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Study on Requirements of Bioequivalence for Registration of Pharmaceutical Products in India, South-Africa and Australia

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ABSTRACT

The present study was aimed to study the requirements of bioequivalence for registration of pharmaceutical products in various countries. It is essential for pharmaceutical industry to study the guidelines of bioequivalence for respective country where industry would like to apply for ANDA and thus want to enter into generic market. This study gives insight about requirements of bioequivalence with study parameters such as study design, fasting or fed state studies, volunteers recruitment, study dose, sampling points, analytical method validation parameters, moieties to be measured in plasma, pharmacokinetic parameters, criteria for bioequivalence, GCP requirements etc. which are needed for pharmaceutical industry to carry out bioequivalence studies and to file ANDA. Test products for these bioequivalence studies are usually manufactured by a sponsor or manufacturer while reference is provided by the government laboratories of respective countries. Sampling points also varies with respect to the regulatory guidelines of these countries. India follows Indian GCP guidelines, South-Africa MCC GCP guidelines and Australia follows ICH GCP guidelines. Criteria of bioequivalence, for India and South-Africa is 90% CI 80-125% for C_{max}, AUC_t, $AUC_{o\text{-}inf}$. for Australia 90% CI 80-125% for C_{max}, $hAUC_t$, $AUC_{o\text{-}inf}$.

Keywords: Bioequivalence, Bioavailability, ANDA, Pharmacokinetics

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INTRODUCTION

In pharmacokinetics bioequivalence is a term used to assess the expected in vivo biological equivalence of two proprietary preparations of a drug. If two products are said to be bioequivalent it means that they would be likely to be, for all intents and purposes, the same. Two pharmaceutical products are bioequivalent if they are pharmaceutically equivalent and their bioavailability (rate and extent of availability) after administration in the same molar dose are similar to such a degree that their effects, with respect to both efficacy and safety, can be predictable to be essentially the same. Pharmaceutical equivalence implies the same amount of the same active substance(s), in the same dosage form, for the same route of administration and gathering the same or comparable standards. 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[1].

As per United States Food and Drug Administration (FDA) bioequivalence is the absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study[2].

In vivo certification of equivalence is needed when there is a risk that probable differences in bioavailability may result in therapeutic nonequivalence. Some of examples are critical use medicines, narrow therapeutic range (efficacy/safety margins), steep dose-response curve, pharmacokinetics complicated by variable or incomplete absorption window, nonlinear pharmacokinetics, pre-systemic elimination or high first pass metabolism (>70%), unfavorable physicochemical properties, e.g., low solubility, instability, meta-stable modifications, poor permeability, documented evidence for bioavailability problems related to the drug or drugs of similar chemical structure or formulations, where high ratio of excipients to active ingredients exists, non-oral, non-parenteral pharmaceutical products designed to act systemically (such as transdermal patches, suppositories, nicotine chewing gum, testosterone gel and skin inserted contraceptives), Modified release pharmaceutical products designed to act systemically, fixed combination products with systemic action, where at least one of the API requires an in vivo study, non-solution pharmaceutical products, which are for non-systemic use (e.g. for oral, nasal, ocular, dermal, rectal or vaginal application) and are intended to act without systemic absorption. In these cases, the equivalence is established through comparative clinical or pharmacodynamic,

dermato-pharmacokinetic studies and/or in vitro studies. In certain cases, measurement of the concentration of the API may still be required for safety reasons, i.e. in order to assess unintended systemic absorption, 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].

Bioequivalence Study Requirements for Registration of Pharmaceutical Product in India

Bioequivalence studies are required in India for the new drugs as per the requirement detailed in schedule Y of the Drug and Cosmetics Rules and its amendments.

The study should be premeditated in such a way 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. Other 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 are sufficient. Standardization of the study environment, diet, fluid intake, post-dosing postures, exercise, sampling schedules etc. is important in all studies. Conformity to these standardizations should be stated in the protocol and reported at the end of the study, in order to restore confidence that all variability factors involved, except that of the products being tested, have been minimized. Unless the study design requires, subjects should withdraw 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.

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[4].

Bioequivalence Study Requirements for Registration of Pharmaceutical Product In South Africa

The study should be premeditated in such a manner that the formulation effect can be notable from other effects. If the number of formulations to be compared is two, a balanced two periods, two sequence crossover designs is considered to be the design of choice. However, under certain conditions and provided the study design and the statistical analysis are scientifically sound, alternatively well recognized designs such as parallel designs for very long half-life substances, could be considered. In general, single dose studies will suffice, but there are situations in which

steady state studies may be required in which case the steady state study design should be provoked. To avoid carry over effects, treatments should be separated by adequate washout periods[5].

For bioavailability studies, measurement of individual enantiomers may be important. For bioequivalence studies, this guidance recommends measurement of the racemate using an achiral assay. Measurement of individual enantiomers in BE studies are recommended only when the enantiomers exhibit different pharmacokinetic and pharmacodynamic characteristics, primary efficacy and safety activity resides with the minor enantiomers and non-linear absorption is present (as expressed by a change in the enantiomer concentration ratio with change in the input rate of the drug/API) for at least one of the enantiomers. In such cases, bioequivalence factors are applied to the enantiomers separately[6].

Bioequivalence Study Requirements for Registration of Pharmaceutical Product in Australia

Where bioequivalence is a requirement, a generic medicine must be shown to be bioequivalent to the corresponding strength of a leading brand (normally the innovator product) as marketed in Australia. The actual batch of reference product (comparator) used in a bioequivalence study for a generic medicine should normally be obtained in Australia. This is the TGA's strong preference. However, in certain circumstances, the TGA may accept bioequivalence studies carried out using a batch of reference product obtained from outside Australia, provided the sponsor can sustain this with convincing evidence that the formulation of the product used is the same as the formulation marketed in Australia. The sponsor might argue that the overseas sourced innovator product is identical to that available in Australia because they are marketed under the same brand name, by the same sponsor, in both countries. However, this argument is not accepted because the TGA is conscious that multinational companies sometimes market different formulations of a medicinal product in different countries under the same or different brand names. In the European Union, it is tolerable for an application for registration of a generic product to be based upon a bioequivalence study where the reference product was obtained in another associate state. However, member states of the EU are able to share information concerning the formulation and other characteristics of the innovator brands registered in their particular countries, in order to establish unequivocally the identity of reference products from different member states. This is not possible in Australia. In order to establish that the overseas reference product is matching to the leading Australian brand, a generic sponsor may be able to provide a declaration from the innovator company that it markets the same product that is

identical in all compliments, including formulation and method of manufacture in both countries. The TGA would refer such a declaration to the local subordinate if the declaration was not provided by the local subordinate company or licensee. Where such a statement cannot be provided the following requirements must meet unless otherwise justified. Otherwise the sponsor should express equivalence of the generic product to the Australian sourced reference product by conducting an appropriate bioequivalence study or studies[1].

The present TGA policy is awaiting there is agreement on the majority appropriate method of statistical analysis of chronological designs; sequential (add-on) studies are discouraged. If a chronological study is used it must be foreshadowed in the study protocol. It is not adequate to analyze the results of a study and then to decide to enroll more subjects because the study was underpowered and bioequivalence criteria were not met. If a sequential study is planned in the protocol then the initial statistical analysis must be modified to meet more careful necessities (even if, ultimately, a second group of subjects is not used). In the absence of consensus on the most appropriate method of statistical analysis, the most conservative of the approaches proposed in the literature, the Bonferroni correction, should be applied. This corresponds to the calculation of 95%, rather than 90%, confidence intervals[3].

STUDY PARAMETERS

Study Design

The basic design of an in-vivo bioavailability study is determined by considering, what are the scientific questions to be answered, the nature of the reference material and the dosage form to be tested, the availability of analytical methods, risk-benefit 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[7].

Selection of the Number of Subjects

The number of subjects required for a study should be statistically significant and is determined by considering the error variance associated with the primary characteristic to be studied as

estimated from a pilot experiment, from previous studies or from published data. The significance level desired usually 0.05. The expected deviation from the reference product compatible with bioequivalence. The required (discriminatory) power, normally equal to 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. 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 subject or reasons for not including partial data as well as the unenclosed data must be documented in the final report[8].

Selection Criteria for Subjects

To reduce intra and inter individual variation subjects should be standardized as much as possible and acceptable. The studies should be normally performed on healthy adult volunteers with the aim to minimize 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[9].

Study Conditions

Standardization of the study environment, diet, fluid intake, post-dosing postures, exercise, sampling schedules etc. is important in all studies. Compliance to these standardizations 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 minimized. 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[10].

Selection of Blood Sampling Points

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 shortened 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[7].

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 measured 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[11].

Steady State Studies

Steady state study is considered where the drug has a long terminal elimination half-life and blood concentrations after a single dose cannot be followed for a sufficient time. Where assay sensitivity is inadequate to follow the terminal elimination phase for an adequate period of time. 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. Modified-release products it is necessary to assess the fluctuation in plasma concentration over a dosage interval at steady state. Drugs inducing its own metabolism and shows large intra-individual variability. In case of enteric-coated preparations when coating is innovative. Drugs exhibiting non-linear (i.e. dose or time dependent) pharmacokinetics, drug is likely to accumulate in the body. In steady state studies, the dosing schedule should follow the clinically recommended dosage regimen[11].

Bioanalytical methodology

The bioanalytical methods used to determine the drug and its metabolites in plasma, serum, blood or urine or any other suitable matrix must be well characterized, standardized, 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. 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. 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[11, 12].

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.

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 cycle's representative of actual sample handling. The absence of any sorption by the sampling containers and stoppers should also be established[2].

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 drugs under study[12].

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."

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.

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.

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.

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[12].

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 reanalysis. 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[13].

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[12, 14].

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 or laboratory standard operating procedures[5].

STATISTICAL EVALUATION

Data analysis

The primary concern in bioequivalence 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.

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 analyzed using non-parametric methods. In addition to above, summary statistics such as minimum, maximum and ratio should be given.

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 narrow therapeutic index, serious dose-related toxicity and steep dose response curve.

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[15].

DOCUMENTATION

Table 1: Describes documents required with respect to the conduct of bioequivalence/bioavailability study.

Table 1: Documents required for conduct of bioequivalence/bioavailability study

S.N.	Document Title	Description required
1	Clinical Data	All relevant documents as required to be maintained for compliance with GCP Guidelines
2	Analytical method validation	✓ System suitability test ✓ Linearity range ✓ Lowest limit of quantitation ✓ QC sample analysis ✓ Stability sample analysis ✓ Recovery experiment result
3	Analytical data of volunteers plasma samples	✓ Validation data of analytical methods used ✓ Chromatograms of all volunteers, including any aberrant chromatograms ✓ Inter-day and intra-day variation of assay results ✓ Details including chromatograms of any repeat analysis performed ✓ Calibration status of the instruments
4	Raw data	If any
5	A copy of the final report	Bioequivalence or bioavailability report should give the complete documentation of its protocol, conduct and evaluation

Study Report

The bioequivalence or bioavailability report should give the complete documentation of its protocol, conduct and evaluation. Table 2 shows the contents of study report.

Table 2: Contents of study report

S.N.	Description required
1	Table of contents
2	Title of the study
3	Names and credentials of responsible investigators
4	Signatures of the principal and other responsible investigators authenticating their respective sections of the report
5	Site of the study and facilities used
6	The period of dates over which the clinical and analytical steps were conducted
7	Names and batch numbers of the products compared
8	A signed declaration that this was identical to that intended for marketing

9	Results of assays and other pharmaceutical tests (e.g., physical description, dimensions, mean weight, weight uniformity, and comparative dissolution) carried out on the batches of products compared
10	Full protocol for the study including a copy of the ICF and criteria for inclusion/exclusion or withdrawal of subjects
11	Report of protocol deviations, violations
12	Documentary evidence that the study was approved by an independent ethics committee and was carried out in accordance with GCP/GLP
13	Demographic data of subjects
14	Names and addresses of subjects
15	Details of and justifications for protocol deviations
16	Details of dropout and withdrawals from the study should be fully documented and accounted.
17	Details of analytical methods used, full validation data, quality control data and criteria for accepting or rejecting assay results
18	Representative chromatograms covering the whole concentration range for all, standard and quality control samples as well as specimens analyzed
19	Sampling schedules and deviations of the actual times from the schedules
20	Details of how pharmacokinetic parameters were calculated
21	Documentation related to statistical analysis ✓ Randomization schedule ✓ Volunteer wise plasma concentration and time points for test and reference products ✓ Volunteer wise AUC_{0-t}, AUC_{0-8}, C_{max}, T_{max}, K_{el}, and $t_{½}$ for test and reference products ✓ Logarithmic transformed measures used for BE demonstration ✓ ANOVA for AUC_{0-t}, AUC_{0-8}, C_{max} ✓ Inter-subject, intra-subject and/or total variability if possible ✓ Confidence intervals for AUC_{0-t}, AUC_{0-8}, 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 ✓ Geometric mean, arithmetic mean, ratio of means for AUC_{0-t}, $AUC_{0-\infty}$, C_{max} ✓ Partial AUC, only if it is used C_{min}, C_{max}, C_{pd}, AUC_{0-t}, degree of fluctuation [(C_{max} - C_{min})/ C_{avg}] and swing [(C_{max} – C_{min})/C_{min}], if steady state studies are employed

Several companies may produce and market similar formulations to the original marketed product following patent expiration, provided they can demonstrate bioequivalence to the original product. Generic substitution has thus provided a means of supplying the market with inexpensive, efficacious, and safe drug products without the need to repeat an entire clinical and clinical pharmacology development package following patent expiration. Any off patent drugs can be marketed as generic drug after its bioequivalence is proved. Any formulation in the market is well safe and effective in the treatment and cure of the disease for which it is prepared. Therefore, any new formulation before marketing it is necessary to fulfill the criteria for marketing. Following are the product requisites before conducting bioequivalence studies. The formulation should have same amount of the active ingredient and the formulation should have same dissolution profile.

Table 3: Comparative assessment of study parameters between India, South-Africa and Australia

S.N.	Parameters	India	South-Africa	Australia
1	Study design	Non-replicated, randomized, crossover studies	Non-replicated, randomized, crossover studies	Non-replicated, randomized, crossover studies
2	Fasting/Fed state studies	Fasting	Fasting	Fasting
3	Volunteers	>16 subjects	Min 80% Power of acceptance criteria	Min 80% Power of acceptance criteria
4	Study Test dose Reference	Test product made by the manufacturer Any internationally available product or Already approved Indian product	Test product made by the manufacturer US/Europe or South African reference product	Test product made by the manufacturer US/Europe or Australian reference product
5	Sampling points	3 sample points during absorption phase, 3-4 at projected T_{max}, 4 samples during elimination phase.	12 to 18 samples per subject/dose	At least 2 samples before expected T_{max}, 3-4 terminal log-linear phase
6	Analytical method validation parameters	Selectivity, accuracy, precision, recovery. Lower limit of quantification (LLOQ) and calibration curve. Stability of analyte in spiked samples	Specificity, accuracy, precision, stability of analyte, limit of detection and quantification, response function, robustness and ruggedness	Specificity, recovery, precision and accuracy, response function, limit of quantification (LOQ)
7	Moieties to be measured in plasma	Active Drug / Metabolites if applicable	Active Drug / Metabolites if applicable	Active Drug / Metabolites if applicable
8	Pharmacokinetic parameters	C_{max}, T_{max}, AUC_{0-t}, $AUC_{0-\infty}$, $t_{1/2}$, λ_z	C_{max}, T_{max}, AUC_{0-t}, $AUC_{0-\infty}$, $t_{1/2}$, λ_z	C_{max}, T_{max}, AUC_{0-t}, $AUC_{0-\infty}$, $t_{1/2}$, λ_z
9	Criteria for Bioequivalence	90% CI 80-125% for C_{max}, AUC_t, AUC_{0-inf}	90% CI 80-125% for C_{max}, AUC_t, AUC_{0-inf}	90% CI 80-125% for C_{max}, $hAUC_t$, AUC_{0-inf}
10	GCP Requirements	Indian GCP Guidelines	MCC GCP Guidelines	ICH GCP Guidelines

India

In a progress to ensure standards of quality, efficacy and safety in medical products, India's Central Drug Standard Control Organization (CDSCO) has revised its guidelines on bioequivalence and bioavailability for pharmaceuticals. The revisions will become part of

Schedule Y of the Drugs and Cosmetics Act and new drug applications will have to meet these necessities. The revisions indicate how a relative study should be executed, the design requirements, study population, the characteristics that need to be studied, facts of the bioanalytical methodology required, and parameters for statistical evaluation of the results.

South-Africa:

Medicines Control Council of South-Africa follows MCC GCP guidelines for bioequivalence focuses on the equivalence of release of the active pharmaceutical ingredient from the pharmaceutical product and its successive absorption into the systemic circulation. Comparative studies using clinical or pharmacodynamic end points may also be used to demonstrate bioequivalence. The 90 % confidence interval for the test/reference ratio should lie within the acceptance interval of 0.80-1.25 (80-125%).

Australia:

In Australia, the Therapeutics Goods Administration (TGA) considers preparations to be bioequivalent if the 90% confidence intervals (90% CI) of the distorted natural log ratios, between the two preparations, of C_{max} and AUC lie in the range 0.80-1.25. T_{max} should also be similar between the products. There are tighter requirements for drugs with a narrow therapeutic index and/or saturable metabolism thus no generic products exist on the Australian market for digoxin or phenytoin for instance.

CONCLUSION

During the last few years, there is a major progress in policies and procedures concerning the determination of bioavailability and bioequivalence. Presently, there is international harmonization of regulatory requirements for bioequivalence studies. Comparative assessment of study parameters between India, South-Africa and Australia revealed that India and South-Africa follow GCP guidelines of respective countries; Australia follows ICH GCP guidelines. Sampling points and number of samples as well as analytical method validation parameters are well defined for India. Moieties to be measured in plasma, pharmacokinetic parameters and criteria for bioequivalence are same for all these countries. However, the trend in the near future appears towards achieving the appropriate choice of clinically relevant bioequivalence ranges based on therapeutic ranges, rate of absorption metrics, designs to resolve the issue of intra and inter subject variability etc.

REFERENCES

1. Roger L. Nation, Lloyd N. Sanso. Bioequivalence requirements for generic products.

Pharmacological Therapy. 1994; 62: 41-55

2. Tomasz Grabowski, Monika Marczak, Jerzy Jan Jaroszewski, Monica Whitmire. Comparison of bioequivalence study regulatory requirements for human and veterinary drugs. Regulatory Toxicology and Pharmacology. 2012; 64: 233–242
3. Bruce E. Strober, Katherine Armour, Ricardo Romiti, Catherine Smith et al. Biopharmaceuticals and bio-similar in psoriasis: what the dermatologist needs to know. Journal of American Academy of Dermatology. 2012; 66(2): 317-322
4. Government of India, Ministry of Health and Family Welfare. Guidelines for bioavailability and bioequivalence studies. Central Drug Standards and Control Organization, New Delhi. 2005: 1-33
5. Merrick Zwarenstein, Jan H Schoeman, Caesar Vundule, Carl J Lombard, et al. Randomised controlled trial of self-supervised and directly observed treatment of tuberculosis. The Lancet. 1998; 352: 1340-1343.
6. Vishal S. Narang, Amar Lulla, Geena Malhotra, Shrinivas Purandare. Bioequivalence evaluation of two marketed brands of stavudine 40 mg capsules in healthy human South African volunteers. Pharmacological Res. 2004; 50: 511–516
7. Yan-Mei Liu, Kanyin E. Zhang, Yun Liu, Hai-Chen Zhang et al. Pharmacokinetic properties and bioequivalence of two sulfadoxine/pyrimethamine fixed-dose combination tablets: a parallel-design study in healthy Chinese male volunteers. Clinical Therapeutics. 2012; 34(11): 2212-2220
8. Shashank Patel, Vijay Chauhan, Jayanta Mandal, Samir Shah et al. Single dose, two-way crossover, bioequivalence study of mycophenolate mofetil 500 mg tablet under fasting conditions in healthy male subjects. Clinical Therapeutics. 2011; 33(3): 378-390
9. Muzaffar Iqbal, Arshad Khuroo, Lakhvinder S. Batolar, Monika Tandon, and et al. Pharmacokinetics and bioequivalence study of three oral formulations of valsartan 160 mg: a single-dose randomized, open-label, three-period crossover comparison in healthy Indian male volunteers. Clinical Therapeutics. 2010; 32(3): 588-596
10. Yun Liu, Meng-qi Zhang, Jian-min Zhu, Jing-ying Jia et al. Bioequivalence and pharmacokinetic evaluation of two formulations of glimepiride 2 mg: a single-dose, randomized-sequence, open-label, two-way crossover study in healthy Chinese male volunteers. Clinical Therapeutics. 2010; 32(5): 986-995
11. Shrutidevi Agrawal, Inderjit Singh, Kanwal Jit Kaur et al. Bioequivalence trials of rifampicin containing formulations: extrinsic and intrinsic factors in the absorption of

Rifampicin. Pharmacological Res 2004; 50: 317–327

12. Ravi Kumar Konda, Babu Rao Chandu, B. R. Challa, Chandrasekhar B. Kothapalli. Bio-analytical method development and validation of rasagiline by high performance liquid chromatography tandem mass spectrometry detection and its application to pharmacokinetic study. J Pharma Analysis. 2012; 2(5): 342–349
13. Islah Ahmed, Ben Kaspar, Uma Sharma. Biosimilars: impact of biologic product life cycle and European experience on the regulatory trajectory in the United States. Clinical Therapeutics. 2012;34(2): 400-419
14. R.K. Verbeeck, I. Kanfer, R.B. Walker. Generic substitution: the use of medicinal products containing different salts and implications for safety and efficacy. Eur J Pharm Sci. 2006; 28: 1-6
15. Senthil Kumar Krishnan, M. Mozhiarasi, Chirag Kapoor, Mahashree Meganathan. Bioequivalence studies in pharmacokinetics of drugs. Int J Universal Pharm and Bio Sci 2012; 1(2):103-109