GUIDELINES FOR BIOAVAILABILITY & BIOEQUIVALENCE STUDIES

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These guidelines should be read in conjunction with Schedule Y to the Drugs and Cosmetic Rules, GCP Guidelines issued by CDSCO, Ministry of Health and Family Welfare, GLP and the Ethical Guidelines for Biomedical research on human subjects issued by Indian Council of Medical Research. All provisions described in above documents shall appropriately apply to the conduct of bioavailability and bioequivalence studies.

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1. INTRODUCTION

Ensuring uniformity in standards of quality, efficacy and safety of pharmaceutical products is the fundamental responsibility of CDSCO. Reasonable assurance has to be provided that various products, containing same active ingredients, marketed by different licensees, are clinically equivalent and interchangeable.

Accordingly, the bioavailability of an active substance from a pharmaceutical product should be known and reproducible. In most cases, it is cumbersome and unnecessary to assess this by clinical studies. Bioavailability and bioequivalence data is therefore required to be furnished with applications for new drugs, as required under Schedule Y, depending on the type of application being submitted.

Both bioavailability and bioequivalence focus on the release of a drug substance from its dosage form and subsequent absorption into the systemic circulation. For this reason, similar approaches to measuring bioavailability should generally be followed in demonstrating bioequivalence.

Bioavailability can be generally documented by a systemic exposure profile obtained by measuring drug and/or metabolite concentration in the systemic circulation over time. The systemic exposure profile determined during clinical trials in the early drug development can serve as a benchmark for subsequent BE studies.

Bioequivalence studies should be conducted for the comparison of two medicinal products containing the same active substance. The studies should provide an objective means of critically assessing the possibility of alternative use of them. Two products marketed by different licensees, containing same active ingredient(s), must be shown to be therapeutically equivalent to one another in order to be considered interchangeable. Several test methods are available to assess equivalence, including:

i comparative bioavailability (bioequivalence) studies, in which the active drug substance or one or more metabolites is measured in an accessible biological fluid such as plasma, blood or urine
ii comparative pharmacodynamic studies in humans
iii comparative clinical trials
iv in-vitro dissolution tests

The guidelines describe when bioavailability or bioequivalence studies are necessary and describe requirements for their design, conduct, and evaluation. The possibility of using *in vitro* instead of *in vivo* studies with pharmacokinetic end points is also envisaged.

For classes of products, including many biologicals such as vaccines, animal sera, and products derived from human blood and plasma, and product manufactured by biotechnology, the concept of interchangeability raises complex which may be addressed by the applicant on the basis of contemporary scientific rationale.

In vivo bioequivalence/bioavailability studies recommended for approval of modified release products should be designed to ensure that

i the product meets the modified release label claims

ii the product does not release the active drug substance at a rate and extent leading to dose dumping

iii there is no significant difference between the performance of the modified release product and the reference product, when given in dosage regimes to arrive at the steady state.

iv there must be a significant difference between the performance of modified release product and the conventional release product when used as reference product.

It is appreciated that pharmacokinetic studies can be conducted during any phase of a clinical trial for New Chemical Entities (NCEs). While these guidelines deal with pharmacokinetic / pharmacodynamic studies vis-à-vis bioavailability or bioequivalence studies for a generic drug, the principles described herein, are applicable for any pharmacokinetic / pharmacodynamic study.

2. DEFINITIONS

BIOAVAILABILITY
Bioavailability refers to the relative amount of drug from an administered dosage form which enters the systemic circulation and the rate at which the drug appears in the systemic circulation.

BIOEQUIVALENCE
Bioequivalence of a drug product is achieved if its extent and rate of absorption are not statistically significantly different from those of the reference product when administered at the same molar dose.

CLINICAL TRIAL
A clinical trial is a systematic study of pharmaceutical products in human subject(s), in order to discover or verify the clinical, pharmacological (*including pharmacodynamic / pharmacokinetic*), and/or adverse effects, with the object of determining their safety and/or efficacy.

GOOD CLINICAL PRACTICE (GCP) GUIDELINES:
Good Clinical Practice Guidelines issued by Directorate General of Health Services, Ministry of Health & Family Welfare, Government of India.

MODIFIED RELEASE DOSAGE FORMS
Modified-release dosage forms are those for which the drug-release characteristics of time course and/or drug-release location are chosen to accomplish such therapeutic or convenience objectives that are not offered by immediate-(conventional) release dosage forms.

PHARMACEUTICAL EQUIVALENTS
Pharmaceutical equivalents are drug products that contain identical amounts of the identical active drug ingredient, i.e., the same salt or ester of the same therapeutic moiety, in identical dosage forms, but not necessarily containing the same inactive ingredients.

PHARMACEUTICAL ALTERNATIVES
Pharmaceutical alternatives are drug products that contain the identical therapeutic moiety, or its precursor, but not necessarily in the same amount or dosage form or as the same salt or ester.

PHARMACODYNAMIC EVALUATION
Pharmacodynamic evaluation is measurement of the effect on a patho-physiological process as a function of time, after administration of two different products to serve as a basis for bioequivalence assessment.

PHARMACOKINETICS

Pharmacokinetics deals with the changes of drug concentration in the drug product and changes of concentration of a drug and/or its metabolite(s) in the human or animal body following administration of the drug product, i.e., the changes of drug concentration in the different body fluids and tissues in the dynamic system of liberation, absorption, distribution, body storage, binding, metabolism, and excretion.

NON-LINEAR PHARMACOKINETICS

Nonlinear kinetics or saturation kinetics refers to a change of one or more of the pharmacokinetic parameters during absorption, distribution, metabolism, and excretion by saturation or overloading of processes due to increased dose sizes.

REFERENCE PRODUCT

For purpose of these guidelines, the reference product is a pharmaceutical product which is identified by the Licensing Authority as “Designated Reference Product” and contains the same active ingredient(s) as the new drug. The Designated Reference Product will normally be the global innovator’s product. An applicant seeking approval to market a generic equivalent must refer to the Designated Reference Product to which all generic versions must be shown to be bioequivalent. For subsequent new drug applications in India the Licensing Authority may, however, approve another Indian product as Designated Reference Product.

SUPRA-BIOAVAILABILITY

This is a term used when a test product displays an appreciably larger bioavailability than the reference product.

SUSTAINED RELEASE DOSAGE FORM

These are modified release dosage forms where the liberation (drug release) rate constant is smaller than the unrestricted absorption rate constant.

STEADY STATE

Steady state is the state when the plasma concentration of drug at any time point during any dosing interval should be identical to the concentration at the same time during any other dosing interval. The steady state drug concentrations fluctuate (oscillate) between a maximum and a minimum steady state concentration within each of the dosing intervals.

THERAPEUTIC EQUIVALENTS

Therapeutic equivalents are drug products that contain the same active substance or therapeutic moiety and, clinically show the same efficacy and safety.

PHARMACOKINETIC TERMS

C_{max}

This is the maximum drug concentration achieved in systemic circulation following drug administration.

C_{min}

This is the minimum drug concentration achieved in systemic circulation following multiple dosing at steady state.

C_{pd}

This is the pre-dose concentrations determined immediately before a dose is given at steady state.

T_{max}

It is the time required to achieve maximum drug concentration in systemic circulation.

AUC_{0-t}

Area under the plasma concentration - time curve from 0 h to the last quantifiable concentration to be calculated using the trapezoidal rule

$AUC_{0-\infty}$

Area under the plasma concentration - time curve, from zero to infinity to be calculated as the sum of AUC_{0-t} plus the ratio of the last measurable concentration to the elimination rate constant

$AUC_{0-\tau}$

Area under the plasma concentration - time curve over one dosing interval following single dose for modified release products.

$AUC_{0-\tau(ss)}$

Area under the plasma concentration - time curve over one dosing interval in multiple dose study at steady state.

K_{el}

Apparent first-order terminal elimination rate constant calculated from a semi-log plot of the plasma concentration versus time curve.

$T_{1/2}$

Elimination half life of a drug is the time necessary to reduce the drug concentration in the blood, plasma, or serum to one-half after equilibrium is reached.

3. SCOPE OF THE GUIDELINES

Bioavailability and Bioequivalence studies are required by regulations to ensure therapeutic equivalence between a pharmaceutically equivalent test product and a reference product. Several in vivo and in vitro methods are used to measure product quality.

3.1 When bioequivalence studies are necessary and types of studies required

3.1.1 *In vivo* studies

For certain drugs and dosage forms, *in vivo* documentation of equivalence, through either a bioequivalence study, a comparative clinical pharmacodynamic study, or a comparative clinical trial, is regarded as especially important. These include:

a. Oral immediate release drug formulations with systemic action when one or more of the following criteria apply:

   i indicated for serious conditions requiring assured therapeutic response;

   ii narrow therapeutic window/safety margin; steep dose-response curve;

   iii pharmacokinetics complicated by variable or incomplete absorption or absorption window, nonlinear pharmacokinetics, pre-systemic elimination/high first-pass metabolism >70%;

   iv unfavourable physicochemical properties, e.g., low solubility, instability, meta-stable modifications, poor permeability, etc.;

   v documented evidence for bioavailability problems related to the drug or drugs of similar chemical structure or formulations;

   vi where a high ratio of excipients to active ingredients exists.

b. Non-oral and non-parenteral drug formulations designed to act by systemic absorption (such as transdermal patches, suppositories, etc.).

c. Sustained or otherwise modified release drug formulations designed to act by systemic absorption.

d. Fixed-dose combination products with systemic action.

e. Non-solution pharmaceutical products which are for non-systemic use (oral, nasal, ocular, dermal, rectal, vaginal, etc. application) and are intended to act without systemic absorption. In these cases, the bioequivalence concept is not suitable and comparative clinical or pharmacodynamic studies are required to prove equivalence. There is a need for drug concentration measurements in order to assess unintended partial absorption.

Bioequivalence documentation is also needed to establish links between:

i early and late clinical trial formulations

ii formulations used in clinical trials and stability studies, if different

iii clinical trial formulations and to be marketed drug products

iv other comparisons, as appropriate

In each comparison, the new formulation or new method of manufacture shall be the test product and the prior formulation (or respective method of manufacture) shall be the reference product.

3.1.2 *In vitro* studies

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

a. Drugs for which the applicant provides data to substantiate all of the following:

   i. highest dose strength is soluble in 250 ml of an aqueous media over the pH range of 1-7.5 at 37°C

   ii. at least 90% of the administered oral dose is absorbed on mass balance determination or in comparison to an intravenous reference dose

   iii. 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 100 rpm in a volume of 900 ml or less in each of the following media:

      1. 0.1 N hydrochloric acid or artificial gastric juice (without enzymes)

      2. a pH 4.5 buffer

      3. a pH 6.8 buffer or artificial intestinal juice (without enzymes)

b. Different strengths of the drug manufactured by the same manufacturer, where all of the following criteria are fulfilled:

   i. the qualitative composition between the strengths is essentially the same;

   ii. the ratio of active ingredients and excipients between the strengths is essentially the same, or, in the case of small strengths, the ratio between the excipients is the same;

   iii. the method of manufacture is essentially the same;

   iv. an appropriate equivalence study has been performed on at least one of the strengths of the formulation (usually the highest strength unless a lower strength is chosen for reasons of safety); and

v. 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.

3.2 When bioequivalence studies are not necessary

In following formulations and circumstances, bioequivalence between a new drug and the reference product may be considered self-evident with no further requirement for documentation:

a. When new drugs are to be administered parenterally (e.g., intravenous, intramuscular, subcutaneous, intrathecal administration etc.) as aqueous solutions and contain the same active substance(s) in the same concentration and the same excipients in comparable concentrations;

b. When the new drug is a solution for oral use, and contains the active substance in the same concentration, and does not contain an excipient that is known or suspected to affect gastro-intestinal transit or absorption of the active substance;

c. When the new drug is a gas;

d. When the new drug is a powder for reconstitution as a solution and the solution meets either criterion (a) or criterion (b) above;

e. When the new drug is an otic or ophthalmic or topical product prepared as aqueous solution and contains the same active substance(s) in the same concentration(s) and essentially the same excipients in comparable concentrations;

f. When the new drug is an inhalation product or a nasal spray, tested to be administered with or without essentially the same device as the reference product, prepared as aqueous solutions, and contain the same active substance(s) in the same concentration and essentially the same excipients in comparable concentrations. Special *in vitro* testing is required to document device performance comparison between reference inhalation product and the new drug product.

For (e) and (f) above, the applicant is expected to demonstrate that the excipients in the new drug are essentially the same and in comparable concentrations as those in the reference product. In the event this information about the reference product cannot be provided by the applicant, *in vivo* studies need to be performed.

4. DESIGN AND CONDUCT OF STUDIES

4.1. Pharmacokinetic Studies:

4.1.1. Study Design:

The basic design of an in-vivo bioavailability study is determined by the following:

i What is the scientific question(s) to be answered.
ii The nature of the reference material and the dosage form to be tested.
iii The availability of analytical methods.
iv Benefit-risk ratio considerations in regard to testing in humans.

The study should be designed in such a manner that the formulation effect can be distinguished from other effects. Typically, if two formulations are to be compared, a two-period, two-sequence crossover design is the design of choice with the two phases of treatment separated by an adequate washout period which should ideally be equal to or more than five half life's of the moieties to be measured.

Alternative study designs include the parallel design for very long half-life substances or the replicate design for substances with highly variable disposition.

Single-dose studies generally suffice. However situations as described below may demand a steady-state study design:

i Dose or time-dependant pharmacokinetics.
ii Some modified release products (in addition to single dose investigations)
iii Where problems of sensitivity preclude sufficiently precise plasma concentration measurements after single-dose administration.
iv If intra-individual variability in the plasma concentration or disposition precludes the possibility of demonstrating bioequivalence in a reasonably sized single-dose study and this variability is reduced at steady state.

4.1.2. Study Population:

1. Selection of the Number of Subjects

The number of subjects required for a study should be statistically significant and is determined by the following considerations:

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

iv The required (discriminatory) power, normally ≥80% to detect the maximum allowable difference (usually± 20%) in primary characteristics to be studied.

The number of subjects recruited should be sufficient to allow for possible withdrawals or removals (dropouts) from the study. It is acceptable to replace a subject withdrawn/drop out from the study once it has begun provided the substitute follows the same protocol originally intended for the withdrawn subject and he/she is tested under similar environmental and other controlled conditions.

However, the minimum number of subjects should not be less than 16 unless justified for ethical reasons.

Sequential or add-on studies are acceptable in specific cases e.g. where a large number of subjects are required or where the results of the study do not convey adequate statistical significance. In all cases the final statistical analysis must include data of all subjects or reasons for not including partial data as well as the un-included data must be documented in the final report.

2. Selection Criteria for Subjects

To minimize intra and inter individual variation subjects should be standardised as much as possible and acceptable. The studies should be normally performed on healthy adult volunteers with the aim to minimise variability and permit detection of differences between the study drugs. Subjects may be males or females; however the choice of gender should be consistent with usage and safety criteria.

Risks to women of childbearing potential should be considered on an individual basis. Women should be required to give assurance that they are neither pregnant, nor likely to become pregnant until after the study. This should be confirmed by a pregnancy test immediately prior to the first and last dose of the study. Women taking contraceptive drugs should normally not be included in the studies.

If the drug product is to be used predominantly in the elderly attempt should be made to include as many subjects of 60 years of age or older as possible. If the drug product is intended for use in both sexes attempt should be made to include similar proportions of males and females in the studies.

For a drug representing a potential hazard in one group of users, the choice of subjects may be narrowed, e.g., studies on teratogenic drugs should be conducted only on males.

For drugs primarily intended for use in only males or only females – volunteers of only respective gender should be included in the studies.

For drugs where the risk of toxicity or side effects is significant, studies may have to be carried out in patients with the concerned disease, but whose disease state is stable.

They should be screened for suitability by means of a comprehensive medical examination including clinical laboratory tests, an extensive review of medical history including medication history, use of oral contraceptives, alcohol intake, and smoking, use of drugs of abuse.

Depending on the study drugs therapeutic class and safety profile, special medical investigations may need to be carried out before, during and after the study.

3. Genetic Phenotyping

Phenotyping and/or genotyping of subjects should be considered for exploratory bioavailability studies and all studies using parallel group design. It may also be considered in crossover studies (e.g. bioequivalence, dose proportionality, food interaction studies etc.) for safety or pharmacokinetic reasons. If a drug is known to be subject to major genetic polymorphism, studies could be performed in panels of subjects of known phenotype or genotype for the polymorphism in question. While designing a study protocol, adequate care should be taken to consider Pharmacogenomic issues in the context of Indian population.

4.1.3. Study Conditions

Standardisation of the study environment, diet, fluid intake, post-dosing postures, exercise, sampling schedules etc. is important in all studies. Compliance to these standardisations should be stated in the protocol and reported at the end of the study, in order to reassure that all variability factors involved, except that of the products being tested, have been minimised. Unless the study design requires, subjects should abstain from smoking, drinking alcohol, coffee, tea, xanthine containing foods and beverages and fruit juices during the study and at least 48 hours before its commencement.

1. Selection of Blood Sampling Points/Schedules

The blood-sampling period in single-dose trials of an immediate release product should extend to at least three-elimination half-lives. Sampling should be continued for a sufficient period to ensure that the area extrapolated from the time of the last measured concentration to infinite time is only a small percentage (normally less than 20%) of the total AUC. The use of a truncated AUC is undesirable except in certain circumstances such as in the presence of entero-hepatic recycling where the terminal elimination rate constant cannot be calculated accurately.

There should be at least three sampling points during the absorption phase, three to four at the projected T_{max}, and four points during the elimination phase. The number of points used to calculate the terminal elimination rate constant should be preferably determined by eye from a semi-logarithmic plot.

Intervals between successive data/sampling points used to calculate the terminal elimination rate constant should, in general, not be longer than the half-life of the study drug.

Where urinary excretion is measured in a single-dose study it is necessary to collect urine for seven or more half-lives.

2. Fasting and Fed State Considerations

Generally, a single dose study should be conducted after an overnight fast (at least 10 hours), with subsequent fast of 4 hours following dosing. For multiple dose fasting state studies, when an evening dose must be given, two hours of fasting before and after the dose is considered acceptable.

However, when it is recommended that the study drug be given with food (as would be in routine clinical practice), or where the dosage form is a modified release product, fed state studies need to be carried out in addition to the fasting state studies.

Fed state studies are also required when fasting state studies make assessment of C_{max} and T_{max} difficult.

Studies in the fed state require the consumption of a high-fat breakfast before dosing. Such a breakfast must be designed to provide 950 to 1000 KCals. At least 50% of these calories must come from fat, 15 to 20% from proteins and the rest from carbohydrates. The vast ethnic and cultural variations of the Indian sub-continent preclude the recommendation of any single standard high fat breakfast. Protocol should specify the suitable and appropriate diet. The high fat breakfast must be consumed approximately 15 minutes before dosing.

3. Steady State Studies

In following cases – an additional “steady state study” is considered appropriate:

i Where the drug has a long terminal elimination half-life and blood concentrations after a single dose cannot be followed for a sufficient time.
ii Where assay sensitivity is inadequate to follow the terminal elimination phase for an adequate period of time.
iii For drugs, which are so toxic that ethically they should only be administered to patients for whom they are a necessary part of therapy, but where multiple dose therapy is required, e.g. many cytotoxics.
iv For modified-release products where it is necessary to assess the fluctuation in plasma concentration over a dosage interval at steady state.

v For those drugs which induce their own metabolism or show large intra-individual variability.
vi For enteric-coated preparations where the coating is innovative.
vii For combination products where the ratio of plasma concentration of the individual drugs is important.
viii For drugs that exhibit non-linear (i.e., dose- or time- dependent) pharmacokinetics.
ix Where the drug is likely to accumulate in the body.

In steady state studies, the dosing schedule should follow the clinically recommended dosage regimen.

4.1.4. Characteristics to be investigated during bioavailability / bioequivalence studies

In most cases evaluations of bioavailability and bioequivalence will be based upon the measured concentrations of the active drug substance(s) in the biological matrix. In some situations, however, the measurements of an active or inactive metabolite may be necessary. These situations include (a) where the concentrations of the drug(s) may be too low to accurately measure in the biological matrix, (b) limitations of the analytical method, (c) unstable drug(s), (d) drug(s) with a very short half-life or (e) in the case of prodrugs.

Racemates should be measured using an achiral assay method. Measurement of individual enantiomers in bioequivalence studies is recommended where all of the following criteria are met:

(a) the enantiomers exhibit different pharmacodynamic characteristics
(b) the enantiomers exhibit different pharmacokinetic characteristics
(c) primary efficacy / safety activity resides with the minor enantiomer
(d) non-linear absorption is present for at least one of the enantiomers

The plasma-time concentration curve is mostly used to assess the rate and extent of absorption of the study drug. These include pharmacokinetic parameters such as the C_{max}, T_{max}, AUC_{0-t} and $AUC_{0-\infty}$.

For studies in the steady state $AUC_{0-\tau}$, C_{max}, C_{min} and degree of fluctuation should be calculated.

4.1.5. Bioanalytical methodology:

The bioanalytical methods used to determine the drug and/or its metabolites in plasma, serum, blood or urine or any other suitable matrix must be well characterised, standardised, fully validated and documented to yield reliable results that can be satisfactorily interpreted.

Although there are various stages in the development and validation of an analytical procedure, the validation of the analytical method can be envisaged to consist of two distinct phases:

1. The pre-study phase which comes before the actual start of the study and involves the validation of the method on biological matrix human plasma samples and spiked plasma samples.

2. The study phase in which the validated bioanalytical method is applied to the actual analysis of samples from bioavailability and bioequivalence studies mainly to confirm the stability, accuracy and precision.

1. Pre-study Phase

The following characteristics of the bioanalytical method must be evaluated and documented to ensure the acceptability of the performance and reliability of analytical results:

i. Stability of the drug/metabolites in the biological matrix:

Stability of the drug and/or active metabolites in the biological matrix under the conditions of the experiment (including any period for which samples are stored before analyses) should be established. The stability data should also include the influence of at least three freezing and thawing cycles representative of actual sample handling. The absence of any sorption by the sampling containers and stoppers should also be established.

ii. Specificity/Selectivity:

Data should be generated to demonstrate that the assay does not suffer from interference by endogenous compounds, degradation products, other drugs likely to be present in study samples, and metabolites of the drug(s) under study.

iii. Sensitivity:

Sensitivity is the capacity of the test procedure to record small variations in concentration. The analytical method chosen should be capable of assaying the drug/metabolites over the expected concentration range. A reliable lowest limit of quantification should be established based on an intra- and inter-day coefficient of variation usually not greater than 20 percent. The limit of detection (the lowest concentration that can be differentiated from background levels) is usually lower than the limit of quantification. Values between limit of quantification and limit of detection should be identified as "Below Quantification Limits."

iv. Precision and Accuracy:

Precision (the degree of reproducibility of individual assays) should be established by replicate assays on standards, preferably at several concentrations. Accuracy is the degree to which the ‘true’ value of the concentration of drug is estimated by the assay. Precision and accuracy should normally be documented at three concentrations (low, medium, high) where ‘low’

is in the vicinity of the lowest concentration to be measured, 'high' is a value in the vicinity of C_{max} and 'medium' is a suitable intermediate value.

Intra-assay precision (within days) in terms of coefficient of variation should be no more than 15%, although no more than 20% may be more realistic at values near the lower limit of quantification. Inter-assay precision (between days) may be higher than 15% but not more than 20%.

Accuracy can be assessed in conjunction with precision and is a measure of the extent to which measured concentrations deviate from true or nominal concentrations of analytical standards. In general, an accuracy of ±15% should be attained.

v. Recovery:

Documentation of extraction recovery at high, medium and low concentrations is essential since methods with low recovery are, in general, more prone to inconsistency. If recovery is low, alternative methods should be investigated. Recovery of any internal standard used should also be assessed.

vi. Range and linearity:

The quantitative relationship between concentration and response should be adequately characterized over the entire range of expected sample concentrations. For linear relationships, a standard curve should be defined by at least five concentrations. If the concentration response function is non-linear, additional points would be necessary to define the non-linear portions of the curve. Extrapolation beyond the standard curve is not acceptable.

vii. Analytical System Stability:

To assure that the analytical system remains stable over the time course of the assay, the reproducibility of the standard curve should be monitored during the assay. A minimal design would be to run analytical standards at the beginning and at the end of the analytical run.

2. Study Phase:

In general, with acceptable variability as defined by validation data, the analysis of biological sample can be done by single determination without a need for a duplicate or replicate analysis. The need for duplicate analysis should be assessed on a case-by-case basis. A procedure should be developed that documents the reason for re-analysis.

A standard curve should be generated for each analytical run for each analyte and should be used to calculate the concentration of the analyte in the unknown samples assayed with that run. It is important to use a standard curve that will cover the entire range of concentrations in the unknown samples. Estimation of

unknowns by extrapolations of standard curves below the lowest standard concentration or above the highest standard concentration is not recommended. Instead, it is suggested that standard curve should be redetermined or sample should be re-assayed after dilution. Quality control sample should be used to accept or reject the run.

3. Quality Control Samples:

Quality control samples are samples with known concentration prepared by spiking drug-free biological fluid with drug. These samples should be prepared in low, medium and high concentration. To avoid possible confusion between quality control samples and standard solutions during the review process, preparation of quality control samples at concentrations different from those used for the calibration is recommended. For stable analytes, quality control samples should be prepared in the fluid of interest at the time of pre-study assay validation or at the time of study sample collection, and stored with the study samples. For less stable analytes, daily or weekly quality control samples may have to be prepared.

A quality control sample for each concentration should be assayed on each occasion that study samples are assayed, and the concentration determined by reference to that day's calibration standards. If the concentration values determined for the controls are not within ±15% of the expected concentrations, the batch should be considered for re-analysis.

4. Repeat Analysis:

In most studies some samples will require re-analysis because of aberrant results due to processing errors, equipment failure or poor chromatography. The reasons for re-analysis of such samples should be stated. The criteria for repeat analyses should be determined prior to running the study and recorded in the protocol / laboratory standard operating procedures.

4.1.6. Statistical Evaluation

1. Data analysis:

The primary concern in bio-equivalence assessment is to limit the consumer’s risk i.e., erroneously accepting bioequivalence and also at the same time minimizing the manufacture’s risk i.e., erroneously rejecting bioequivalence. This is done by using appropriate statistical methods for data analysis and adequate sample size.

2. Statistical analysis:

The statistical procedure should be specified in the protocol itself. In case of bioequivalence studies the procedures should lead to a decision scheme which is symmetrical with respect to the two formulations (i.e. leading to the same decision whether the new formulation is compared to the reference product or the reference product to the new formulation).

The statistical analysis (e.g. ANOVA) should take into account sources of variation that can be reasonably assumed to have an effect on the response.

The 90% confidence interval for the ratio of the population means (Test/reference) or two one sided-t tests with the null hypothesis of non-bioequivalence at the 5% significance level for the parameter under consideration are considered for testing bioequivalence.

To meet the assumption of normality of data underlying the statistical analysis, the logarithmic transformation should be carried out for the pharmacokinetic parameters C_{max} and AUC before performing statistical analysis. However, it is recommended not to verify the assumptions underlying the statistical analysis before making logarithmic transformation.

The analysis of T_{max} is desirable if it is clinically relevant. The parameter T_{max} should be analysed using non-parametric methods. In addition to above, summary statistics such as minimum, maximum and ratio should be given.

3. Criteria for bioequivalence:

To establish Bioequivalence, the calculated 90% confidence interval for AUC and C_{max} should fall within the bioequivalence range, usually 80-125%. This is equivalent to the rejection of two one sided-t tests with the null hypothesis of non-bioequivalence at 5% level of significance. The non-parametric 90% confidence interval for T_{max} should lie within a clinically acceptable range.

Tighter limits for permissible differences in bioavailability may be required for drugs that have:

i A narrow therapeutic index.
ii A serious, dose-related toxicity.
iii A steep dose/effect curve, or
iv A non-linear pharmacokinetics within the therapeutic dose range.

A wider acceptance range may be acceptable if it is based on sound clinical justification.

In case of supra-bioavailability, a reformulation followed by a fresh bioequivalence study will be necessary. Otherwise, clinical trial data on new formulation will be required to support the application, especially dosage recommendations. Such formulations are usually not be accepted as therapeutically equivalent to the existing reference product. The name of the new product should preclude confusion with the earlier approved product.

4. Deviations from the study plan

The method of analysis should be defined in the protocol. The protocol should specify methods for handling drop-outs and for identifying biologically implausible outliers. Post hoc exclusion of outliers is not recommended. A scientific explanation should be provided to justify the exclusion of a volunteer from the analysis.

4.1.7. Special considerations for modified-release drug products

For the purpose of these guidelines modified release products include:

i delayed release
ii sustained release
iii mixed immediate and sustained release
iv mixed delayed and sustained release
v mixed immediate and delayed release

Generally, these products should:

i act as modified-release formulations and meet the label claim
ii preclude the possibility of any dose dumping effect
iii there must be a significant difference between the performance of modified release product and the conventional release product when used as reference product.
iv provide a therapeutic performance comparable to the reference immediate-release formulation administered by the same route in multiple doses (of an equivalent daily amount) or to the reference modified-release formulation;
v produce consistent pharmacokinetic performance between individual dosage units; and
vi produce plasma levels which lie within the therapeutic range (where appropriate) for the proposed dosing intervals at steady state.

If all of the above conditions are not met but the applicant considers the formulation to be acceptable, justification to this effect should be provided.

i. Study Parameters

Bioavailability data should be obtained for all modified release drug products although the type of studies required and the pharmacokinetic parameters which should be evaluated may differ depending on the active ingredient involved. Factors to be considered include whether or not the formulation represents the first market entry of the drug substance, and the extent of accumulation of the drug after repeated dosing.

If the formulation is the first market entry of the drug substance, the product's pharmacokinetic parameters should be determined. If the formulation is a second or subsequent market entry then comparative bioavailability studies using an appropriate reference product should be performed.

ii. Study design

Study design will be single dose or single and multiple dose based on the modified release products that are likely to accumulate or unlikely to accumulate both in the fasted and non-fasting state. If the effect of food on the reference product is not known (or it is known that food affects its absorption), two separate two-way cross-over studies, one in the fasted state and the other in the fed state, may be carried out. If it is known with certainty (e.g. from published data) that the reference product is not affected by food, then a three-way cross-over study may be appropriate with:

a. the reference product in the fasting state
b. the test product in the fasted state, and
c. the test product in the fed state.

iii. Requirements for modified release formulations unlikely to accumulate

This section outlines the requirements for modified release formulations which are used at a dose interval that is not likely to lead to accumulation in the body ($AUC_{0-\tau}/AUC_{0-\infty} \geq 0.8$).

When the modified release product is the first market entry of that type of dosage form, the reference product should normally be the innovator's immediate-release formulation. The comparison should be between a single dose of the modified release formulation and doses of the immediate-release formulation which it is intended to replace. The latter must be administered according to the established dosing regimen.

When the modified release product is the second or subsequent entry on the market, comparison should be with the reference modified release product for which bioequivalence is claimed.

Studies should be performed with single dose administration in the fasting state as well as following an appropriate meal at a specified time.
The following pharmacokinetic parameters should be calculated from plasma (or relevant biological matrix) concentrations of the drug and/or major metabolite(s): $AUC_{0-\tau}$, AUC_{0-t}, $AUC_{0-\infty}$, C_{max} (where the comparison is with an existing modified release product), and k_{el}

The 90% confidence interval calculated using log transformed data for the ratios (Test:Reference) of the geometric mean AUC (for both $AUC_{0-\tau}$ and AUC_{0-t}) and C_{max} (where the comparison is with an existing modified release product) should generally be within the range 80 to 125% both in the fasting state and following the administration of an appropriate meal at a specified time before taking the drug.

The pharmacokinetic parameters should support the claimed dose delivery attributes of the modified-release dosage form.

iv. Requirements for modified release formulations likely to accumulate

This section outlines the requirements for modified release formulations that are used at dose intervals that are likely to lead to accumulation ($AUC_{0-\tau}/AUC_{0-\infty} < 0.8$).

When a modified release product is the first market entry of the modified release type, the reference formulation is normally the innovator's immediate-release formulation. Both a single dose and steady state doses of the modified release formulation should be compared with doses of the immediate-release formulation which it is intended to replace. The immediate-release product should be administered according to the conventional dosing regimen.

Studies should be performed with single dose administration in the fasting state as well as following an appropriate meal. In addition, studies are required at steady state. The following pharmacokinetic parameters should be calculated from single dose studies: $AUC_{0-\tau}$, AUC_{0-t}, $AUC_{0-\infty}$, C_{max} (where the comparison is with an existing modified release product), and k_{el}. The following parameters should be calculated from steady state studies: $AUC_{0-\tau(ss)}$, C_{max}, C_{min}, C_{pd} and degree of fluctuation.

When the modified release product is the second or subsequent modified release entry, single dose and steady state comparisons should normally be made with the reference modified release product for which bioequivalence is claimed.

The 90% confidence interval for the ratio of geometric means (Test:Reference drug) of AUC (for both $AUC_{0-\tau}$ and AUC_{0-t}) and C_{max} (where the comparison is with an existing modified release product) determined using log-transformed data should generally be within the range 80 to 125% when the products are compared after single dose administration in both the fasting state and the fed state.

The 90% confidence interval for the ratio of geometric means (Test:Reference drug) for $AUC_{0-\tau(ss)}$, C_{max}, and C_{min} determined using log-transformed data should generally be within the range 80 to 125% when the formulations are compared at steady state.

The pharmacokinetic parameters should support the claimed attributes of the modified-release dosage form.

Pharmacodynamic data may reinforce or clarify interpretation of differences in the plasma concentration data.

Where these studies do not show bioequivalence, comparative efficacy and safety data may be required for the new product.

4.2. Pharmacodynamic Studies:

Studies in healthy volunteers or patients using pharmacodynamic parameters may be used for establishing equivalence between two pharmaceutical products. These studies may become necessary if quantitative analysis of the drug and/or metabolite(s) in plasma or urine cannot be made with sufficient accuracy and sensitivity. Furthermore, pharmacodynamic studies in humans are required if measurements of drug concentrations cannot be used as surrogate endpoints for the demonstration of efficacy and safety of the particular pharmaceutical product e.g., for topical products without an intended absorption of the drug into the systemic circulation.

In case, only pharmacodynamic data is collected and provided, the applicant should outline what other methods were tried and why they were found unsuitable.

The following requirements should be recognised when planning, conducting and assessing the results from a pharmacodynamic study:

i The response measured should be a pharmacological or therapeutic effect which is relevant to the claims of efficacy and/or safety of the drug.

ii The methodology adopted for carrying out the study should be validated for precision, accuracy, reproducibility and specificity.

iii Neither the test nor the reference product should produce a maximal response in the course of the study, since it may be impossible to distinguish differences between formulations given in doses that produce such maximal responses. Investigation of dose-response relationship may become necessary.

iv The response should be measured quantitatively under double-blind conditions and be recorded in a instrument-produced or instrument-recorded fashion on a repetitive basis to provide a record of pharmacodynamic events which are a substitute for plasma concentrations. If such measurements are not possible, recordings on visual-analog scales may be used. In instances, where data are limited to qualitative (categorized) measurements, appropriate special statistical analyses will be required.

v Non-responders should be excluded from the study by prior screening. The criteria by which responders *versus* non-responders are identified must be stated in the protocol.

vi Where an important placebo effect can occur, comparison between products can only be made by a priori consideration of the placebo effect in the study design. This may be achieved by adding a third period/phase with placebo treatment, in the design of the study.

vii A crossover or parallel study design should be used, as appropriate.

viii When pharmacodynamic studies are to be carried out on patients, the underlying pathology and natural history of the condition should be

considered in the study design. There should be knowledge of the reproducibility of the base-line conditions.

ix In studies where continuous variables could be recorded, the time course of the intensity of the drug action can be described in the same way as in a study where plasma concentrations are measured. From this, parameters can be derived which describe the area under the effect-time curve, the maximum response and the time when the maximum response occurred.

x Statistical considerations for the assessments of the outcomes are in principle, the same as in pharmacokinetic studies.

xi A correction for the potential non-linearity of the relationship between dose and area under the effect-time curve should be made on the basis of the outcome of the dose ranging study.

The conventional acceptance range as applicable to pharmacokinetic studies and bioequivalence is not appropriate (too large) in most cases. This range should therefore be defined in the protocol on a case-to-case basis.

4.3 Comparative Clinical Studies

In several instances (For example, section 3.1.1(e) above), the plasma concentration time-profile data may not be suitable to assess equivalence between two formulations. Whereas in some of the cases pharmacodynamic studies can be an appropriate tool for establishing equivalence, in other instances this type of study cannot be performed because of lack of meaningful pharmacodynamic parameters which can be measured and a comparative clinical study has to be performed in order to demonstrate equivalence between two formulations. Comparative clinical studies may also be required to be carried out for certain orally administered drug products when pharmacokinetic and pharmacodynamic studies are not feasible. However, in such cases, the applicant should outline what other methods were tried and why they were found unsuitable.

If a clinical study is considered as being undertaken to prove equivalence, the appropriate statistical principles should be applied to demonstrate bioequivalence. The number of patients to be included in the study will depend on the variability of the target parameters and the acceptance range, and is usually much higher than the number of subjects in bioequivalence studies.

The following items are important and need to be defined in the protocol in advance:

a. The target parameters which usually represent relevant clinical end-points from which the intensity and the onset, if applicable and relevant, of the response are to be derived.
b. The size of the acceptance range has to be defined case-to- case taking into consideration the specific clinical conditions. These include, among others, the natural course of the disease, the efficacy of available treatments and the chosen target parameter. In contrast to bioequivalence studies (where a

conventional acceptance range is applied) the size of the acceptance range in clinical trials cannot be based on a general consensus on all the therapeutic classes and indications.

c. The presently used statistical method is the confidence interval approach. The main concern is to rule out that the test product is inferior to the reference product by more than the specified amount. Hence, a one-sided confidence interval (for efficacy and/or safety) may be appropriate. The confidence intervals can be derived from either parametric or nonparametric methods.
d. Where appropriate, a placebo leg should be included in the design.
e. In some cases, it is relevant to include safety end-points in the final comparative assessments.

4.4 In Vitro studies

In certain situations a comparative *in vitro* dissolution study may be sufficient to demonstrate equivalence between two drug products (See Section 3).

The test methodology adopted should be in line with the pharmacopoeial requirements unless those requirements are shown to be unsatisfactory. Alternative methods may be acceptable provided they have sufficient discriminatory power.

Dissolution studies should generally be carried out under mild agitation conditions at 37±0.5°C and at physiologically relevant pH. More than one batch of each formulation should be tested. Comparative dissolution profiles, rather than single point dissolution test data, should be generated. The design should include:

i Individually testing of at least twelve dosage units (e.g., tablets, capsules) of each batch. Mean and individual results should be reported along with their standard deviations or standard errors.
ii Measuring the percentage of nominal content released at a number of suitably spaced time points to provide a profile for each batch, e.g. at 10, 20 and 30 minutes or as appropriate to achieve virtually complete dissolution.
iii Determining the dissolution profile in at least three aqueous media covering the pH range of 1.0 to 6.8 or in cases where considered necessary, pH range of 1.0 to 8.0.
iv Conducting the tests on each batch using the same apparatus and, if possible, on the same or consecutive days.

Comparisons of the dissolution profiles may be made by any of the established model-independent or model-dependent methods.

5. DOCUMENTATION

With respect to the conduct of bioequivalence/bioavailability studies following important documents must be maintained:

i. Clinical Data:
   a. All relevant documents as required to be maintained for compliance with GCP Guidelines

ii. Details of the analytical method validation including the following:
   a. System suitability test
   b. Linearity range
   c. Lowest limit of quantitation
   d. QC sample analysis
   e. Stability sample analysis
   f. Recovery experiment result

iii. Analytical data of volunteer plasma samples which should include the following:
   a. Validation data of analytical methods used
   b. Chromatograms of all volunteers, including any aberrant chromatograms
   c. Inter-day and intra-day variation of assay results
   d. Details including chromatograms of any repeat analysis performed
   e. Calibration status of the instruments

iv. Raw data

v. All comments of the chief investigator regarding the data of the study submitted for review.

vi. A copy of the final report

STUDY REPORT

The bioequivalence or bioavailability report should give the complete documentation of its protocol, conduct and evaluation.

The report should include (as a minimum) the following information:

a. Table of contents

b. Title of the study

c. Names and credentials of responsible investigators

d. Signatures of the principal and other responsible investigators authenticating their respective sections of the report

e. Site of the study and facilities used

f. The period of dates over which the clinical and analytical steps were conducted

g. Names and batch numbers of the products compared

h. A signed declaration that this was identical to that intended for marketing.

i. Results of assays and other pharmaceutical tests (e.g., physical description, dimensions, mean weight, weight uniformity, comparative dissolution) carried out on the batches of products compared

j. Full protocol for the study including a copy of the ICF and criteria for inclusion/exclusion or withdrawal of subjects

k. Report of protocol deviations, violations

l. Documentary evidence that the study was approved by an independent ethics committee and was carried out in accordance with GCP/GLP.

m. Demographic data of subjects

n. Names and addresses of subjects

o. Details of and justifications for protocol deviations

p. Details of dropout and withdrawals from the study should be fully documented and accounted for

q. Details of analytical methods used, full validation data, quality control data and criteria for accepting or rejecting assay results

r. Representative chromatograms covering the whole concentration range for all, standard and quality control samples as well as specimens analysed

s. Sampling schedules and deviations of the actual times from the scheduled

t. Details of how pharmacokinetic parameters were calculated

u. Documentation related to statistical analysis:

    i. Randomization schedule

    ii. Volunteer wise plasma concentration and time points for test and reference products

    iii. Volunteer wise AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, T_{max}, K_{el}, and $t_{1/2}$ for test and reference products

    iv. Logarithmic transformed measures used for BE demonstration

    v. ANOVA for AUC_{0-t}, $AUC_{0-\infty}$, C_{max}

vi. Inter-subject, intra-subject and/or total variability if possible

vii. Confidence intervals for AUC_{0-t}, $AUC_{0-\infty}$, C_{max} (Confidence interval (CI) values should not be rounded off; therefore, to pass a CI range of 80 to 125, the values should be at least 80.00 and not more than 125.00

viii. Geometric mean, arithmetic mean, ratio of means for AUC_{0-t}, $AUC_{0-\infty}$, C_{max}

ix. Partial AUC, only if it is used

x. C_{min}, C_{max}, C_{pd}, $AUC_{0-\tau}$, degree of fluctuation $[(C_{max} – C_{min})/C_{av}]$ and swing $[(C_{max} – C_{min})/C_{min}]$, if steady state studies are employed

6. FACILITIES FOR CONDUCTING BIOAVAILABILITY AND/OR BIOEQUIVALENCE STUDIES

6.1 Legal identity:

The organization, conducting the bioequivalence / bioavailability studies, or the parent organization to which it belongs, must be a legally constituted body with appropriate statutory registrations.

6.2 Impartiality, confidentiality, independence and integrity:

The organization shall:

a. have managerial staff with the authority and the resources needed to discharge their duties.
b. have arrangements to ensure that its personnel are free from any commercial, financial and other pressures which might adversely affect the quality of their work.
c. be organized in such a way that confidence in its independence of judgment and integrity is maintained at all times.
d. have documented policies and procedures, where relevant, to ensure the protection of its sponsors' confidential information and proprietary rights.
e. not engage in any activity that may jeopardize the trust in its independence of judgement and integrity
f. have documented policies and procedures for the safety of human rights and the use of human subjects in research consistent with Schedule Y (refer Drugs & Cosmetics Act and Rules) and GCP Guidelines
g. have documented policies and procedures for scientific integrity including procedures dealing with and reporting possible scientific misconduct.

6.3 Organisation and management:

The study site organization must include the following:

a. An Investigator who has the overall responsibility to provide of the human subjects. The Investigator(s) should possess appropriate medical qualifications and relevant experience for conducting pharmacokinetic studies.
b. The site should have identified adequately qualified and trained personnel to perform the following functions:
   i Clinical Pharmacological Unit (CPU) management
   ii Analytical laboratory management
   iii Data handling and interpretation
   iv Documentation and report preparation
   v Quality assurance of all operations in the centre

6.4 Documented Standard Operating Procedures

The center shall establish and maintain a quality system appropriate to the type, range and volume of its activities. All operations at the site must be conducted as per the authorized and documented standard operating procedures. These documented procedures should be available to the respective personnel for ready reference. The procedures covered must include those that ensure compliance with all aspects of:

a. GCP Guidelines
b. Good laboratory practice guidelines issued by Ministry of Health & Family Welfare

A partial list of procedures for which documented standard operating procedures should be available includes:

a. maintenance of working standards (pure substances) and respective documentation.
b. withdrawal, storage and handling of biological samples.
c. maintenance, calibration and validation of instruments.
d. managing medical as well as non-medical emergency situations
e. handling of biological fluids
f. managing laboratory hazards
g. disposal procedures for clinical samples and laboratory wastes
h. documentation of clinical pharmacology unit observations, volunteer data and analytical data
i. obtaining informed consent from volunteers
j. volunteer screening and recruitment and management of ineligible volunteers
k. volunteer recycling (using the same volunteer for more than one study
l. randomization code management
m. study subject management at the site (including check-in and check-out procedures)
n. recording and reporting protocol deviations
o. recording, reporting and managing scientific misconduct
p. monitoring and quality assurance

Wherever possible, disposable (sterile, wherever applicable) medical devices must be used for making subject interventions.

If services of a laboratory or a facility other than those available at the site (whether with in India or outside the country) are to be availed – its/their name(s), address(s) and specific services to be used should be documented.

6.5 Clinical Pharmacological Unit

It must have adequate space and facilities to house at least 16 volunteers. Adequate area must be provided for dining and recreation of volunteers, separate from their sleeping area.

Additional space and facilities should also be provided for the following:

a. Office and administrative functions
b. Sample collection and storage
c. Control sample storage
d. Wet chemical laboratory
e. Instrumental Laboratory
f. Library
g. Documentation archival room
h. Facility for washing, cleaning and Toilets
i. Microbiological laboratory (Optional)
j. Radio Immuno – Assay room (optional)

7. MAINTENANCE OF RECORDS OF BA/BE STUDIES

All records of in vivo or in vitro tests conducted on any marketed batch of a drug product to assure that the product meets a bioequivalence requirement shall be maintained by the Sponsor for at least 2 years after the expiration date of the batch and submitted to CDSCO on request.

8. RETENTION OF BA/BE SAMPLES

All samples of test and reference drug products used in bioavailability / bioequivalence study should be retained by the organization carrying out the bioavailability / bioequivalence study for a period of three years after the conduct of the study or one year after the expiry of the drug, whichever is earlier. The study sponsor and/or drug manufacturer should provide to the testing facility batches of the test and reference drug products in such a manner that the reserve samples can be selected randomly. This is to ensure that the samples are in fact representative of the batches provided by the study sponsor and/or drug manufacturer and that they are retained in their original containers. Each reserve sample should consist of a quantity sufficient to carry out twice all the in-vitro and in-vivo tests required during bioavailability / bioequivalence study.

The reserve sample should be stored under conditions consistent with product labelling and in an area segregated from the area where testing is conducted and with access limited to authorized personnel.

9. SPECIAL TOPICS:

9.1 Food effect bioavailability studies

Food effect study is required when there is a possibility to have effect of food on the bioavailability of the drug. Food effect bioavailability studies focus on effects of food on the release of the drug substance from the drug product as well as the absorption of the drug substance. Usually, a single dose crossover study is recommended for BA and BE studies.

9.2 Long half-life drugs

For BE determination of an oral product with long half life, a single dose crossover study can be conducted, provided an adequate wash out period is used. If due to longer periods, chances of drop outs as well as intra subject variation are higher with routine cross over designs; parallel group designs can be used. In all cases, blood sampling period should be adequate to describe the plasma concentration time profile. C_{max} and a suitably truncated AUC can be used to characterize peak and total drug exposure, respectively. For drugs, demonstrating high intra-subject variability in distribution and clearance, AUC truncation warrants caution. In such cases, sponsors and/or applicants should consult the regulatory authority.

9.3 Early Exposure

In general, bioequivalence may be demonstrated by measurements of peak and total exposure for an immediate release product. However, in situations such as rapid onset of an analgesic effect or to avoid an excessive hypotensive action of an antihypertensive, an early exposure measure may be informative on the basis of appropriate clinical efficacy/safety trials and/or pharmacokinetic / pharmacodynamic studies that call for better control of drug absorption into the systemic circulation. In these situations, use of partial AUC is recommended as an early exposure measure. The partial area should be truncated at T_{max} values for the reference formulation. At least two quantifiable samples should be collected before the expected peak time to allow adequate estimation of the partial area.

Individual and population bioequivalence:

The current practice of evaluating bioequivalence has been termed as ″average bioequivalence″. Whereas in individual bioequivalence, determination of the intra subject variation of drug response is important. By “population bioequivalence” we mean a bioequivalence criterion that requires the distribution of the formulation to be sufficiently similar to that of the reference in some appropriate population. Average bioequivalence is a special case of population bioequivalence.

The average bioequivalence of the two formulations is important in the case of prescribability. However, Individual bioequivalence is required in case of switchability.

Assessment of individual bioequivalence is an interesting and exciting alternative to the current practice of evaluating average bioequivalence. The evaluation of individual bioequivalence requires values of intra-subject variability of the test and reference formulations. Hence the assessment of individual bioequivalence is done based on three or four period designs. Replicate study designs provide such information.

Up till now, bioequivalence studies are designed to evaluate average bioequivalence. Experience with population and individual bioequivalence studies is limited. Hence no specific recommendation is proposed on this matter. However, for highly variable drugs, individual bioequivalence can be considered.