Comparison of Bioequivalence Requirements Between The Different Regulatory Agencies

Manan Bhalawat

Regulatory Bodies

US : Food And Drug Administration (FDA)

Europe : The European Agency for Evaluation of medicinal Products (EMEA)

Canada : Health Products and Food Branch (HPFB) Therapeutic Product Directorate (TPD)

Australia : Therapeutic Goods Administration (TGA)

Brazil : Agência Nacional de Vigilância Sanitária Brazilian Sanitary Survillance Agency (ANVISA)

India : Central Drugs standard Control Organization (CDSCO)

WHO : World Health Organization

South Africa : Medicines Control Council (MCC)

- Despite a common scientific basis supporting bioequivalence, no hormonization between the different regulatory agencies.
- There are also major discrepancies within Europe, even though common EMEA Guidance documents are followed.
- EMEA Guidance documents are somewhat vague with regards to many aspects related to study design, conduct and PK and statistical analysis of BE studies.

Retention period

- EMEA: for one year in excess of the accepted shelf life or two years after completion of trial or until approval whichever is longer to allow re-testing (in-vitro & invivo), if it is requested by authorities.
- TGA: Same as EMEA
- SA: Same as EMEA
- CDSCO: 3 yrs after the conduct of study or one yr after expiry of drug whichever is earlier
- Quantity sufficient to carry out twice all in-vitro & invivo tests
- WHO: ----------
- SA: should be kept for one year in excess of the accepted shelf-life, or two years after completion of the trial or until approval, whichever is longer, in order to allow re-testing if required by the MCC.

Drug content / potency

- US: The drug content of the test product cannot differ from that of the reference listed product by more than 5 percent.
- CA: Potency correction seems to have very limited impact on study outcome.
  - However, if potency correction is not required, we should consider including specific recommendation about the acceptable difference between the test and reference formulations used in the BE study.
  - Potency correction results may otherwise be required
- AN: The drug content of the test product should not differ from that of the reference listed product by more than 5 percent.

No. of subjects

US	CDSCO	WHO, AN, CA	EMEA,TGA	SA
12 pilot	NLT 16 unless justified for ethical reasons	NLT 12	NLT 12, If less, justify	NLT 12, NLT 20 for MR

- WHO: The number of recruited subjects should always be justified with the sample size calculation provided in the study protocol.
- CDSCO: No. of subjects recruited should be sufficient to allow for possible withdrawn/dropout. CA: justification for the sample size calculation in protocol is must.

Inclusion criteria

Gender :

- **AN: Male, female or both, in which case the number of men and women must be the same and distributed equally between the sequences.**
- **SA: Same as ANVISA**
- **EMEA: Subjects could belong to either sex, however, the risk of childbearing potential should be considered on an individual basis.**
- **TGA: Same as EMEA**
- **US: Individuals representative of the general population, taking into account age, sex, and race. If the drug product is intended for use in both sexes, attempt to include similar proportions.**
- **CA: Can usually be tested in normal, healthy volunteers.**
  - **The investigators should ensure that female volunteers are not pregnant or likely to become pregnant during the study.**

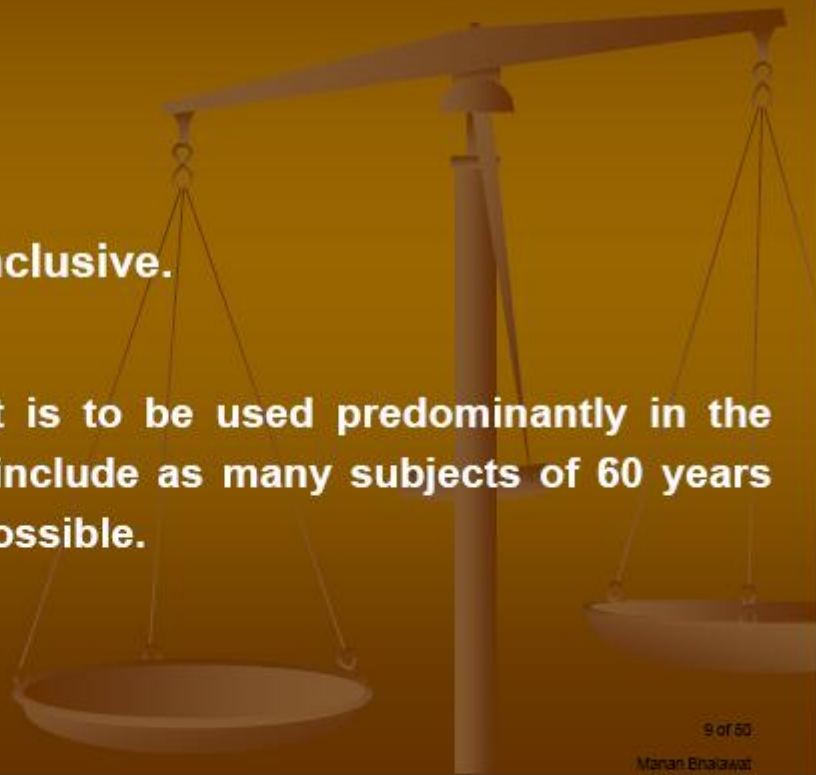
Inclusion Criteria

- CDSCO: Subjects may be males or females; however choice of gender s'be consistent with usage & safety criteria
  - Risk of women of childbearing potential s'be considered on individual basis. Women taking contraceptives should normally not be included in studies.
  - If product is intended for use in both sexes attempt s'be made to include similar proportions.
  - For drug representing a potential hazard in one group of users, the choice of subjects may be narrowed e.g. studies on teratogenic drugs s'be conducted only on males. E.g. Enalapril
  - For drugs primarily intended for use in only males or only females-volunteers of only respective gender s'be included.
- WHO: If product is intended for use in both genders, the sponsor may wish to include both.

Inclusion Criteria

Age:

- AN: 18 and 50 yrs
- TGA: 18 and 55 yrs
- EM: 18 and 55 yrs
- SA: 18 and 55 yrs
- CA: 18 to 55 years, inclusive.
- US: 18 or older
  - If the drug product is to be used predominantly in the elderly, attempt to include as many subjects of 60 years of age or older as possible.
- CDSCO: same as US



Inclusion Criteria

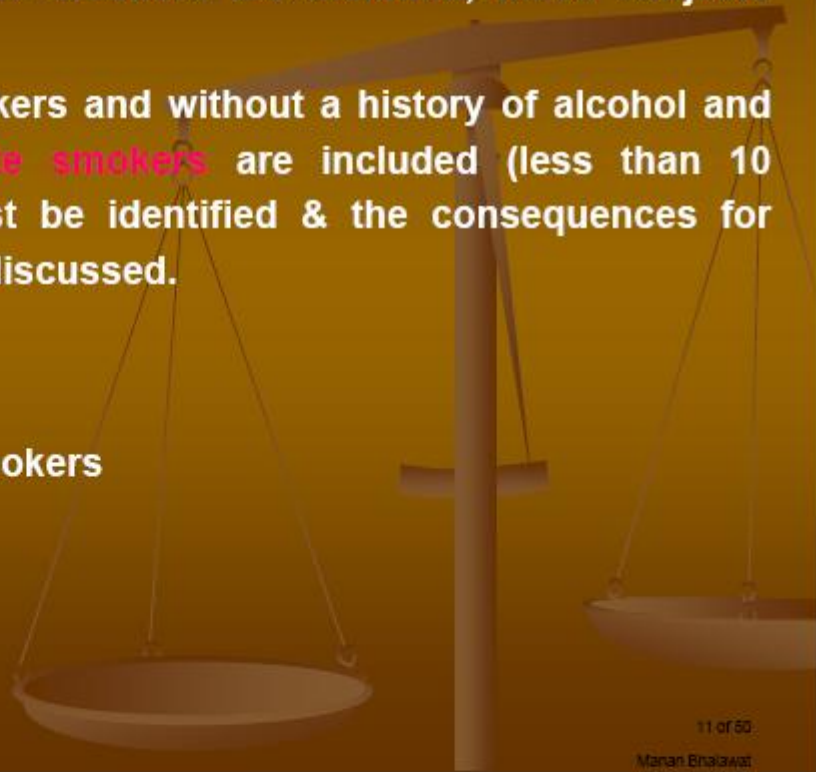
Weight:

- AN: Within a limit of ± 15% of the weight considered normal for men and women
- EM: Normal range according to accepted normal values for BMI
- TGA: Same as EMEA
- CA: Within 15 percent of the normal range- in current Ciba-Geigy or Metropolitan Life Insurance tables.
- SA: Body mass within the normal range according to accepted normal values for the Body Mass Index (BMI = mass in kg divided by height in meters squared, i.e. kg/m2), or within 15 % of the ideal body mass, or any other recognized reference
- USA: NA
- CDSCO: NA
- WHO: normal range according to accepted life tables

Inclusion Criteria

Smoker:

- AN: Smokers and subjects with a history of alcohol and drug abuse must be avoided. In case smokers are included, these subjects must be identified.
- EM: Preferably non-smokers and without a history of alcohol and drug abuse. If moderate smokers are included (less than 10 cigarettes per day) must be identified & the consequences for study results should be discussed.
- TGA: Same As EMEA
- SA: Same As EMEA
- USA: Smokers or non smokers
- CDSCO: NA



Water quantity

Why water quantity is important?

As fluid intake may profoundly influence the gastric transit of orally administered dosage forms.

US: 8 ounces (240 milliliters) of water

CA: water of a standard volume (e.g., 150 mL)

AN: standard (usually 200mL) liquid (generally water)

EMEA: the volume of fluid (at least 150 mL)

WHO: water of a standard volume (usually 150-250 ml).

SA: volume of fluid should be constant (e.g. 200 ml).

TGA: Standardisation of diet, fluid intake & exercise

CDSCO: Standardisation of diet, fluid intake & exercise

Water and food restriction

Water	US	EM	CA	WHO
Pre	1 hr	1	2	1
Post	1 hr	2	2	2

- US, CA, WHO, EMEA: **Fast for 10 hours before drug administration. Four hours after drug administration, a standard meal may be taken.**
- **ANVISA: Fast for 8 hours before drug administration**
  - **Abstain from alcohol for 24 hours before each study period and until after the last sample from each period is collected.**

EMEA: ad *libitum*.

Water restriction and food restriction

- CA: A fast means that no food or solids are to be consumed, although alcohol-free and xanthine-free clear fluids are permissible the night prior to the study. On the morning of the study, up to 250 mL of water may be permitted up to two hours before drug administration. Two hours after drug administration, 250 mL of xanthine-free fluids are permitted.
- Posture and Physical Activity: For most drugs, 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.
- CDSCO: Standard diet, fluid intake, post-dosing postures, exercise

Water restriction and food restriction

- SA: Period of fasting prior to dosing should be standardised and supervised. All meals and fluids taken after dosing should also be standardised in regard to composition and time of administration and in accordance with any specific requirements for each study.

- WHO: However, alcohol-free and xanthine-free clear fluids are permissible during the night prior to the study. All meals should be standardized and of similar composition and quantity during each study period.

Fed study

- US:
  - In addition to a BE study under fasting conditions, we recommend a BE study under fed conditions for all orally administered immediate-release drug products, except:
  - BCS Class I
  - DOSAGE AND ADMINISTRATION section of the RLD label states that the product should be taken only on an empty stomach, or
  - RLD label does not make any statements about the effect of food on absorption or administration.
  - For MR: both

- EMEA: If SPC (Summary of Product Characteristics) of the reference product contains specific recommendations in relation with food intake related to food interaction effects the study should be designed accordingly.

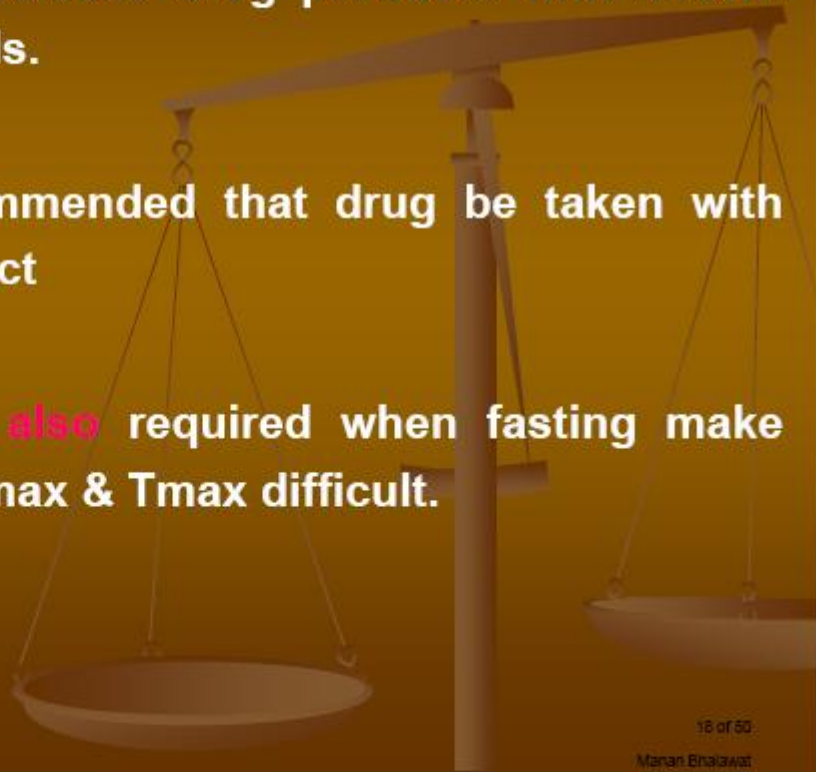
- WHO:
  - Fasted state studies are generally preferred. When the product is known to cause gastrointestinal disturbances if given in the fasted state, or if labelling restricts to administration to the fed state only, then the fed state pharmacokinetic bioequivalence study becomes the preferred method.

Fed study

- TGA: Same as EMEA
- CA: Some drugs are given with food to reduce gastrointestinal side effects. Studies of such drugs should include studies with standard meals.
- SA: For IR: fasting, unless food effects influence bioavailability. If the reference product dosage directions specifically state administration with food, the study should be designed taking in consideration any possible food effects.
- For MR, the influence of food should be demonstrated to exclude any possibility of dose dumping; hence, both fed and fasted studies are required.

Fed study

- AN: studies with feeding must be carried out for modified release forms (in addition to the fasting study) and for immediate release drug products with known interaction with foods.
- CDSCO:
  - When it is recommended that drug be taken with food or MR product
  - Both fast & fed
  - Fed studies are also required when fasting make assessment of Cmax & Tmax difficult.



Minimum sample points

US, SA, AN, CA	WHO	EMEA, TGA
12 -18	Atleast 7	-

- US: This sampling can continue for at least three or more terminal half lives of the drug.
- CA: Four or more points be determined during the terminal log-linear phase of the curve.
- CDSCO: At least 3 during absorption phase, 3-4 at the projected Tmax & 4 points during elimination phase
- WHO: At least 1-2 points before Cmax, 2 points around Cmax and 3-4 points during the elimination phase. Consequently at least seven sampling points

Length of blood sampling time

HALF-LIVES TO BE CONSIDERED WHILE DESIGNING TIME-POINTS

US, CDSCO, EMEA, TGA, CA, WHO	AN
at least 3	equal or greater than 3-5

Washout (half-lives)

US	CDSCO, EMEA	WHO	SA	AN	TGA	Canada
> 5	≥ 5	NLT 5	7-10	Atleast 7	Adequate	NLT 10

The interval between study days should be long enough to permit elimination of essentially all of the previous dose from the body.

- To avoid carry-over effects, treatments should be separated by adequate wash-out periods.
- CA & WHO: Normally, the interval between study days should not exceed three to four weeks.
- AN: drugs must be administered at approximately same time & where possible, the same day of the week.

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Metabolite

US	EMEA	Canada
Not required, except: Parent drug not measurable Or Significantly active & pre-systemic metabolism (presented as supportive data only)	Not required, except: Parent drug not measurable Or Significantly active Or Non-linear PK	Not required, except: Parent drug not measurable

CA: Assessment on the metabolite (rather than the parent compound) may no longer be valid. If the assessment is to be based on the metabolite, a rationale for doing so should be provided.

- **US: For BA studies, both the parent drug and its major active metabolites should be measured, if analytically feasible.**

Metabolites

- US:
  - Measurement of a metabolite may be preferred when parent drug levels are too low to allow reliable analytical measurement in blood, plasma, or serum for an adequate length of time.
  - If there is a clinical concern related to efficacy or safety for the parent drug, sponsors and/or applicants should contact the appropriate review division to determine whether the parent drug should be measured and analyzed statistically.
  - Metabolite may be formed as a result of gut wall or other presystemic metabolism.
  - If the metabolite contributes meaningfully to safety and/or efficacy, the metabolite and the parent drug should be measured.
  - When the relative activity of the metabolite is low and does

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Metabolites

- EM:
  - In some situations, measurements of active or inactive metabolite may be necessary instead of parent compound.
  - If Conc. of active substance is too low to be accurately measured in biological matrix (e.g. major difficulty in analytical method, product unstable in biological matrix or half-life of parent compound too short)
- CDSCO: In some situations, measurements of active or inactive metabolite may be necessary instead of parent compound. These include (a) If Conc. of active substance is too low to be accurately measured in biological matrix, (b) major difficulty in analytical method (c) product unstable (d) very short half-life or (e) in case of prodrugs
- SA: Same as CDSCO
- TGA: Same as CDSCO

- **AN:**

The unaltered drug must always be quantified. The metabolites must be quantified in the cases of analytical limitations for quantification of the unaltered drug, or when the unaltered drugs are active, significantly contributing to the effectiveness and safety of the product, having

Enantiomers Versus Racemates

- US:

  Recommends measurement of the racemate using an achiral assay.

  Measurement of individual enantiomers in BE studies is recommended only when all of the following conditions are met:

  - Different pharmacodynamic characteristics,
  - Different pharmacokinetic characteristics,
  - Primary efficacy and safety activity resides with the minor enantiomer, and
  - Nonlinear absorption is present (as expressed by a change in the enantiomer concentration ratio with change in the input rate of the drug) for at least one of the enantiomers. In such cases, BE criteria should be applied to the

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Enantiomers Versus Racemates

- CA: ---------
- AN: ---------
- EMEA: Products containing chiral active substances should be based upon enantiomeric bio-analytical methods unless both products contain
  - Same stable single enatiomer as an active substance or contain the racemate and
  - Both enantiomers: linear pharmacokinetics.
- TGA: Same as EM
- WHO: case when systemic availability of different enantiomers is demonstrated to be non-linear. (Very different pharmacological or metabolic profiles)
- SA: --------
- CDSCO: Same as US

When is steady state necessary?

- US: Multiple-dose studies are generally not recommended, even in instances where non-linear pharmacokinetics are present.
- EM: Steady state studies may be required
  - For dose or time dependent PK
  - Some modified release products (In addition to single dose investigations)

  OR can be considered in case of:
  - if problem of sensitivity preclude sufficiently precise plasma conc. measurements after single dose administration.
  - if the intra-individual variability in plasma conc. or disposition precludes the possibility of demonstrating bioequivalence in a reasonably sized single dose study & this variability is reduced at steady state.
- TGA: Certain situations

When is steady state necessary?

- **CDSCO:** If problem of sensitivity preclude sufficiently precise plasma conc. measurements after single dose administration
  - i) may be due to its drugs long half-life.
  - ii) Where assay sensitivity is inadequate to follow elimination phase for 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
  - some modified release products where it is necessary to assess fluctuation in plasma conc. over dosage interval at steady state.
  - For those drugs which induce their own metabolism or show large intra-individual variability.
  - For enteric-coated preparations where coating is innovative.
  - For combination products where ratio of plasma conc. of individual drugs is imp.
  - For drugs that exhibit dose or time dependent PK,
  - Where drug is likely to accumulate in body.

When is steady state necessary?

- CA: In addition to the single-dose studies described, a comparison should be made between the first market entry MR formulation and equivalent doses of the conventional formulation over the dosing interval (claimed for the MR product) at steady state.
  - Generally, steady-state studies should be performed under fasting conditions.
- AN: ------
- WHO:
  - Multiple dose studies in patients are most useful in cases where the medicine is considered to be too potent and/or too toxic to be administered to healthy volunteers, even in single doses.
  - Drugs that exhibit non-linear kinetics at steady state (e.g. saturable metabolism, active secretion);
  - Cases where the assay sensitivity is too low to adequately characterize pharmacokinetic profile after single dose;

Drugs with non-linear PK

US	EMEA	Canada
not addressed in guidances. refer to RLD	Use strength with largest sensitivity to identify differences in formulation. Steady-state study may be required Measurement of active metabolite required Chiral assay needed if racemate	Use strength with largest sensitivity to identify differences in formulation. Fed study required Steady-state may be required

Drugs with non-linear PK

- EM: If metabolites significantly contribute to the net activity of an active substance and the PK of the system is non linear, it is necessary to measure both parent drug and active metabolite plasma concentrations and evaluate them separately
- TGA: same as EM
- CDSCO: Steady state study
- AN: ------
- WHO: Multiple dose study
- SA:-----

Long half life

- US: Parallel or truncated
  - For drugs that demonstrate low intrasubject variability in distribution and clearance, an AUC truncated at 72 hours (AUC0-72 hr) can be used in place of AUC0-t or AUC0-œ.
  - For drugs demonstrating high intrasubject variability, AUC truncation warrants caution.
- CDSCO: If due to longer half-life, chances of drop outs & intra subject variation are higher with crossover design, parallel design can be used.
  - Suitably truncated AUC can be used. For drugs demonstrating high intrasubject variability, AUC truncation warrants caution.
- CA: Truncated or parallel
- EMEA: Parallel, not explained on truncated
- TGA: Same as EM

Long half life

- WHO: (i) where low concentrations occur in the terminal portion of the plasma concentration versus time curve, which may not be quantifiable by means of an adequately validated, sensitive analytical method; and (ii) for products of active pharmaceutical ingredients with long half-lives.
- SA: parallel designs for very long half-life substances.For long half-life drugs (> 24 hours) the study should cover a minimum of 72 hours unless 80 % is recovered before 72 hours.
- AN: in the case of drugs presenting long elimination half life (over 24 hours), an alternative collection schedule may be used, of up to 72 hours, allowing the determination of the area under the fragmented curve (ASC0-72), or a parallel study.

Narrow therapeutic drugs

- US: Traditional BE limit of 80 to 125% for non-narrow therapeutic range drugs remain unchanged for the bioavailability measures (AUC and Cmax) of narrow therapeutic range drugs.
  - Digoxin, lithium, phenytoin, theophylline, and warfarin.
- EM: AUC_t, C_{max}: Acceptance interval need to be tightened
- TGA: same as EM
- WHO: same as EM
- SA: 80 – 125 % will apply
- CDSCO: Tighter limit for: narrow therapeutic index
  - serious, dose-related toxicity
  - steep dose/effect curve or
  - non-linear pharmacokinetics within therapeutic dose range
- CA: Cmax, AUC: 80 – 125 %
- AN:-------

Highly variable drug products

US	EMEA	Canada
Absence of specific recommendation	Possibility of conducting replicate design (but no specific recommendation about use of individual BE) Possibility of conducting steady-state	Absence of specific recommendation

CDSCO	SA
Wider acceptance range may be acceptable if it is based on sound clinical justification	a wider interval or other appropriate measure may be acceptable, but should be stated *a priori* and justified in the protocol

Modified release formulation

US	EMEA	Canada
Total of 2 studies:	Total of 2 studies:	Total of 2 studies:
1 single dose, Fasted	1 single dose, Fasted	1 single dose, Fasted
1 single dose, Fed	1 single dose, Fed	1 single dose, Fed
	1 steady-state study (SR products)	1 steady-state study if drug accumulation

Statistical criteria

- US: AUC_t, AUC_{inf}, C_{max}: 90% C.I. must be between 80.00-125.00%
- CA: AUC_t: 90% C.I. must be between 80-125 %
  - C_{max}: Ratio must be between 80-125 %
  - Need to pass also on potency corrected data
- EMEA: AUC_t: 90% C.I. must be between 80-125 %
  - Cmax: 90% C.I. must be between 80-125 %
  - Wider interval may be acceptable (e.g. 75-133%) with proper efficacy and safety justification
  - In specific cases of narrow therapeutic range the acceptance interval may need to be tightened
- TGA: same as EM

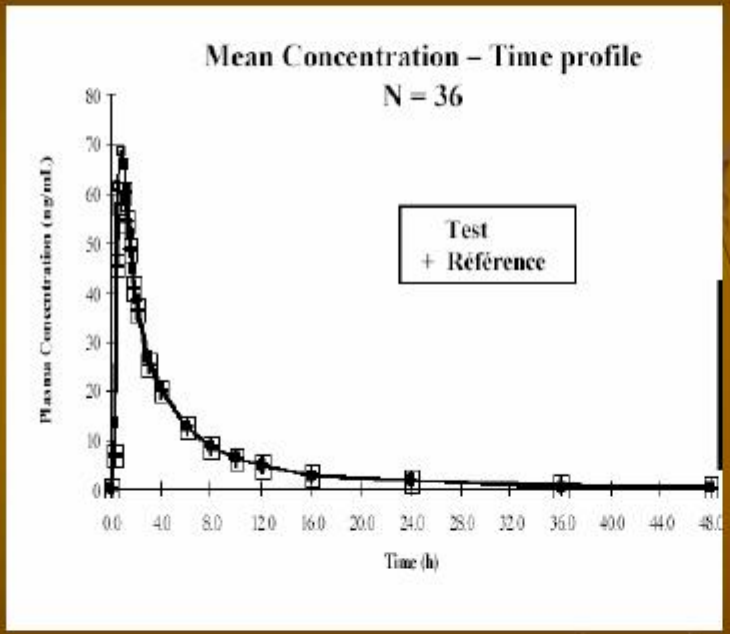
Statistical criteria

- WHO: In general acceptance limit 0.80-1.25 should be applied to the Cmax-ratio. However, this measure of relative bioavailability is inherently more variable than, for example, the AUC-ratio, and in certain cases a wider acceptance range (e.g. 0.75-1.33) may be acceptable.
- AUC0-t and Cmax are considered to be the most relevant parameters for assessment of bioequivalence.
- SA: AUC_t: 90% C.I. should lie within 80-125 %

  C_{max}: 90% C.I. should lie within 75-133 %

  ANVISA: the exclusion of more than 5% of the subjects from the study or the lack of over 10% of the values for blood concentration of the drug resulting from administration of each drug product will not be accepted.

Statistical criteria



	AUCinf	Cmax
Ratio	108.7%	117.6%
90% CI	104.1 - 113.6%	106.4 - 129.9%

Not acceptable for FDA
May be acceptable for EMEA
acceptable for HPFB

Dropout

- US: Sufficient number of subjects in the study to allow for dropouts. Because replacement of subjects during the study could complicate the statistical model and analysis, dropouts generally should not be replaced. Sponsors who wish to replace dropouts during the study should indicate this intention in the protocol. The protocol should also state whether samples from replacement subjects, if not used, will be assayed.
- EMEA: Protocol should also specify methods of handling drop-outs .
- CDSCO: SAME AS EMEA
- AN: SAME AS EMEA

Dropout

- CA: More subjects than the sample size calculation
  - Fixed number (one or two for each sequence) of subjects are added to the sample-size number.
  - Fixed number of subjects are added into the study.
  - Extras
  - Only if there is a drop-out will the appropriate extra subject's blood samples be assayed.
  - Method of accounting for dropouts must be outlined in the protocol.
- CDSCO: It is acceptable to replace drop out/subject withdrawn from study once it has begun provided the substitute follows the same protocol
- SA: ----------
- AN: Consider Dropout rate during experimental design

- WHO:
  - Same as US.
  - Sponsors who wish to replace drop-outs during the study or consider an add-on design should indicate this intention in the protocol. It is more appropriate to recruit into the study more subjects than the sample size calculation requires.
  - These subjects are designated as extras. The protocol should state whether samples from extra subjects will be assayed if not required for statistical analysis.

Outlier

Outliers: are defined as observations that appear to be inconsistent with the rest of the data. They can be identified as the values, which completely distort descriptive statistics. Subjects who exhibit extremely high or low bioavailability relative to the reference formulation are detected using statistical method namely Lund's Method using statistical package SAS® 9.1. A valid clinical or physiological reason will be explored for such an outlier, if found, and will be reported. However, to avoid the biasedness in the results, the statistical analysis will be performed on both the data sets i.e. including as well as excluding the outliers if the outlier is justified clinically as well.

Outlier

- EMEA: Protocol should also specify methods for identifying biologically implausible outliers. Post hoc exclusion of outliers is generally not acceptable.
- WHO: SAME AS EMEA
- CDSCO: SAME AS EMEA
- TGA: Considers that the most acceptable way of dealing with dropouts is to dose several more than the required number of subjects in the first phase and to specify in the protocol how the requisite number of subjects is to be chosen for dosing in the second phase, from those remaining in the study.
- SA:----------
- US: ----------
- CA:----------

Outlier

- AN: There are basically three possible outlier types in bioequivalence studies:
  - Unexpected values in plasma concentration curve versus time for given collection times;
  - Extremely high or low values for a given formulation
  - Uncommon individuals that show too high or too low a bioavailability relative to the reference medication, i.e., a too large difference for curve behavior between the two formulations, which implies distinct values for all pharmacokinetic measures evaluated.

(Add-on) Designs

- US: If the dropout rate is high and sponsors wish to add more subjects, a modification of the statistical analysis may be recommended
- Additional subjects should not be included after data analysis unless the trial was designed from the beginning as a sequential or group sequential design.
- CA: If the study is run with the appropriate size and the standards are not met, the sponsor may add more subjects (a minimum of 12). If this option is chosen, it must be stated in the study protocol.
- SA: The provision for add-ons should be made in the protocol a priori clearly reflecting the maximum number of subjects to be included.
- AN: ---------
- EMEA: ----------
- TGA: ----------
- CDSCO: ----------

Sequential (Add-on) Designs

- **WHO:**
  - If the bioequivalence study was performed with the appropriate size but bioequivalence cannot be demonstrated because of a result of a larger than expected random variation or a relative difference, an add-on subject study can be performed using not less than half the number of subjects in the initial study. Combining is acceptable only in the case when the same protocol was used and preparations from the same batches were used. **Add-on designs must be carried out strictly according to the study protocol and SOPs**, and must be given appropriate statistical treatment, including consideration of consumer risk.
  - *suppose to be mentioned in protocol*
  - It is not acceptable 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.

Retention period for sample

- US: 5 years
- amount that constitutes the five times quantity from the sponsor and/or drug manufacturer. For solid oral dosage forms (e.g., tablets, capsules), an upper limit of 300 units can be considered sufficient to meet the five times quantity.
- CA: 3 years after conduct of the study or one year after expiry of the drug, whichever is earlier.
  - Twice to conduct all invivo and invitro test required during BA BE studies.
- AN: The minimum period of retention of the batches will correspond to the expiry date of the product plus year, having as parameter the expiry date of the most recent product.
  - The minimum quantity of retention of test and reference drugs will be sufficient to repeat the study.

Retention period

- EMEA: for one year in excess of the accepted shelf life or two years after completion of trial or until approval whichever is longer to allow re-testing (in-vitro & invivo), if it is requested by authorities.
- TGA: Same as EMEA
- SA: Same as EMEA
- CDSCO: 3 yrs after the conduct of study or one yr after expiry of drug whichever is earlier
- Quantity sufficient to carry out twice all in-vitro & invivo tests
- WHO: ----------
- SA: should be kept for one year in excess of the accepted shelf-life, or two years after completion of the trial or until approval, whichever is longer, in order to allow re-testing if required by the MCC.