Review Article on Regulatory Requirements for Conducting Bioequivalence/Bioavailability Studies in USA, Europe, Canada and India.

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***Abstract*- This article highlights the regulatory requirements for conducting bioavailability/bioequivalence (BA/BE) studies, which aim to ensure therapeutic equivalence between a test drug and a generic or reference drug. The study review emphasizes adherence to standards of quality, efficacy, and safety comparable to the innovator's product. While international harmonization of regulatory requirements for bioequivalence are lacking, there are partial harmonization in the bioequivalence range and statistical analysis. However, discrepancies exist in the subject selection, study design for immediate release and modified release formulations, food effect assessment, application of multiple-dose studies, in-vitro dissolution study and retention of innovator and reference products.**

This review offers a concise overview of the relevant regulatory guidelines for bioequivalence studies in the United States, Europe, Canada, and India including a comparative analysis of differences in study design and specifications. Importantly, the conduction of BA/BE studies in these countries aligns with the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) Good Clinical Practice (GCP) Guidance.

***Key Words*- Bioequivalence, BA/BE studies, Steady-state, Single-dose, Multiple-dose, EMA, USFDA, CDSCO, Health Canada, ICH GCP.**

I.INTRODUCTION:

The pharmaceutical industry operates within a highly regulated environment, governed by a multitude of rules and regulations implemented by governments worldwide to safeguard public health and well-being. As such, the industry strives to identify and develop generic drug products that can be tailored to meet diverse market demands.

Each country has its own regulatory system in place to assess the quality, safety, and efficacy of imported drug products. National governments take on the responsibility of establishing robust regulatory authorities, equipped with stringent guidelines for ensuring quality assurance and regulation within their respective territories.

Recognizing the growing movement towards harmonization and the establishment of a unified medicine market within Europe, officials from prominent regulatory bodies such as the United States Food and Drug Administration (USFDA), the European Medicines Agency (EMA), Health Canada, and the Central Drugs Standard Control Organization (CDSCO) convened during the International Conference of Drug Regulatory Authorities (ICDRA), organized by the World Health Organization (WHO). This gathering highlighted the need for broader harmonization efforts in the field.

In the absence of an internationally harmonized guideline specifically addressing bioequivalence studies, individual countries and regional organizations have independently established their own regulations and guidelines. For instance, notable guidelines include those formulated by USFDA in 2021, EMA in 2010, and Health Canada in 2018, CDSCO in 2005.

Over the past two decades, the expiration of patents and exclusivity periods for various pharmaceutical products has led to a significant surge in the generic drug market. This trend has been observed not only in developed nations but also in developing countries, resulting in substantial growth and expansion of the generic drug industry on a global scale.

This review provides a concise summary of regulatory guidelines for bioequivalence (BA/BE) studies in the United States, Europe, Canada, and India. It includes a comparative analysis of the study design and specifications, highlighting key differences.

The standard for BE studies varies from the country with respect to the following:

1. Number of volunteers required for the study.
2. Selection criteria.
3. Physical fitness of volunteers before the study.
4. Dietary restrictions during the study.
5. Dosing of the Investigational product.
6. Drawing of blood samples for analysis.
7. Presentation of data.
8. Calculation of BA/BE data pharmacokinetic parameters.
9. Data documentation.
10. Final review and conclusion.
11. Limitation of the studies.
12. Management of untoward effects during the studies.

II.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.

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

Steady-state: Steady state is the state in which the plasma concentration of the 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.

Genotype: A person's genotype is their unique sequence of DNA. More specifically, this term is used to refer to the two alleles a person has inherited for a particular gene.

Phenotype: the set of observable characteristics of an individual resulting from the interaction of its genotype with the environment.

Highly variable drug products: Highly variable drug products (HVDP) are those whose intra-subject variability for a parameter is larger than 30%.

Generic medicinal product: A generic medicine is a medicine that is developed to be the same as a medicine that has already been authorized. Its authorization is based on efficacy and safety data from studies on the authorized medicine.

III.STUDY DESIGN

i.General Study Design:

This section suggests the study design with respect to blinding, periods, treatment, sequences, dose, study conditions, etc.
The type of study design required for determining bioequivalence depends on the physicochemical characteristics of the substance, its pharmacokinetic properties and proportionality in composition and/or strengths and should be justified accordingly. There are two types of study designs Viz., Standard Study Design and Alternative Design.

A. Standard study design:

If two formulations are compared, a randomized, two-period, two-sequence, single-dose, crossover design using either healthy subjects or other populations, as appropriate is recommended by all regulatory guidelines.

B. Alternative study design:

a. Parallel Study design:

For USFDA:
For an oral immediate-release product with a long elimination half-life drug (> 24 hours), applicants can conduct a single-dose, crossover study, provided an adequate washout period is used. If the crossover study is problematic, applicants should conduct a BE study with a parallel design.

For EMA, Health Canada and CDSCO:
The guidelines of EMA, Health Canada and CDSCO state that parallel study design can be considered for substances with very long half-lives.

b. Replicate Study Design:

- **For Highly Variable Drugs:**

USFDA suggest a replicate crossover study design (either partial or fully replicate) is appropriate for drugs whether the reference product is a highly variable drug or not.
EMA and CDSCO suggest a replicate design e.g. for substances with highly variable pharmacokinetic characteristics. It is acceptable to apply either a 3-period or a 4-period crossover scheme in the replicate design study for EMA.
Health Canada Suggests that replicated cross-over designs may also be used, where the formulations are tested more than once on the same subjects.

- **For Narrow Therapeutic Index Drugs:**

USFDA Suggest a replicate design is advantageous over a non-replicate design for non-narrow therapeutic index (NTI) drugs with high intrasubject variability. Either a partial or fully replicate design may be used.

c. Multiple dose study design:

EMA Guidance suggests the conduct of a multiple-dose study in patients is acceptable if a single-dose study cannot be conducted in healthy volunteers due to tolerability reasons, and a single-dose study is not feasible in patients. In the rare situation where problems of sensitivity of the analytical method preclude sufficiently precise plasma concentration measurements after single-dose administration and where the concentrations at steady state are sufficiently high to be reliably measured, a multiple-dose study may be acceptable as an alternative to the single-dose study.

Moreover, the EMA guidance on Modified release dosage forms recommends the requirement of a multiple-dose study for the determination of the bioequivalence of modified release formulations.

IV.BLINDING:

Health Canada recommends double-blinded studies to avoid study bias. Comparative bioavailability studies should be conducted in such a way that the subjects are not aware of which product (test or reference) is being administered. Furthermore, the persons checking for adverse reactions and those conducting the bioanalysis of samples should not know the treatment sequence.

V.THE NUMBER OF SUBJECT / SELECTION OF SUBJECTS:

The number of subjects to be included in the bioequivalence study should be based on the calculation of sample size. According to USFDA, the total number of subjects in a study should be sufficient to provide adequate statistical power for a BE demonstration in the proposed study design.

The number of evaluable subjects in a bioequivalence study should not be less than 12 according to EMA and Health Canada. For CDSCO, the number of subjects should not be less than 16 unless justified for ethical reasons.

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 /dropout from the study once it has begun provided the substitute follows the same protocol originally intended for the withdrawn subjects and he/she is tested under similar environmental and other controlled conditions.

VI.SELECTION OF SUBJECT:

USFDA recommends if a drug product is used in both sexes, then similar proportions of males and females should be included in the study.

According to EMA, Health Canada and CDSCO recommendations, the subject can belong to either sex. EMA, Health Canada and CDSCO consider the risk to women of childbearing potential when conducting bioequivalence studies, but do not prohibit their participation if there is no risk. In addition to the previous recommendations, CDSCO adds that women taking contraceptives should not be included in the study.

Women should be required to give assurance that they are not pregnant, nor likely to become pregnant after the study.

Selection criteria: Some of the selection criteria are mentioned as follows for different guidelines.

i. Subjects are chosen based on criteria such as age and body mass index (BMI)
ii. Subjects should preferably be non-smokers and without a history of alcohol or drug abuse.
iii. Phenotyping and/or genotyping of subjects may be considered for safety or pharmacokinetic reasons.
iv. Volunteers should generally be between 18 to 55 years of age
v. Subjects of 60 years of age or more in case the drug product is to be used predominantly in the elderly.
vi. BMI Criteria for Europe, India and Canada recommended 18.5 and 30 kg/m^2, In USFDA the BMI criteria is not specified.
vii. Bioequivalence studies should be conducted in healthy subjects.
viii. The health of volunteers should be determined by the supervising physician through a medical examination including a review of medical history
ix. Phenotyping and genotyping of subjects may be considered for safety or pharmacokinetic reasons.

Note: Other selection criteria should be added or followed as per their regulatory requirements and the specifications mentioned in the respective molecules 'Prescribing Information'.

VII.STRENGTHS TO BE INVESTIGATED:

According to USFDA in most cases, the highest strengths are suggested, however in a few cases conducting the study on lower strength may be appropriate for reasons of safety, providing the following conditions.

i. Linear elimination kinetics has been shown over the therapeutic dosage range.
ii. All active and inactive ingredients are in similar proportion between different strengths
iii. For drug products that meet the following criteria: (1) the total weight of the dosage form remains nearly the same for all strengths (within +/- 10 percent of the total weight of the strength on which a bio study was performed), (2) the same inactive ingredients are used for all strengths, and (3) the change in any strength is obtained by altering the amount of the active ingredients and one or more of the inactive ingredients.
iv. Active and inactive ingredients that are not in similar proportion between different strengths can be considered proportionally similar with adequate justification.

EMA recommend for the drug with linear pharmacokinetics use of the highest strength is preferred. For drugs with non-linear pharmacokinetics, the establishment of BE studies both at the highest and at the lower strength is required.

For Health Canada, for Products in which the proportion of excipients and dissolution characteristics are similar, comparative bioavailability studies may not be required for all strengths. Further guidance will be found in the Therapeutic Products Directorate Policy: Bioequivalence of Proportional Formulations - Solid Oral Dosage Forms. It suggests if different strengths are proportional in formulation, or have only "minor" differences in the proportion of ingredients, a comparative bioavailability study is required on only one strength (preferably the highest). If different strengths have differences in the proportion of ingredients which exceed, but within the progression of strengths the changes are incremental, a comparative bioavailability study is required on the lowest and highest strengths.

CDSCO suggest 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).

VIII. SINGLE DOSE/ MULTIPLE DOSES:

USFDA recommends the use of single-dose pharmacokinetic studies for both immediate and modified-release drug products to demonstrate BE as they are generally more sensitive than steady-state studies in assessing differences in the release of the drug substance from the drug product into the systemic circulation. multiple-dose studies are generally not recommended.

As per EMA, the single-dose study is recommended for immediate release formulation and the conduct of a multiple-dose study in patients is acceptable if a single-dose study cannot be conducted in healthy volunteers due to tolerability reasons and a single-dose study is not feasible in patients.

However, the multiple-dose study is less sensitive in detecting differences in C_{max}, this will only be accepted if the applicant can adequately justify that the sensitivity of the analytical method cannot be improved and when it becomes difficult to rely on the measurement of parent compound after the single administration. Hence, the use of a multiple-dose study instead of a single-dose study, due to the limited sensitivity of the analytical method, will only be accepted in exceptional cases.

According to CDSCO, single-dose studies are generally recommended. However, there are some situations where the steady state study design is required such as

i. Drug with dose and time-dependent pharmacokinetics
ii. Some modified-release products
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 a steady state.

IX. ENDOGENOUS SUBSTANCES:

USFDA recommends that applicants measure and approximate the baseline endogenous concentrations in blood (plasma) or urine and subtract these concentrations from the total concentrations measured from each subject after the drug product is administered to achieve an estimate of the actual drug availability from the drug product. When the body produces the compound, it is recommended that applicants measure multiple baseline concentrations from each individual subject in the time period before administration of the study drug. When there is a dietary intake of the compound, it is recommended that applicants strictly control the intake both before and during the study. Subjects should be housed at the clinic before the study and served standardized meals.

According to EMA, for endogenous substances, the sampling schedule should allow the characterization of the endogenous baseline profile for each subject in each period. Often, a baseline is determined from 2-3 samples taken before the drug products are administered.

In bioequivalence studies with endogenous molecules, it cannot be directly assessed whether carryover has occurred, so extra care should be taken to ensure that the washout period is of adequate duration.

CDSCO and Health Canada did not provide any recommendations regarding the endogenous molecules.

X. PARENT DRUG OR METABOLITE:

USFDA generally recommends that the applicants measure only the parent drug, rather than metabolites because the concentration-time profile of the parent drug is more sensitive to change in formulation performance than the metabolite, which is more reflective of metabolite formation, distribution and elimination. when the parent drug concentration is too low to allow reliable analytical measurement in blood, plasma or serum for an adequate length of time. Therefore, the metabolite contributes meaningfully to safety and/or efficacy.

EMA guidance suggests evaluation of bioequivalence should be based on measured concentrations of the parent compound. The reason for this is that the C_{max} of a parent compound is usually more sensitive to detect differences between formulations in absorption rate than the C_{max} of a metabolite. However, some prodrugs may have low plasma concentration and be quickly eliminated resulting in difficulties in the demonstration of the bioequivalence of the parent compound. In this situation, it is acceptable to demonstrate bioequivalence for the main active metabolite without measurement of the parent compound. The use of a metabolite as a surrogate for an active parent compound is not encouraged.

Health Canada recommends determination of comparative bioavailability should be based on data for the parent drug. If the parent drug is not detectable due to rapid biotransformation. In such instances, the use of metabolite data may be acceptable. The choice of using the metabolite instead of the parent drug is to be clearly stated, a priori, in the objective of the study in the study protocol.

CDSCO suggest evaluations of bioavailability and bioequivalence will be based on the measured concentrations of the active drug substances in the biological matrix. In the case of the concentrations of the drugs may be too low to accurately measure in the

biological matrix or limitations of the analytical method or the case of prodrugs. the measurements of an active or inactive metabolite may be necessary.

XI.ENANTIOMERS:

USFDA, EMA and CDSCO recommend using an achiral assay to measure the racemate. It is recommended to measure individual enantiomers in BE studies only when all the following conditions have been met:

i. The enantiomers exhibit different pharmacodynamic characteristics.
ii. The enantiomers exhibit different pharmacokinetic characteristics.
iii. The primary efficacy and safety activity reside with the minor enantiomer.
iv. Nonlinear absorption is present for at least one of the enantiomers.

XII.POSTURE AND PHYSICAL ACTIVITY:

USFDA did not suggest any criteria regarding posture conditions. EMA recommends as the bioavailability of an active moiety from a dosage form could be dependent upon gastrointestinal transit times and regional blood flows, posture and physical activity may need to be standardized. Therefore, it is recommended to standardize exercise. Health Canada suggests subjects should not be allowed to recline until at least two hours after drug ingestion. Physical activity and posture should be standardized as much as possible to limit effects on gastrointestinal blood flow and motility. Moreover, CDSCO recommends standardization of the study environment, post-dosing postures and exercise.

XIII.EMESIS OR VOMITING:

USFDA recommend that data from subjects who experience vomiting during a BE study for immediate-release products be deleted from statistical analysis if that vomiting occurred at or before 2 times the median T_{max}. For modified-release products, it recommends deleting data from the analysis if a subject vomits during a period of time less than or equal to the dosing interval stated in the labelling of the product. The concentration data for the subject who vomited should be reported.

According to EMA, events such as vomiting and diarrhea are the reasons to exclude the subjects from the study as these may render the plasma concentration-time profile unreliable.

As per Health Canada the subjects who vomit should be evaluated for continued participation in the study based on the potential impact of vomiting on the integrity of the study results and the evaluation should take place as soon as possible after the episodes of analysis of the study samples

For CDSCO there have been no recommendations provided.

XIV.FASTING AND FLUID INTAKE:

USFDA recommends the test or reference product should be administered with about 8 ounces (240 milliliters) of water to subjects under fasting conditions (i.e., after an overnight fast of at least 10 hours). In the case of fed breakfast, it recommends that subjects should start the recommended meal 30 minutes before administration of the test or reference product following an overnight fast of at least 10 hours. Study subjects should finish eating this meal in 30 minutes or less, and the drug product should be administered 30 minutes after the start of the meal. The subjects are allowed water as desired except for 1 hour before and after the drug administration.

EMA and Health Canada recommend Subjects should fast for at least 8 hours prior to administration of the products unless otherwise justified. As fluid intake may influence gastric passage for oral administration forms, the test and reference products should be administered with a standardized volume of fluid (at least 150 ml). It is recommended that water is allowed as desired except for one hour before and one hour after drug administration and no food is allowed for at least 4 hours post-dose. Meals taken after dosing should be standardized regarding composition and time of administration during an adequate period of time (e.g. 12 hours).

CDSCO suggest generally, a single dose study should be conducted after an overnight fast (at least 10 hours), with the 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.

XV.CALORIE COUNT FOR FED STUDIES:

USFDA, EMA and Health Canada recommend a high-fat (approximately 50 % of the total caloric content of the meal), high-calorie (approximately 800 to 1000 kilocalories) test meal for fed BE studies. This test meal should derive approximately 150, 250, and 500-600 kcal from protein, carbohydrate, and fat, respectively. The composition of the meal should be described with regard to protein, carbohydrate and fat content (specified in grams, calories and relative caloric content [%]). The test meal should be consumed within a 30-minute interval prior to administration of the drug product.

For CDSCO, 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 protein and the rest from carbohydrates. The high-fat breakfast must be consumed approximately 15 minutes before dosing.

XVI.SAMPLING TIME POINTS:

USFDA recommends drawing blood samples at appropriate times to describe the absorption, distribution, and elimination phases of the drug. For most drugs, collecting 12 to 18 samples, including a pre-dose sample, per subject, per dose. This sampling should continue for at least three or more terminal elimination half-lives of the drug. EMA recommends the sampling schedule should include frequent sampling around predicted t_{max} to provide a reliable estimate of peak exposure. In particular, the sampling schedule

should be planned to avoid C_{max} being the first point of a concentration-time curve. At least three to four samples are needed during the terminal log-linear phase in order to reliably estimate the terminal rate constant. Health Canada suggests a minimum of 12 samples should be collected per subject per dose.
CDSCO suggest the blood-sampling period in single-dose trials of an immediate-release product should extend to at least three 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 of the total AUC. There should be at least three sampling points during the absorption phase, three to four at the projected T_{max} and four points at the elimination phase.

XVII.WASHOUT PERIOD:

USFDA, EMA and CDSCO recommend the treatment periods should be separated by a washout period sufficient to ensure that drug concentrations are below the lower limit of bioanalytical quantification in all subjects at the beginning of the second period. An adequate washout period (e.g., more than five half-lives of the moieties to be measured) should separate each treatment. In the case of Health Canada, the minimum time between treatments should be the same for all subjects and, to account for variability in elimination rate between subjects, normally should be not less than 10 times the mean terminal half-life of the drug. Normally, the interval between study days should not exceed three to four weeks.

XVIII.STATISTICAL PARAMETER:

USFDA recommend the determination of bioequivalence after a single the parameters to be analyzed are $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$, C_{max}, T_{max}, Kel and $t_{1/2}$. At steady state $AUC_{0\text{-}tau}$, $C_{max,ss}$, $C_{min,ss}$, C_{avss}, the degree of fluctuation, swing and T_{max} shall be determined.
EMA recommend in studies to determine bioequivalence after a single dose, the parameters to be analyzed are $AUC_{(0\text{-}t)}$ or when relevant $AUC_{(0\text{-}72h)}$, and C_{max}. For studies to determine the bioequivalence of immediate release formulations at steady state, $AUC_{(0\text{-}\tau)}$ and $C_{max,ss}$ should be analyzed using the same acceptance interval as stated above.
A statistical evaluation of tmax is not required. However, if rapid release is claimed to be clinically relevant and of importance for onset of action or is related to adverse events, there should be no apparent difference in median tmax and its variability between test and reference product.
Health Canada suggests if single dose studies are conducted AUC_T, AUC_I, AUC_T/AUC_I, C_{max}, T_{max}, λ and $t_{1/2}$. Also, $AUC_{Reftmax}$ should be measured. Where multiple dose studies are conducted parameters such as C_{min}, C_{pd} and C_{tau}, pAUC should be measured.
In the case of CDSCO, 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}, $AUC_{0\text{-}t}$ and $AUC_{0\text{-}\infty}$. For studies in the steady state $AUC_{0\text{-}\tau}$, C_{max}, C_{min} and degree of fluctuation should be calculated.

XIX.RETENTION SAMPLE OF INVESTIGATIONAL PRODUCT:

USFDA recommend each reserve sample shall be retained for a period of at least 5 years following the date on which the application or supplemental application is approved, or if such application or supplemental application is not approved, at least 5 years following the date of completion of the bioavailability study in which the sample from which the reserve sample was obtained was used.
EMA and Health Canada recommend retention samples from each batch of the finished product should be retained for at least one year after the expiry date.
CDSCO Suggests All samples of test and reference drug products used in the 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.

Table 1: Comparison of bioequivalence guidelines of the USA, Europe, Canada and India.

Sr. No.	Criteria	US-FDA	EMA	Health Canada	CDSCO
1.	**Strength to be Investigated**	The BE studies generally should be conducted on the highest strength of the drug product	The bioequivalence study should in general be conducted at the highest strength. For drugs with linear Pharmacokinetics, the use of the highest strength is preferred. For drugs with non-linear pharmacokinetics, the establishment of BE studies both at the highest and at the lower strength is required.	Bioequivalence studies may not be required for all strengths of drug products with similar proportions of excipients and dissolution characteristics.	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).

2.	**Standard Design**	Single-dose, two-period, two-treatment, two-Sequence, cross-over study design.	Single-dose, two-period, two-treatment, two-Sequence, cross-over study design.	Single-dose, two-period, two-treatment, two-Sequence, cross-over study design.	Single-dose, two-period, two-treatment, two-Sequence, cross-over study design.
3.	**Alternative Design:**				
I	**Long Half-Life:**	Single dose crossover parallel study design.	Single dose crossover parallel study design.	Single dose crossover parallel study design.	Single dose crossover parallel study design.
II	**Highly Variable Drug:**	A replicate cross-over study design (either partial or fully replicate).	Replicate design for substances with highly variable pharmacokinetic characteristics.	Replicate cross-over design may also be used for highly variable substances.	Replicate design for substance with highly variable deposition.
III	**Narrow Therapeutic Index Drug**	Either a partial or fully replicate design may be used.	Not Defined	Not Defined	Not Defined
4.	**Blinding**	Not Defined	Not Defined	Double-blind study where the subject person recording adverse drug reaction and the person conducting bio-analysis should not be aware of sample/ products as well as about the treatment sequence.	Not Defined
5.	**Number of Subjects**	The total number of subjects in a study should be sufficient to provide adequate statistical power for a BE demonstration in the proposed study design.	The number of evaluable subjects in a bioequivalence study should not be less than 12.	The minimum number of subjects is 12, but a larger number is usually required.	The minimum number of subjects should not be less than 16 unless justified for ethical reasons.
6.	**Sex of subjects**	Male/Female; If a drug product is intended for use in both sexes, the attempt should be made to include similar proportions of females and males in the study.	Male and/or Female	Male and/or Female	Male/Female; If a drug product is intended for use in both sexes, the attempt should be made to include similar proportions of females and males in the study
7.	**Female Subjects**	Females should not be pregnant or lactating, and, if applicable, should practice abstention or contraception	Risks to women of childbearing potential should be considered.	Female volunteers are not pregnant, lactating, or likely to become pregnant during the study. Confirmation regarding pregnancy should be obtained by urine or serum tests prior to drug administration in each period.	Women taking contraceptive drugs should normally not be included in the studies. Women are required to give assurance that they are not pregnant, nor likely to become pregnant until after the study and this should be confirmed by the pregnancy test immediately prior to the first and last dose of the study. Furthermore, women

					taking contraceptive drugs should normally not be included in the study.
8.	**Extra subjects for replacement on Withdrawal and/or Dropout**	Not Defined	The data from all treated subjects should be treated equally. It is not acceptable to have a protocol which specifies that 'spare' subjects in the study	A fixed number of subjects, in addition to the number estimated by the sample size calculation, should be recruited into the study. This strategy allows for possible drop-outs.	The number of subjects recruited should be sufficient to allow for possible withdrawals or removals (dropouts) from the study.
9.	**Age**	18 years or older	18 years or older	Age range of 18 to 55 years, (inclusive)	Healthy adult volunteers.
10.	**Body Mass Index**	Not Defined	18.5 and 30 kg/m^2.	18.5 and 30 kg/m^2.	Not Defined
11.	**Single/ Multiple doses**	Usually recommend single-dose PK studies for both immediate- and modified-release drugs. However, steady-state studies are conducted only wherever required.	The multiple-dose study is only in cases where the single-dose study is not acceptable to carry out.	Not Defined	Single-dose studies are preferred except for some special situations, the conduct of steady-state studies is acceptable.
12.	**Endogenous molecule**	Applicants should measure and approximate the baseline endogenous concentrations in blood (plasma) or urine. When the body produces the compound, it is recommended that applicants measure multiple baseline concentrations and when there is a dietary intake of the compound, it is recommended that applicants strictly control the intake.	the sampling schedule should allow the characterization of the endogenous baseline profile for each subject in each period. Often, a baseline is determined from 2-3 samples taken before the drug products are administered.	Not Defined	Not Defined
13.	**Parent drug/ Metabolite**	Recommend that measure only the parent drug, rather than metabolites. If the parent drug concentrations are too low to allow reliable analytical measurement in blood, plasma, or serum for an adequate length of time, then the metabolite data is	Concentrations of the parent Compound should be measured. However, some pro-drugs may have low plasma concentrations and be quickly eliminated resulting in difficulties in demonstrating bioequivalence for the parent compound. In this situation, it is acceptable to	Parent compounds are to be measured except for cases when the parent drug is not detectable, then their active detectable metabolite could be considered.	The concentration of the active drug substances should be measured. In some situations, however, the measurement of an active or inactive metabolite may be necessary.

		obtained from the study.	demonstrate bioequivalence for the main active metabolite without measurement of the parent compound		
14.	**Physical activity/ posture**	Not Defined	posture and physical activity may need to be Standardized.	Not allowed to recline until at least two hours after drug ingestion.	post-dosing postured and exercise should be standardized.
15.	**Vomiting**	Subjects who experience vomiting during a BE study for immediate-release products be deleted from statistical analysis if that vomiting occurred at or before 2 times the median T_{max}. Furthermore, in the case of modified-release drug products, the subjects should be excluded from the study if they experience emesis.	Subjects experiencing vomiting should be excluded from the study.	The evaluation of subjects for continued participation in the study should be done after the episodes of vomiting and before the analysis of the study samples.	Not Defined
16.	**Fasting study**	Pre-dose 10.00 hours and 4.00 hours post-dose drug administration.	Pre-dose 8.00 hours and 4.00 hours post-dose administration of drug product, unless otherwise justified.	Pre-dose 8.00 hours and 4.00 hours post-dose drug administration.	For a single dose, at least 10.00 hours overnight fasting and 4.00 hours after dosing. Multiple doses: 2.00 hours before and after the dose.
17.	**Fed Calories**	High-fat (approximately 50 percent of the total caloric content of the meal) high-calorie (approximately 800 to 1000 kilocalories) test meal for fed BE studies test meal should derive approximately 150, 250, and 500 to 600 kilocalories from protein, carbohydrate, and fat, respectively	High-fat (approximately 50 percent of the total caloric content of the meal) and high-calorie (approximately 800 to 1000 kcal) meals test meal should derive approximately 150, 250, and 500-600 kcal from protein, carbohydrate, and fat, respectively composition of the meal should be described with regard to protein, carbohydrate and fat content (specified in grams, calories and relative caloric content (%)).	A high-fat (approximately 50% of the total caloric content of the meal) and high-calorie (approximately 800 to 1000 kilocalories) the meal should derive approximately 150, 250, and 500-600 kilocalories from protein, carbohydrate, and fat, respectively. One example of a high-fat, high-calorie test meal is the following breakfast: 2 eggs fried in butter, 2 strips of bacon, 2 slices of toast with butter, 120 grams of hash browns and 240 milliliters of whole milk	High breakfast before dosing must be designed to provide 950-1000 Kcal. At least 50% of these calories must come from fat 15 to 20% from proteins and the rest from carbohydrates.

18.	**Fluid intake**	The test or reference product should be administered with about 8 ounces (240 milliliters) of water. The subjects are allowed water as desired except for 1 hour before and after the drug administration.	The test and reference products should be administered with a standardized volume of fluid (at least 150 ml). It is recommended that water is allowed as desired except for one hour before and one hour after drug administration	The dose should be taken with water of a standard volume (150 to 250 milliliters) and at a standard temperature. water should not be administered from one hour prior to dosing, concurrent with dosing and up to one-hour post-dosing.	The drug should be administered with the standard quantity of fluid.
19.	**Sampling Time Points**	For most drugs, collecting 12 to 18 samples, including a pre-dose sample, per subject, per dose.	At least three to four samples are needed during the terminal log-linear phase in order to reliably estimate the terminal rate constant.	Health Canada suggests a minimum of 12 samples should be collected per subject per dose.	There should be at least three sampling points during the absorption phase, three to four at the projected T_{max} and four points at the elimination phase.
20.	**Washout**	treatment periods should be separated by a washout period sufficient to ensure that drug concentrations are below the lower limit of bioanalytical quantification in all subjects. washout period should be more than five successive half-lives of the moieties to be measured.	treatment periods should be separated by a washout period sufficient to ensure that drug concentrations are below the lower limit of bioanalytical quantification in all subjects at the beginning of the second period. Normally at least 5 elimination half-lives are necessary to achieve this.	The interval between study days should be long enough to permit the elimination of essentially all of the previous doses from the body, normally should be not less than 10 times the mean terminal half-life of the drug.	two phases of treatment separated by an adequate washout period which should ideally be equal to or more than five half-lives of the moieties to be measured.
21.	**Statistical Parameters**	bioequivalence after a single the parameters to be analyzed are AUC_{0-t}, AUC_{0-inf}, C_{max}, T_{max}, Kel and $t_{1/2}$. At steady state AUC_{0-tau}, $C_{max,ss}$, $C_{min,ss}$, C_{avss}, the degree of fluctuation, swing and T_{max} shall be determined.	For a single-dose study $AUC_{(0-t)}$ or when relevant $AUC_{(0-72h)}$, and C_{max} will be analyzed. at steady state, $AUC_{(0-\tau)}$ and $C_{max,ss}$ should be analyzed.	AUC_T, AUC_I, AUC_T/AUC_I, C_{max}, T_{max}, λ and $t_{1/2}$. Also, $AUC_{Reftmax}$ should be measured for single-dose studies. C_{min}, C_{pd} and C_{tau}, pAUC should be measured for multiple dose studies.	C_{max}, T_{max}, AUC_{0-t} and $AUC_{0-\infty}$ should be measured for single-dose. For studies in the steady state $AUC_{0-\tau}$, C_{max}, C_{min} and degree of fluctuation should be calculated
22.	**Acceptance criteria**	90% confidence interval between 80-125%.	90% confidence interval between 80-125%. AUC should be tightened to 90-111.11% for narrow therapeutic range drugs and C_{max} should be widened to 69.84%-143.19% for highly variable drugs.	90% confidence interval between 75.41%-103.74% (AUC ratio) and C_{max} is 61.94-107.06%.	90% confidence interval between 80-125%. No specifications on narrow therapeutic drugs.

23.	**Retention of investigational product**	Each reserve sample shall be retained for a period of at least 5 years following the date on which the application or supplemental application is approved.	retention samples from each batch of the finished product should be retained for at least one year after the expiry date.	retention samples from each batch of the finished product should be retained for at least one year after the expiry date.	Samples should be retained for a period of three years after the conduct of the study or one year after the expiry of the drug.

XX.ABBREVIATIONS:

- $AUC_{(0-t)}$: Area under the plasma concentration curve from administration to last observed concentration at time t;
- $AUC_{(0-\infty)}$: Area under the plasma concentration curve extrapolated to infinite time;
- $AUC_{(0-\tau)}$: AUC during a dosage interval at a steady state;
- $AUC_{(0-72h)}$: Area under the plasma concentration curve from administration to 72h;
- C_{max}: Maximum plasma concentration;
- $C_{max,ss}$: Maximum plasma concentration at steady state;
- residual area: Extrapolated area ($AUC_{(0-\infty)}$ - $AUC_{(0-t)}$)/ $AUC_{(0-\infty)}$;
- $R_{max:}$ Maximal rate of urinary excretion;
- t_{max}: Time until C_{max} is reached;
- $t_{max,ss}$: Time until C_{max},ss is reached;
- $t_{1/2}$: Plasma concentration half-life;
- λz: Terminal rate constant.

XXI.CONCLUSION:

In conclusion, conducting bioequivalence/bioavailability studies in the United States, Europe, Canada, and India requires adherence to specific regulatory requirements. While there are variations among these regions, the overarching goal remains the same: ensuring the safety, efficacy, and quality of generic drugs.

The US Food and Drug Administration (USFDA) in the United States, the European Medicines Agency (EMA), Health Canada in Canada, and the Central Drugs Standard Control Organization (CDSCO) in India play crucial roles in establishing guidelines and standards for these studies. Each regulatory authority emphasizes the importance of study design, data analysis, and reporting.

Harmonization efforts among these regions have led to increased alignment in guidelines, simplifying the process for pharmaceutical companies conducting bioequivalence/bioavailability studies across multiple regions. This alignment enhances efficiency and facilitates the availability of affordable and high-quality generic drugs worldwide.

Understanding and complying with these regulatory requirements is essential for pharmaceutical companies seeking approval for generic drugs. Compliance ensures credibility and reliability, benefiting patients and the pharmaceutical industry as a whole. Continued collaboration and harmonization among regulatory authorities will further streamline the drug approval process and promote global access to safe and effective generic drugs.

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