalence in India for long, it is more robust at present. The implementation of stringent laws and guidelines regarding patency has further brought a significant improvement. Among the health care costs, drug cost is an important constituent that causes an economic burden in the society. The concept of generic medicines thus came into existence that benefited the patients to procure the drugs at affordable prices with quality comparable to the branded drugs. They also aim to remove inequities in the drug cost in diseases like cancer & AIDS which have a huge financial impact in developing countries.

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