Egyptian Guideline for Conducting Bioequivalence Studies for Marketing Authorization of Generic Products

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SECTION 1

OVERVIEW

1.1 Objectives

This guideline is intended to provide recommendations to sponsors and Contract Research Organizations (CROs) on the requirements for approval of generic pharmaceutical products in Egypt to assure interchangeability, safety, quality and efficacy of these products.

Two medicinal products containing the same active substance(s) are considered bioequivalent if they are pharmaceutically equivalent or pharmaceutical alternatives and their bioavailabilities (rate and extent of drug absorption) after administration in the same molar dose lie within acceptable predefined limits. These limits are set to ensure comparable in vivo performance, i.e. similarity in terms of safety and efficacy.

It is the objective of this guideline to specify the requirements for the design, conduct, and evaluation of bioequivalence studies. The possibility of using in vitro instead of in vivo studies is also addressed.

1.2 Introduction

Generic pharmaceutical products need to conform to the same standards of quality, safety and efficacy of the innovator / reference listed product(s). In addition, they should be clinically interchangeable with equivalent marketed products.

A generic drug is bioequivalent to the reference listed product if the rate and extent of absorption of the drug do not show a significant difference from the rate and extent of absorption of the reference listed product when administered at the same molar dose of the therapeutic ingredient(s) under similar experimental conditions in either a single dose or multiple doses.

This guideline is generally applicable to orally administered generic products, as well as to non-orally administered pharmaceutical products for which systemic exposure measures are suitable for documenting bioequivalence (e.g. transdermal delivery systems and certain parenteral, rectal, vaginal and nasal pharmaceutical products). Other classes of products, including many biologicals such as vaccines, animal sera, and products derived from human blood and plasma, and products manufactured by biotechnology, are excluded from consideration in this guideline.

To ensure interchangeability, the generic product must be therapeutically equivalent to the reference listed product. Therapeutic equivalence can be assured when the generic product is both pharmaceutically equivalent / alternative and bioequivalent.

In such a pharmacokinetic study any statement about the safety and efficacy of the test product will be a prediction based on measurement of systemic concentrations, assuming that essentially similar plasma concentrations of the drug will result in essentially similar concentrations at the site of action, and thus an essentially similar therapeutic outcome.

Bioequivalence studies should be performed in compliance with the general regulatory requirements and good practices recommendations as specified in Good Clinical Practice (GCP) and Good Laboratory Practice (GLP) guidelines.

1.3 Glossary

Accuracy

The accuracy of an analytical procedure expresses the closeness of the determined value to the value which is accepted either as a conventional true value or an accepted reference value. Accuracy is defined as (determined value/true value) x100%.

Analyte

A specific chemical moiety being measured, which can be intact drug, biomolecule or its derivative metabolite and/or degradation product in a biologic matrix.

Analytical Procedure

The analytical procedure refers to the way of performing the analysis. It should describe in detail the steps necessary to perform each analysis.

Analytical run

A complete set of analytical and study samples with appropriate number of calibration standards and QC samples for their validation. Several runs may be completed in one day, or one run may take several days to complete.

Anchor calibrators

Anchor calibrators are standards points outside of the range of quantification, used to assist in fitting the non linear regression of the standard curve in ligand-binding assays.

Bioavailability

Bioavailability can be defined as the rate and extent to which the active pharmaceutical ingredient or active moiety is absorbed from a pharmaceutical dosage form and becomes available in the general circulation.

Bioequivalence

Two pharmaceutical products are bioequivalent if they are pharmaceutically equivalent or pharmaceutical alternatives, and their bioavailabilities, in terms of peak (C_{max} and T_{max}) and area under the curve (AUC) after administration of the same molar dose under the same conditions, are similar to such a degree that their effects can be expected to be essentially the same.

Biological matrix

A discrete material of biological origin that can be sampled and processed in a reproducible manner. Examples are blood, serum, plasma, urine, feces, cerebrospinal fluid, saliva, sputum, and various discrete tissues.

Biopharmaceutics Classification System (BCS)

The BCS is a system for classifying active pharmaceutical ingredients based upon their aqueous solubility and intestinal permeability. When combined with the dissolution of the pharmaceutical product, the BCS takes into account three major factors that govern the rate and extent of drug absorption (exposure) from immediate-release oral solid dosage forms: dissolution, solubility, and intestinal permeability.

Biowaiver

The term biowaiver is applied to a regulatory drug approval process based on evidence of equivalence other than through in vivo equivalence testing.

Blank

A sample of a biological matrix to which no analytes have been added, that is used to assess the specificity of the bioanalytical method.

Calibration range

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure meets the requirements for precision, accuracy and response function.

Calibration standard

A matrix to which a known amount of analyte has been added or spiked. Calibration standards are used to construct calibration curves.

Carry over

Carry-over is the appearance of an analyte signal in blank sample after the analysis of samples with a high analyte concentration.

Cross validation

Comparison of validation parameters when two or more bioanalytical methods are used to generate data within the same study or across different studies.

Dosage form

The form of the completed pharmaceutical product, e.g. tablet, capsule, elixir, suppository……etc.

Enantiomers

Two stereoisomers (molecules that are identical in atomic constitution and bonding, but differ in the three-dimensional arrangement of the atoms) that are related to each other by a reflection: they are mirror images of each other, which are nonsuperimposable. Every stereocenter in one has the opposite configuration in the other. Two compounds that are enantiomers of each other have the same physical properties, except for the direction in which they rotate the polarized light and how they interact with different optical isomers of other compounds.

Equivalence requirements

In vivo and /or in vitro testing requirements for approval of a generic pharmaceutical product and marketing authorization.

Equivalence test

A test that determines the equivalence between the generic product and the reference listed product using in vivo and / or in vitro approaches.

Fixed-dose combination (FDC)

A combination of two or more active pharmaceutical ingredients in a fixed ratio of doses. This term is used generically to mean a particular combination of active pharmaceutical ingredients irrespective of the formulation or brand. It may be administered as single-entity products given concurrently or as a finished pharmaceutical product.

Fixed-dose combination finished pharmaceutical product (FDC-FPP)

A finished pharmaceutical product that contains two or more active pharmaceutical ingredients.

Full validation

Establishment of all validation parameters to apply to sample analysis for the bioanalytical method for each analyte.

Generic product

A pharmaceutically equivalent or pharmaceutical alternative product that may or may not be therapeutically equivalent. Generic pharmaceutical products that are therapeutically equivalent are interchangeable.

In other words, It is a product which has the same qualitative and quantitative composition in active substances and the same pharmaceutical form as the reference listed medicinal product, and whose bioequivalence with the reference listed medicinal product has been demonstrated by appropriate bioavailability studies. The different salts, esters, ethers, isomers, mixtures of isomers, complexes or derivatives of an active substance are considered to be the same active substance, unless they differ significantly in properties with regard to safety and/or efficacy. Furthermore, the various immediate-release oral pharmaceutical forms shall be considered to be one and the same pharmaceutical form.

In vitro equivalence test

An in vitro equivalence test is a dissolution test that includes comparison of the dissolution profile between the generic product and the reference listed product in three media: pHs 1.2, 4.5 and 6.8 in addition to the most suitable medium which should be used based on FDA-Recommended Dissolution Methods or pharmacopeial requirements.

Incurred sample reanalysis

The analysis of a portion of the incurred samples to determine whether the original analytical results are reproducible.

Incurred samples

Study samples from dosed subjects.

Interchangeable pharmaceutical product

An interchangeable pharmaceutical product is one which is therapeutically equivalent to a reference listed product and can be interchanged with the reference listed product in clinical practice.

Internal standard

Test compound(s) (e.g. a structurally similar analogue, or stable isotope labelled compound) added to calibration standards, QC samples and study samples at a known and constant concentration to correct for experimental variability during sample preparation and analysis.

Limit of detection (LOD)

The lowest concentration of an analyte that the bioanalytical procedure can reliably differentiate from background noise.

Lower limit of quantification (LLOQ)

The lower limit of quantification of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with pre-defined precision and accuracy.

Ligand Binding Assay (LBA):

A method to analyse an analyte of interest using reagents that specifically bind to the analyte. The analyte is detected using reagents labelled with e.g., an enzyme, radioisotope, fluorophore or chromophore. Reactions are carried out in microtitre plates, test tubes, disks, etc., the validation to be considered according to ICH guideline M10 on bioanalytical method validation and study sample analysis.

Matrix effect

The direct or indirect alteration or interference in response due to the presence of unintended analytes (for analysis) or other interfering substances in the sample.

Nominal concentration

Theoretical or expected concentration.

Partial validation

Series of analytical experiments where only relevant parts of the validation are repeated after modifications are made to the validated bioanalytical method.

Pharmaceutical alternatives

Products are pharmaceutical alternatives if they contain the same molar amount of the same active pharmaceutical moiety(s) but differ in dosage form (e.g. tablets versus capsules), and/or chemical form (e.g. different salts, different esters). Pharmaceutical alternatives deliver the same active moiety by the same route of administration but are otherwise not pharmaceutically equivalent. They may or may not be bioequivalent or therapeutically equivalent to the reference listed product.

Pharmaceutical equivalents

Products are pharmaceutical equivalents if they contain the same molar amount of the same active pharmaceutical ingredient(s) in the same dosage form, if they meet comparable standards, and if they are intended to be administered by the same route. Pharmaceutical equivalence does not necessarily imply therapeutic equivalence, as differences in the excipients and/or the manufacturing process and some other variables can lead to differences in product performance.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained under the prescribed conditions. Precision is defined as the ratio of standard deviation/mean (%).

Processed Sample

The final extract (prior to instrumental analysis) of a sample that has been subjected to various manipulations (e.g., extraction, dilution, concentration).

Quality control (QC) sample

A spiked sample used to monitor the performance of a bioanalytical method and to assess the integrity and validity of the results of the unknown samples analysed in an individual batch.

Racemate

A racemate is optically inactive. Because the two isomers rotate plane-polarized light in opposite directions, they cancel out; therefore, a racemic mixture does not rotate plane-polarized light. In contrast to the two separate enantiomers, which generally have identical physical properties, a racemate often has different properties compared to either one of the pure enantiomers. Different melting points and solubilities are very common, but differing boiling points are also possible. Pharmaceuticals can be available as a racemate or as a pure enantiomer, which might have different potencies.

Recovery

The recovery of an analyte in an assay is the detector response obtained from an amount of the analyte added to and extracted from the biological matrix, compared to the detector response obtained for the true concentration of the analyte in solvent. The extraction efficiency of an analytical process, reported as a percentage of the known amount of an analyte carried through the sample extraction and processing steps of the method.

Reference listed product

The reference listed product is a pharmaceutical product with which the generic product is intended to be interchangeable in clinical practice. The reference listed product will normally be the innovator product (which was first authorized for marketing) for which efficacy, safety and quality have been established.

Reproducibility

The precision between two laboratories. It also represents precision of the method under the same operating conditions over a short period of time.

Response function

Response function is a function which adequately describes relationship between instrument response (e.g. peak area or height ratio) and the concentration (amount) of analyte in the sample. Response function is defined within a given range.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Selectivity

Selectivity is the ability of the bioanalytical method to measure and differentiate the analyte(s) of interest and internal standard in the presence of components which may be expected to be present in the sample.

Specificity

Specificity is the ability to measure the analyte unequivocally in the presence of other compounds, either exogenous or endogenous, in the matrix.

Stability

The chemical stability of an analyte in a given matrix under specific conditions for given time intervals.

Standard curve

The relationship between the experimental response values and the analytical concentrations (also called a calibration curve).

Standard Operating Procedure

Document which describes the regularly recurring operations relevant to the quality of the investigation and enabling to carry out the operations correctly and always in the same manner.

System suitability

Determination of instrument performance (e.g., sensitivity and chromatographic retention) by analysis of a set of reference standards conducted prior to the analytical run. System suitability testing is an integral part of many analytical procedures. The tests are

based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated.

Surrogate Matrix

In some cases, it may be difficult to obtain an identical matrix to that of the study samples (e.g., rare matrices such as tissue, cerebrospinal fluid, bile or in cases where free drug is measured). In such cases, surrogate matrices may be acceptable for analytical method validation.

If the Surrogate Matrix Approach is used, refer to ICH guideline M10 on bioanalytical method validation and study sample analysis.

Therapeutic equivalents

Two pharmaceutical products are considered to be therapeutically equivalent if they are pharmaceutically equivalent or pharmaceutical alternatives and after administration in the same molar dose, their effects, with respect to both efficacy and safety, are essentially the same when administered to patients by the same route under the conditions specified in the labeling. This can be demonstrated by appropriate bioequivalence studies, such as pharmacokinetic, pharmacodynamic, clinical or in vitro studies.

Upper limit of quantification (ULOQ)

The upper limit of quantification of an individual analytical procedure is the highest amount of analyte in a sample which can be quantitatively determined with pre-defined precision and accuracy.

1.4 Abbreviations

- **$Ae_{(0-t)}$:** Cumulative urinary excretion of unchanged drug from administration until time t;
- **ANOVA:** Analysis of variance;

- **$AUC_{(0-t)}$:** 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;
- **$AUC_{(0-\tau)}$:** Area under the plasma concentration curve during a dosage interval at steady state;
- **$AUC_{(0-72h)}$:** Area under the plasma concentration curve from administration to 72h;
- **BCS:** Biopharmaceutics classification system;
- **C_{av}:** Average steady state concentration ($AUC_{\tau/\tau}$);
- **C_{last}:** is the last measurable drug concentration;
- **C_{max}:** Maximum plasma concentration;
- **$C_{max,ss}$:** Maximum plasma concentration at steady state;
- **C_{min}:** Minimum plasma concentration;
- **$C_{min,ss}$:** minimum plasma concentration at steady state;
- **Residual area:** Extrapolated area ($AUC_{(0-\infty)}$ - $AUC_{(0-t)}$)/ $AUC_{(0-\infty)}$;
- **f1:** Dissimilarity (Difference) factor;
- **f2 :** Similarity factor;
- **Fluctuation:** (C_{max}-C_{min})/C_{av};
- **ICH:** International Conference on Harmonization;
- **K_{el}:** Elimination rate constant;
- **R_{max}:** Maximal rate of urinary excretion;
- **R_t:** Cumulative percentage of the drug dissolved at each of the selected time points of the reference listed product;
- **SmPC:** Summary of Product Characteristics;
- **SOPs:** Standard operating procedures;
- **T_{max}:** Time until Maximum plasma concentration is reached;
- **$T_{max,ss}$:** Time until maximum plasma

concentration at steady state is reached;

- **T_t:** Cumulative percentage of the drug dissolved at each of the selected time points of the test product; and
- **$T_{1/2}$:** Plasma concentration half-life.

SECTION 2

BIOEQUIVALENCE STUDY CONDUCTANCE

2.1 Methods to assess equivalence

a) Comparative pharmacokinetic studies in humans, in which the active pharmaceutical ingredient (APIs) and/or its metabolite(s) are measured as a function of time in an accessible biological fluid such as blood, plasma, serum or urine to obtain pharmacokinetic parameters, such as AUC and C_{max} that are reflective of the systemic exposure;

b) Comparative pharmacodynamic studies in humans;

c) Comparative clinical trials; and

d) Comparative in vitro tests.

2.2 Pharmaceutical products exempted from equivalence studies

The following types of generic pharmaceutical products are considered to be equivalent without the need for further documentation:

a) Parenterally administered (e.g. intravenously, subcutaneously or intramuscularly) aqueous solutions;
b) Solutions for oral use (e.g. syrups, elixirs and tinctures). Critical review for the excipients known to affect absorption or stability of the APIs in GIT should be performed;
c) Powders for reconstitution as solution for parenteral or oral administration;
d) pharmaceutical gases;
e) Otic or ophthalmic or nasal products prepared as aqueous solutions;
f) Topical products prepared as aqueous solutions; and
g) Aqueous solution for nebulizer inhalation products or nasal sprays, intended to be administered with essentially the same device.

The generic product should contain the same APIs in the same molar concentration, and the applicant should demonstrate that the product contains the same or similar excipients in comparable concentrations as the reference listed product. Certain excipients may be different provided that it can be shown (if applicable) that the change(s) in the excipients would not affect the safety and/or efficacy of the pharmaceutical products.

2.3 Bioequivalence studies in humans

In vivo documentation of equivalence is needed when there is a risk that possible difference in bioavailability may result in therapeutic inequivalence. ***Examples are listed below:***

a) Oral immediate-release pharmaceutical products with systemic action when one or more of the following criteria apply:

- Critical use medicines;
- Narrow therapeutic range (efficacy / safety margins), steep dose-response curve;
- Documented evidence for bioavailability problems or bioinequivalence related to the APIs or its formulations (unrelated to dissolution problems); and
- Scientific evidence to suggest that polymorphs of APIs, the excipients and / or the pharmaceutical process used in manufacturing could affect bioequivalence.

b) Modified-release pharmaceutical products designed to act systemically.

c) Fixed-combination products with systemic action, where at least one of the APIs requires an in vivo study.

d) Non-oral, non-parenteral pharmaceutical products designed to act systemically (such as transdermal patches, suppositories, chewing gum, and skin-inserted contraceptives).

e) 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, e.g. comparative clinical or pharmacodynamic, dermatopharmacokinetic studies and / or in vitro studies. In certain cases, measurement of the concentration of the APIs may still be required for safety reasons, i.e. in order to assess unintended systemic absorption.

2.4 Ethical considerations

All research involving human subjects should be conducted in accordance with the ethical principles contained in the current version of The World Medical Association (WMA) Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects,

including respect for persons, maximize benefits and do not be harmful, also according to Egyptian laws and regulations which ever represents the greater protection for subjects.

2.5 Study protocol

The number of studies and study design depend on the physico-chemical characteristics of the substance, its pharmacokinetic properties and proportionality in composition, and should be justified accordingly. In particular it may be necessary to address the linearity of pharmacokinetics, the need for studies both in fed and fasting state, the need for enantioselective analysis and the possibility of waiver for additional strengths.

A bioequivalence study should be carried out in accordance with a protocol agreed upon and signed by the investigator and the sponsor. The protocol should state the aim of the study and the procedures to be used, the reasons for proposing the study to be undertaken in humans, the nature and degree of any known risks, assessment methodology, criteria for acceptance of bioequivalence, the groups from which it is proposed that subjects be selected and the means for ensuring that they are adequately informed before they give their consent.

The investigator is responsible for ensuring that the protocol is strictly followed. Any change(s) required must be agreed on and signed by the investigator and sponsor, and included in the final report, except when necessary to eliminate an apparent immediate hazard or danger to a subject.

The protocol and attachments / appendices should be scientifically and ethically appraised by Institutional Review Board (IRB) and / or ethics committee in accordance with Egyptian drug regulatory authority guidelines and in compliance with Good Clinical Practice (GCP) guidelines.

A signed and dated study protocol together with the study report should be presented to the authority in order to obtain the marketing authorization for the generic product.

2.6 Pilot bioequivalence studies

If the applicant chooses, a pilot study in a small number of subjects can be carried out before proceeding with a full (pivotal) bioequivalence study. This pilot study can be used to validate analytical methodology, assess variability, optimize sample collection time intervals, and provide other information.

2.7 Study design

2.7.1 Types of Experimental Design

2.7.1.1 Nonreplicated Designs

A conventional nonreplicated design, such as the standard two-formulation, two-period, two-sequence crossover design, can be used to generate data where an average or population approach is chosen for bioequivalence comparisons. Under certain circumstances, parallel designs can also be used.

2.7.1.2 Replicated Crossover Designs

Replicated crossover designs can be used irrespective of which approach is selected to establish bioequivalence, although they are not necessary when an average or population approach is used. Replicated crossover designs are critical when an individual bioequivalence approach is used to allow estimation of within-subject variances for the test and reference listed products measures and the subject-by-formulation interaction variance component.

- A four-period, two-sequence, two-formulation design is recommended for replicated bioequivalence studies (TRTR – RTRT). For this design, the same lots of the T and R formulations should be used for the replicated administration. Each period should be separated by an adequate washout period.
- Other replicated crossover designs are possible. For example, a three-period design. For this design, A greater number of subjects would be encouraged for the three-period design

compared to the recommended four-period design to achieve the same statistical power.

In general, for a pharmacokinetic bioequivalence study involving a generic and a reference product, a two-period, single-dose, cross-over study in healthy subjects will suffice. However, in certain circumstances, an alternative, well-established and statistically appropriate study design may be adopted.

- The study design should follow the published guidance, in case of no guidance for API the default design is two-way cross over design. Otherwise the study design should be firstly evaluated.

2.7.2 Single dose studies

A comparative randomized, single dose, two period, two sequence, two treatment, open labelled, two-way ANOVA, crossover study design in healthy adult subjects is the first choice for pharmacokinetic bioequivalence studies. Each subject is given the generic and the reference listed product in randomized order. The study should be conducted under fasting conditions unless the intake of the product is recommended to be only in the fed state.

An adequate wash-out period should follow the administration of each product. The interval (wash-out period) between doses of each formulation should be long enough to permit the elimination of essentially all of the previous dose from the body. The wash-out period should be the same for all subjects and should normally be more than five times the terminal half-life of the APIs.

This period should be extended if active metabolites with longer half-lives are produced and under some other circumstances as for example, if the elimination rate of the product has high variability between subjects. Just prior to administration of treatment during the second study period, blood samples are collected and assayed to determine the concentration of the APIs or metabolites. The minimum wash-out period should be at least seven days.

The adequacy of the wash-out period can be estimated from the pre-dose concentration of the

APIs and should be less than 5% of C_{max}. If the predose value is greater than 5 % of C_{max}, it is recommended to drop the subject from all bioequivalence study evaluations. There is no need for blood samples to be collected for more than 72 hours.

2.7.3 Studies on drugs with long elimination half-lives (i.e., longer than 24 hours)

A single-dose, cross-over, pharmacokinetic bioequivalence study of an orally administered product with a long elimination half-life can be conducted provided an adequate wash-out period is used between administrations of the treatments. The interval between study days should be long enough to permit elimination of essentially all of the previous dose from the body. Ideally, the interval should not be less than five terminal elimination half-lives of the active compound or metabolite, if the latter is measured. Normally the interval between study days should not exceed 3 – 4 weeks. If the crossover study is problematic, a pharmacokinetic bioequivalence study with a parallel design may be more appropriate. For both cross-over and parallel-design studies, sample collection time should be adequate to ensure completion of gastrointestinal transit (approximately 2–3 days) of the pharmaceutical product and absorption of the APIs. Blood sampling up to 72 hours following administration should be carried out, unless shorter periods can be justified. The number of subjects should be derived from statistical calculations, but generally more subjects are needed for a parallel study design than for a cross-over study design.

For drugs that demonstrate low intrasubject variability in distribution and clearance, it is recommended to use an AUC truncated at 72 hours (AUC_{0-72}) in place of AUC_{0-t} or $AUC_{0-\infty}$. For drugs demonstrating high intrasubject variability in distribution and clearance, AUC truncation should not be used.

2.7.4 Study designs in patients

For APIs that are very potent or too toxic to administer in the usual dose to healthy subjects

(e.g. because of the potential for serious adverse events, or the trial necessitates a high dose) it is recommended that the study be conducted using the APIs at a lower strength. However, if the pharmacokinetics are not proportional or if the solubility of the APIs is an issue, it will not be appropriate to extrapolate the bioequivalence results of the studies at lower strength to those at higher strengths.

For APIs that show unacceptable pharmacological effects in healthy subjects, a multiple dose, steady-state, cross-over study in patients or a parallel group design study in patients may be required. The alternative study design in patients should be justified by the sponsor who should attempt to recruit patients whose disease process is stable for the duration of the pharmacokinetic bioequivalence study.

2.7.5 Multiple–dose studies

In certain situations, multiple-dose studies may be considered appropriate. Multiple-dose studies in patients are most useful in cases where the medicine being studied is considered to be too potent and / or too toxic to be administered to healthy subjects, even in single doses. In this case, a multiple-dose cross-over study in patients may be performed without interrupting therapy. Evaluation of such studies can be based on either pharmacokinetic or pharmacodynamic end-points, although it is likely that using pharmacodynamic end-points would require a larger number of patients than pharmacokinetic end-points. The dosage regimen used in multiple-dose studies should follow the usual dosage recommendations.

Other situations in which multiple-dose studies may be appropriate are as follows:

- Drugs that exhibit non-linear pharmacokinetics at steady state (e.g. saturable metabolism, active secretion);
- Extended-release dosage forms with a tendency to accumulation (in addition to a single-dose study);
- Cases where the assay sensitivity is too low to adequately characterize the pharmacokinetic

profile after a single dose, and where the concentrations at steady state are sufficiently high to be reliably measured.

However, given that a multiple dose study is less sensitive in detecting differences in C_{max}, this will only be acceptable if the applicant can adequately justify that the sensitivity of the analytical method cannot be improved and that it is not possible to reliably measure the parent compound after single dose administration taking into account also the option of using a supra-therapeutic dose in the bioequivalence study. But due to the recent development in the bioanalytical methodology, it is unusual that parent drug cannot be measured accurately and precisely. Hence, use of a multiple dose study instead of a single dose study, due to limited sensitivity of the analytical method, will only be accepted in exceptional cases.

In contrast to single dose studies, where the two treatment period have to be separated by a sufficiently long washout period without any treatment, such a washout period can be skipped in favor of a direct switch at steady state after the first treatment period. Thus, in steady-state studies the wash-out of the last dose of the previous treatment given in period I can overlap with the build-up of the second treatment given in period II, provided the build-up period is sufficiently long (at least five times the terminal half-life). Appropriate dosage administration and sampling should be carried out to document for the attainment of a steady state.

2.7.6 Studies involving modified –release products

Modified-release products include extended-release products and delayed-release products. Extended-release products are variously known as controlled-release, prolonged-release and sustained-release products.

To establish the bioequivalence of modified-release products, a single-dose, non-replicate cross-over, fasting study comparing the highest strength of the generic and the reference listed product should be performed. Single dose studies are preferred to multiple-dose studies as single-dose studies are considered to provide more sensitive measurements of the

release of APIs from the pharmaceutical product into the systemic circulation. Multiple-dose studies may need to be considered (in addition to a single dose study) for extended-release dosage forms with a tendency to accumulate.

The reference listed product in this study should be a pharmaceutically equivalent modified-release product. The pharmacokinetic bioequivalence criteria for modified-release products are basically the same as for conventional-release dosage forms.

A concern with modified-release products is the possibility that food cause dumping, Therefore, a pharmacokinetic bioequivalence study under fed conditions is generally required, in addition to the study under fasting state, same design for both studies must be the same; Otherwise the study design should be firstly evaluated for orally administered modified-release pharmaceutical products. Omission of either the fed or fasting study should be justified by the applicant. A fed-state pharmacokinetic bioequivalence study should be conducted after the administration of an appropriate standardized high fat meal at a specified time (usually not more than 30 minutes) before taking the medicine. The composition and caloric breakdown of the test meal should be provided in the study protocol and report.

2.8 Subjects

2.8.1 Selection of subjects

Pharmacokinetic bioequivalence studies should generally be performed with healthy subjects. Clear criteria for inclusion and exclusion should be stated in the study protocol. If the pharmaceutical product is intended for use in both genders, the sponsor may wish to include both males and females in the study. *Female subjects that are pregnant or lactating should not be included. Male contraception barrier methods or abstinence) is recommended if drugs (have any embryo-fetal toxicity and pose transferability issues to female partners of reproductive potential*. Confirmation should be obtained by urine and / or blood tests just before

administration of the first and last doses of the product under study. Generally, subjects should be between the ages of 18 and 55 years, and their weight should be within the normal range according to accepted life tables. The subjects should preferably have a Body Mass Index between 18.5 and 30 kg/m^2. They should also have no history of alcohol or drug abuse problems and should preferably be non-smokers.

The subjects should be screened for their suitability using standard laboratory tests, a medical history, and a physical examination. If necessary, special medical investigations may be carried out before and during studies depending on the pharmacology of the individual APIs being investigated, e.g. an electrocardiogram if the APIs has a cardiac effect.

The ability of the subjects to understand and comply with the study protocol has to be assessed. Subjects who are being or have previously been treated for any gastrointestinal problems, convulsive, depressive or hepatic disorders, and in whom there is a risk of a re-currence during the study period, should be excluded. If the aim of the bioequivalence study is to address specific questions (e.g. bioequivalence in a special population) the selection criteria should be adjusted accordingly.

2.8.2 Genetic phenotyping

Phenotyping of subjects can be considered for studies of drugs that show phenotype linked metabolism and for which a parallel group design is to be used, because it allows fast and slow metabolizers to be evenly distributed in the two groups of subjects. Phenotyping could also be important for safety reasons, determination of sampling times and wash-out periods in cross-over design studies.

2.8.3 Number of subjects

The number of subjects to be recruited for the study should be estimated by considering the standards that must be met and calculated by appropriate statistical methods as the following:

- The error variance (coefficient of variation) associated with the primary parameters to be studied, as estimated from a pilot experiment, from previous studies or from published data;
- The significance level desired (5%);
- The statistical power desired;
- The mean deviation from the comparator product compatible with bioequivalence and with safety and efficacy;
- The need for the 90% confidence interval around the geometric mean ratio to be within bioequivalence limits, normally 80‑125%, for log-transformed data.

The number of subjects recruited should always be justified by the sample-size calculation provided in the study protocol. The total number of subjects in a study should be sufficient to provide adequate statistical power for bioequivalence demonstration. A minimum of 24 subjects is required (If otherwise, it should be justified).

2.8.4 Drop-outs and withdrawals

Sponsors should select a sufficient number of study subjects to allow for possible drop-outs or withdrawals. Because replacement of subjects during the study could complicate the statistic model and analysis, drop-outs generally should not be replaced. Reasons for withdrawal (e.g. adverse drug reaction or personal reasons) must be reported.

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 appropriate to recruit into the study more subjects than the sample-size calculation requires. These subjects are designated as extras.

If the bioequivalence study was performed with the appropriate number of subjects but bioequivalence cannot be demonstrated because 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, provided this eventuality was anticipated and provided for in the study protocol. Combining data is acceptable only in the case that 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.

Ideally, all treated subjects should be included in the statistical analysis. However, subjects in a crossover study who do not provide evaluable data for both of the test and reference listed products (or who fail to provide evaluable data for the single period in a parallel group study) should not be included.

The data from all treated subjects should be treated equally. It is not acceptable to have a protocol which specifies that "spare" subjects will be included in the analysis only if needed as replacements for other subjects who have been excluded. It should be planned that all treated subjects should be included in the analysis, even if there are no drop-outs.

In studies with more than two treatment arms (e.g. a three-period study including two references or a four-period study including test and reference in fed and fasted states), the analysis for each comparison should be conducted excluding the data from the treatments that are not relevant for the comparison in question.

2.8.5 Reasons for exclusion

Unbiased assessment of results from randomised studies requires that all subjects are observed and treated according to the same rules. These rules should be independent from treatment or outcome. In consequence, the decision to exclude a subject from the statistical analysis must be made before bioanalysis.

Examples of reasons to exclude the results from a subject in a particular period are events such as vomiting and diarrhea which could render the plasma concentration-time profile unreliable. In exceptional cases, the use of concomitant medication could be a reason for excluding a subject.

It is recommended that data from subjects who experience emesis during the course of a bioequivalence study for immediate release products be deleted from statistical analysis if vomiting occurs at or before 2 times median T_{max}. For modified release products, it is recommended to delete data from the analysis if a subject vomits during a period of time less than or equal to the dosing interval stated in the labeling of the product.

Exclusion of data cannot be accepted on the basis of statistical analysis or for pharmacokinetic reasons alone, because it is impossible to distinguish the formulation effects from other effects influencing the pharmacokinetics. ***The exceptions to this are:***

1) A subject with lack of any measurable concentrations or only very low plasma concentrations for reference listed medicinal product. A subject is considered to have very low plasma concentrations if its AUC is less than 5% of reference listed medicinal product geometric mean AUC (which should be calculated without inclusion of data from the outlying subject). The exclusion of data due to this reason will only be accepted in exceptional cases and may question the validity of the study.

2) Subjects with non-zero baseline concentrations > 5% of C_{max}. Such data should be excluded from bioequivalence calculation (carry-over effects).

The above can, for immediate release formulations, be the result of subject non-compliance and an insufficient wash-out period, respectively, and should as far as possible be avoided by mouth check of subjects after intake of study medication to ensure the subjects have swallowed the study medication and by designing the study with a sufficient wash-out period. The samples from subjects excluded from the statistical analysis should still be assayed and the results listed. As stated, (AUC_{0-t}) should cover at least 80% of ($AUC_{0-\infty}$). Subjects should not be excluded from the statistical analysis if (AUC_{0-t}) covers less than 80% of ($AUC_{0-\infty}$), but if the percentage is less than 80% in more than 20% of the observations then the validity of the study may need to be

discussed. This does not apply if the sampling period is 72 h or more and (AUC_{0-72}) is used instead of (AUC_{0-t}).

2.8.6 Monitoring the health of subjects during the study

During the study, the health of subjects should be monitored so that onset of side-effects, toxicity, or any intercurrent disease may be recorded, and appropriate measures taken.

The incidence, severity, and duration of any adverse reactions and side-effects observed during the study must be reported. The probability that an adverse effect is drug-induced is to be judged by the investigator. Health monitoring before, during and after the study and taking the appropriate measures including any further laboratory tests must be carried out under the supervision of a qualified medical practitioner licensed in the jurisdiction in which the study is conducted.

2.9 Two-stage sequential design

In some situations reliable information concerning the expected variability in the parameters to be estimated may not be available. In such situations a two-stage sequential study design can be employed after submission of scientific rationale such that an accurate estimate of the variability can be determined in the first stage of the study. The number of subjects employed in the first stage is generally based on the most likely intra-subject variance estimate with some added subjects to compensate for dropouts.

The analysis undertaken at the end of the first stage is treated as an interim analysis. If bioequivalence is proven at this point the study can be terminated. If bioequivalence is not proven at the end of the first stage, the second stage is conducted employing an appropriate number of additional subjects as determined based on the variance estimates and point estimate calculated from the stage 1 data. At the end of the second stage, the results from both groups combined are used in the final analysis. In order to use a two-stage design, adjustments

must be made to protect the overall Type 1 error rate and maintain it at 5%. To do this, both the interim and final analyses must be conducted at adjusted levels of significance with the confidence intervals calculated using the adjusted values.

It is recommended that the same alpha for both stages be employed. This gives an alpha of 0.0294 for this case, however, the amount of alpha to be spent at the time of the interim analysis can be set at the study designer's discretion. For example, the first stage may be planned as an analysis where no alpha is spent in the interim analysis since the objective of the interim analysis is to obtain information on the point estimate difference and variability and where all the alpha is spent in the final analysis with the conventional 90% confidence interval. In this case no test against the acceptance criteria is made during the interim analysis and bioequivalence cannot be proven at that point.

The proposed statistical plan must be clearly defined in the study protocol, including the adjusted significance level that is to be employed during each analysis (*as directed in the below mentioned flow charts*).

The plan to use a two-stage sequential approach must be pre-specified in the protocol along with the adjusted significance levels to be used for each of the analyses. A factor for stage should be included in the ANOVA model for the final analysis of the combined data from the two stages. This approach can be employed in both cross-over and parallel study designs.

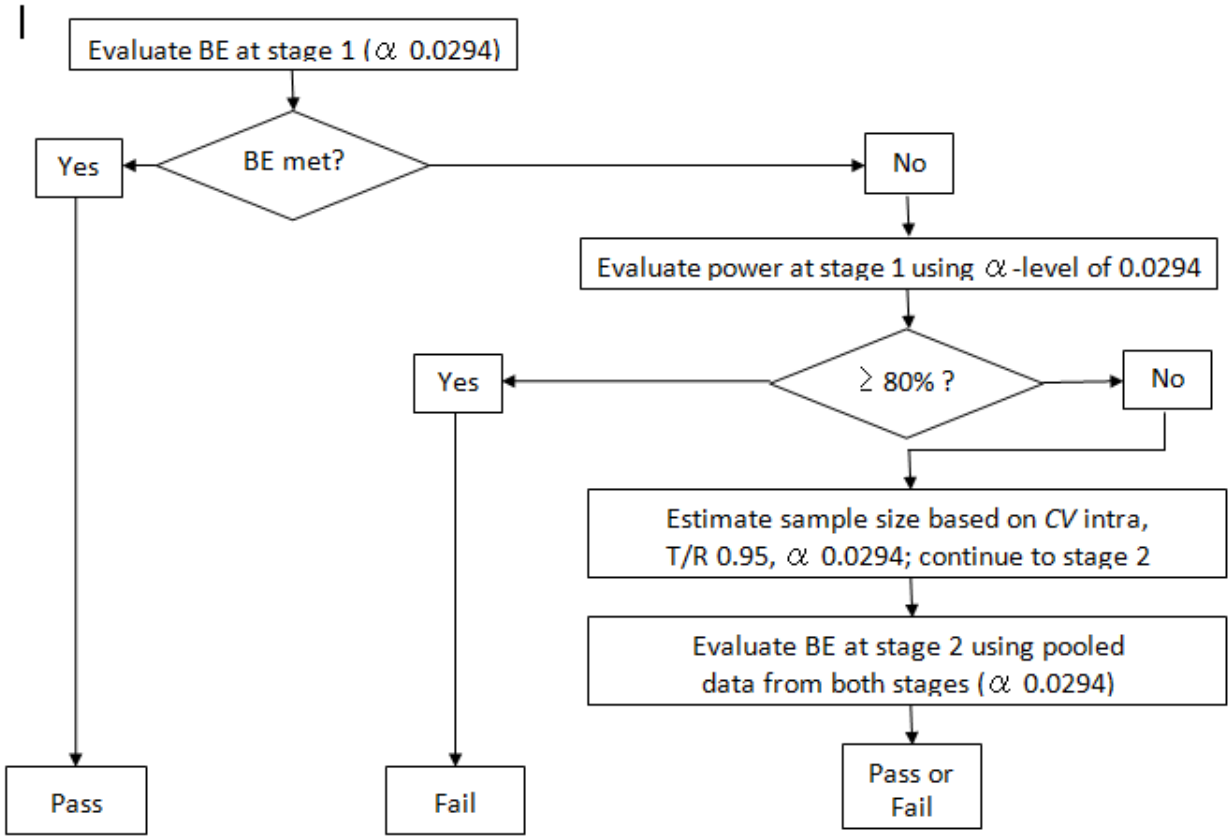
In addition, to account for the fact that the periods in the first stage are different from the periods in the second stage, a term for period within stage is required. Therefore, the expected ANOVA model for analysis of the combined data from a two-stage design would have the following terms: stage, sequence, sequence*stage, subject (sequence*stage), period (stage), formulation. To fit this model it is necessary to have in each stage at least one subject in each sequence – so a minimum of two subjects in each stage of the study - but more if both happen to be randomised to the same sequence.

A model which also includes a term for a formulation*stage interaction would give equal weight to the two stages, even if the number of subjects in each stage is very different. The results can be very misleading hence such a model is not considered acceptable. Furthermore, this model assumes that the formulation effect is truly different in each stage. If such an assumption were true there is no single formulation effect that can be applied to the general population, and the estimate from the study has no real meaning.

This approach can't be done only for multi-arm designs comparing more than two formulations, replicate studies (partial and full replicate study design).

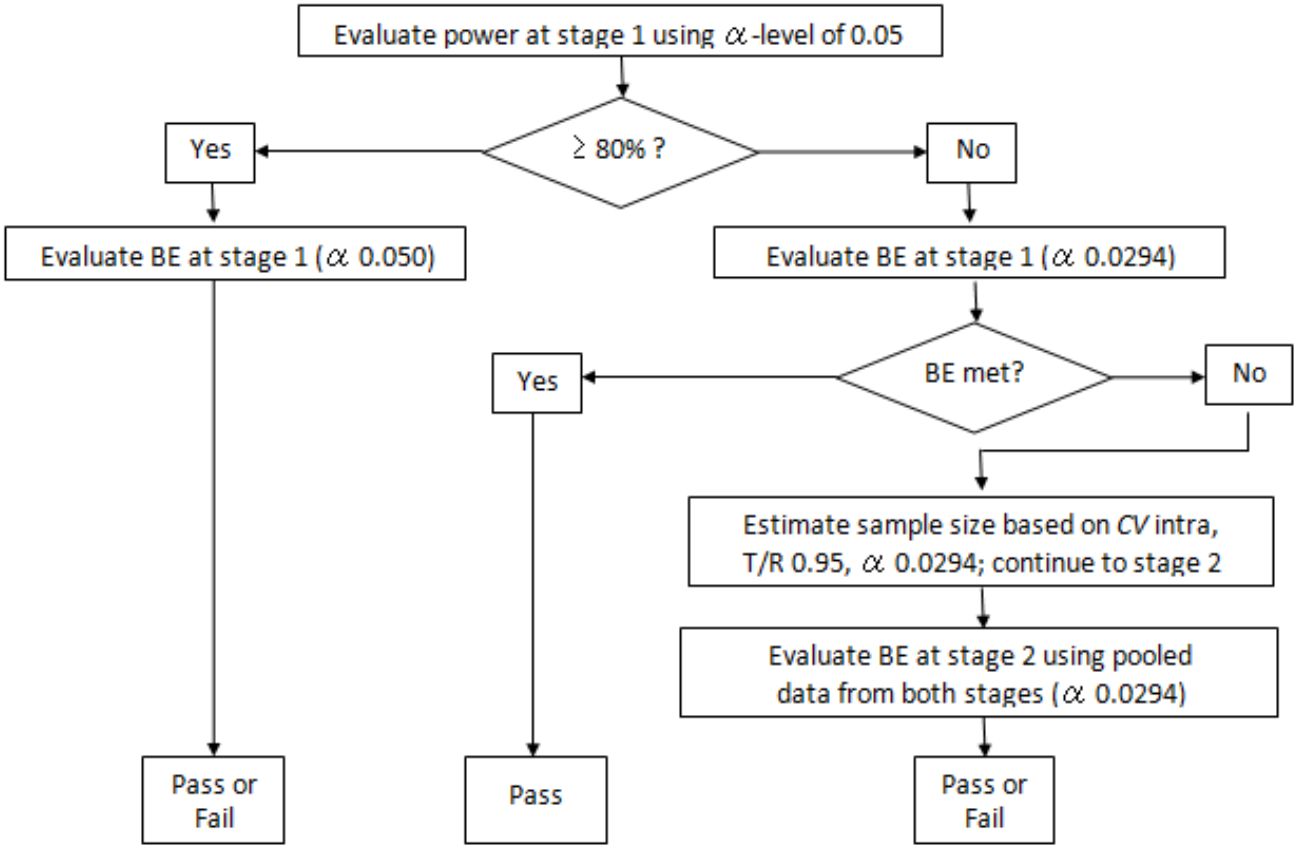
**** Flow chart (1):***

"Potvin et al. (Method B)"



**** Flow chart (2):***

"Potvin et al. (Method C)"



2.10 Fasting or/and fed conditions

In general, a bioequivalence study should be conducted under fasting conditions as this is considered to be the most sensitive condition to detect a potential difference between formulations. Therefore, for the majority of these drug products, BE may be demonstrated in a single study conducted under fasting conditions.

High-risk products (for immediate release products)

High-risk products are those where the drug substance characteristics in combination with the complexity of the formulation design or manufacturing process led to an increased likelihood that in vivo performance will be impacted differently by varying gastrointestinal (GI) conditions between the fasted and fed conditions. For these drug products, performance differences related to differences in formulation and/or manufacturing process may not be detected with a single BE study, i.e., results from a fasting BE study may not be extrapolated to predict fed BE study outcome or vice versa, thus both fasting and fed BE studies should be conducted. For example, some drug products containing low solubility drug substances (as defined by the BCS low solubility criterion described in ICH M9) have complex formulation and/or manufacturing methods, such as solid dispersions, microemulsions, co-processed drug substances, lipid-based formulations, nanotechnologies, or other specialized technologies, to ensure sufficient solubility of the drug substance and dissolution of the drug substance from the drug product or to manage the impact of food. For these high-risk products, BE studies should be conducted under both fasting and fed conditions, irrespective of the drug product labelling with regard to food intake, if safety permits.

<u>Considerations for study design</u>

The design of a BE study with regard to the use of fasting and/or fed conditions depends on the dosing instructions of the comparator product as well as the properties of the drug substance and drug product formulation. A rationale should be provided for the selection of the type of BE study (ies) (fasting or fed or both) and meal type, e.g., fat and calorie content, based on the

understanding of the comparator product and the test product, e.g., high- or non-high-risk.

However, safety-related aspects need to be considered when selecting the appropriate condition for a BE study regarding food intake. If administration of a single dose of the drug product under either fasting or fed conditions raises safety concerns, the BE study should be conducted under the condition with less safety concerns.

If safety permits, for non-high-risk products, the following is recommended:

▪ For a drug product that is labelled to be taken only under fasting conditions or can be taken under fasting or fed conditions, i.e., without regard to food, a single BE study conducted under fasting conditions is recommended to demonstrate BE.

▪ For a drug product that is labelled to be taken only with food due to PK reasons, i.e., enhancing absorption or reducing variability, a single BE study conducted under fed conditions is recommended to demonstrate BE.

▪ For a drug product that is labelled to be taken only with food due to tolerability reasons, e.g., stomach irritation or other non-PK reasons, a single BE study conducted under either fasting or fed conditions is acceptable to demonstrate BE.

- High-risk products, BE studies should be conducted under both fasting and fed conditions, irrespective of the drug product labelling with regard to food intake, if safety permits.

In cases where information is required in both the fed and fasted states, it is acceptable to conduct either two separate two-way cross-over studies or a four-way cross-over study.

If BE studies are conducted under both fasting and fed conditions, *i.e.,* for high-risk products, the BE study conducted under fed conditions should employ a meal that has the potential to cause the greatest effect on GI physiology. The meal should be a high-fat (approximately 50% of total caloric content of the meal) and high-calorie (approximately 900 to 1000 kcal) meal, which should derive approximately 150, 250, and 500-600 kcal from protein, carbohydrate, and fat, respectively. It is recognized that there may be situations where it is appropriate to administer a pre-dose meal with a different caloric/fat content from these recommendations, *e.g.,* for studies performed

in patient populations who cannot tolerate the recommended meal composition.

If, however, only one BE study conducted under fed conditions is needed for a non-high-risk product, either a high-fat, high-calorie meal or a low-fat, low-calorie meal, *e.g.,* a meal of approximately 500 kcal with approximately 25% of calories from fat, may be administered. If the type of meal to be consumed at the time of drug product administration is clearly specified in the comparator product labelling, then this meal should be employed in the BE study.

In these cases, the timing of administration of the drug product in relation to food intake is recommended to be according to the SmPC of the reference listed product. If no specific recommendation is given in the reference listed SmPC, it is recommended that subjects should start the meal 30 minutes prior to administration of the drug product and eat this meal within 30 minutes.

2.11 Study standardization

Standardization of study conditions is important to minimize the magnitude of variability other than in the pharmaceutical products. 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 standardised. Standardization should cover exercise; diet; fluid intake; posture; and the restriction of the intake of alcohol, caffeine, food, drinks, which may interact with circulatory, gastrointestinal, hepatic or renal function (e.g. alcoholic drinks or certain fruit juices such as grapefruit juice), and concomitant medicines for a specified time period before and during the study.

Subjects should not take any other medicine, alcoholic beverages or over-the-counter (OTC) medicines and supplements (including herbal remedies) for an appropriate interval either before or during the study. In the event of emergency, the use of any non-study medicine must be reported (dose and time of administration), and possible effects on the study outcome must be addressed. In rare cases, the use of a concomitant medication is needed for all subjects for

safety or tolerability reasons (e.g. opioid antagonists, anti-emetics). In that scenario, the risk for a potential interaction or bioanalytical interference affecting the results must be addressed.

Medicinal products that according to the reference SmPC are to be used explicitly in combination with another product (e.g. certain protease inhibitors in combination with ritonavir) may be studied either as the approved combination or without the product recommended to be administered concomitantly.

Physical activity and posture should be standardized as far as possible to limit their effects on gastrointestinal blood flow and motility. The same pattern of posture and activity should be maintained for each day of the study. The time of day at which the study drug is to be administered should be specified.

Packaging, labelling and administration of the products to the subjects should therefore be documented in detail. This documentation should include all precautions taken to avoid and identify potential dosing mistakes. The use of labels with a tear-off portion is recommended.

Medicines are usually given after an overnight fast of at least 10 hours, and volunteers are allowed free access to water. On the morning of the study, no water is allowed during the hour prior to drug administration. The dose should be taken with a standard volume of water (*150-250) ml*. *One* hour after drug administration water is again permitted ad libitum. A standard meal is usually provided four hours after drug administration. All meals should be standardized and the composition stated in the study protocol and report. Some medicines are normally given with food to reduce gastrointestinal side effects; in certain cases, coadministration with food increases bioavailability of orally administered preparations.

If the label of a modified release reference listed product states that the product can be administered sprinkled in soft foods, it is recommended for applicants to conduct an additional bioequivalence study. For each treatment arm, the product should be sprinkled on one of the soft foods mentioned in the labelling of the reference listed product. Aside from administration

in the soft food, this additional study should follow the recommendations for the fasting bioequivalence study.

2.12 Pharmaceutical products under test

2.12.1 Generic product

The generic pharmaceutical product used in the bioequivalence studies for registration purposes should be identical to the projected commercial pharmaceutical product. Therefore, not only the composition and quality characteristics (including stability), but also the manufacturing methods (including equipment and procedures) should be the same as those to be used in the future routine production runs. Test products must be manufactured under Good Manufacturing Practice (GMP) regulations. Batch-control results of the generic product, the lot numbers and expiry dates of both generic and reference listed products should be stated. Samples should ideally be taken from production batches. It is recommended that potency and in vitro dissolution characteristics of the generic and the reference listed pharmaceutical products be ascertained prior to performance of an bioequivalence study. Content of the APIs of the reference listed product should be close to the label claim, and the difference between two products should preferably be not more than ± 5%. The minimum no. of dosage units to be tested for test reference listed products should be at least ten units of each unless otherwise specified in the drug monograph.

2.12.2 Reference listed product

The reference listed product (for which marketing authorization has been granted, on the basis of quality, safety and efficacy) should be selected based on the following options (taking into consideration to choose a reference listed product with the same dosage form as the generic product if available on the market):

(1) The product is mentioned in the **FDA - Orange Book** as a Reference Listed Product **(RLD) or** Reference Standard **(RS)**;

URL: http://www.accessdata.fda.gov/scripts/cder/ob/default.cfm

(2) The product is mentioned in the **FDA - Orange Book** as a New Drug Application **(NDA)**;

URL: http://www.accessdata.fda.gov/scripts/cder/daf

(3) The product is mentioned in **ANSM – Affssaps** (Regulatory Authority of France) under: "Décisions portant modification au répertoire des groupes Génériques "as a Reference Product **(R)**;

URL: http://ansm.sante.fr/Mediatheque/Publications/Listes-et-repertoires-Repertoire-des-medicaments-generiques

(4) The product is mentioned in the **MHRA – Public Assessment Reports (PAR)** as a reference product;

URL(s): https://www.gov.uk/government/organisations/medicines-and-healthcare-products-regulatory-agency & http://www.mhra.gov.uk/public-assessment-reports/index.htm

*This can be applied for **Public Assessment Reports (PAR)** from any reference countries' list approved by Egyptian Drug Authority (EDA).

(5) The product is mentioned as a one of **WHO Comparators' List**;

Link: http://apps.who.int/medicinedocs/documents/s19641en/s19641en.pdf

(6) The product is considered as **European reference medicinal product**, where its marketing authorisation has been granted in the EU in accordance with Articles 8(3), 10a, 10b or 10c of Directive 2001/83/EC according to **Volume 2A, Procedures For Marketing Authorisation - Chapter 1, Marketing Authorisation, July 2015**;

Link: *http://ec.europa.eu/health/files/eudralex/vol-2/a/vol2a_chap1_201507.pdf*

• The Chosen reference product from the above mentioned options can be either brought from one of the reference countries' lists approved by Egyptian Drug Authority (EDA))

or from the Egyptian market if registered in Egypt as **(Import or Bulk)** from the sponsor that had licensed the reference listed product,

or if registered & manufactured locally in Egypt **(Under License "Under Authority")** from the sponsor that had licensed the reference listed product;

• In the case that no reference listed product can be identified within the above mentioned context, the choice of the reference listed product must be made carefully and comprehensively justified by the applicant, and a request must be submitted by the sponsor to EDA before using the chosen reference product.

Additionally, “well selected reference” must conform to compendial quality standards, where these exist. The country of origin of the reference product should be reported together with lot number and expiry date.

2.13 Study conduct

2.13.1 Selection of dose

In bioequivalence studies the molar equivalent dose of generic and reference listed product must be used. Generally the marketed strength with the greatest sensitivity to bioequivalence assessment should be administered as a single unit. This will usually be the highest marketed strength.

A higher dose (i.e. more than one dosage unit) may be employed when analytical difficulties exist. In this case the total single dose should not exceed the maximal daily dose of the dosage regimen. In certain cases a study performed with a lower strength can be considered acceptable when this lower strength is chosen for reasons of safety / tolerability or where the drug substance is highly soluble, ***if the following conditions are met:***

- Linear elimination pharmacokinetics has been documented over the therapeutic dose range;
- The higher strengths of the test and reference listed products are proportionally similar to their corresponding lower strength;

- Comparative dissolution testing on the higher strength of the test and reference listed products has been submitted and found to be acceptable.

2.13.2 Sampling times

The exact timing for sample collection depends on the nature of the drug and the rate of input from the administered dosage form. It is recommended to record the actual clock time when samples are drawn as well as the elapsed time related to drug administration. Blood samples should be taken at a frequency sufficient for assessing C_{max}, AUC and other parameters. Sampling points should include a pre-dose sample, at least 2 points before C_{max}, 2 points around C_{max} and 3 – 4 points during the elimination phase.

For most medicines the number of samples necessary will be higher to compensate for between-subject differences in absorption and elimination rate and thus enable accurate determination of the maximum concentration of the APIs in the blood (C_{max}) and terminal elimination rate constant (K_{el}) in all subjects. It is recommended to collect 12 to 18 samples, including a predose sample, per subject, per dose. This sampling should continue for at least three or more terminal elimination half-lives of the drug in order to reliably estimate the terminal rate constant which is needed for a reliable estimate of ($AUC_{0-\infty}$).

Generally, sampling should continue for long enough to ensure that 80% of the ($AUC_{0-\infty}$) can be accrued, but it is not necessary to sample for more than 72 hours. In certain cases the use of partial "truncated" AUC (AUC_{0-72}) could be used instead of the area extrapolated to infinity. This approach is of great value for products of APIs with a long $T_{1/2}$ and in cases where low concentration 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.

The first point of a concentration-time curve in a bioequivalence study, based on blood and /

or plasma measurements, is sometimes the highest point, which raises questions of bias in the estimation of C_{max} because of insufficient early sampling times. A carefully conducted pilot study can enable an applicant to avoid this problem. In the main bioequivalence study, collection of blood samples at an early time point, between 5 and 15 minutes after dosing, followed by additional sample collections (e.g., two to five) in the first hour after dosing is usually sufficient to assess peak drug concentrations. Failure to include early (5-15 minute) sampling times leading to first time-point C_{max} values may result in not considering the data for affected subjects from the analysis.

In multiple-dose studies, the pre-dose sample should be taken immediately before dosing (within 5 minutes) and the last sample is recommended to be taken within 10 minutes of the nominal time for the dosage interval to ensure an accurate determination of ($AUC_{0-\tau}$).

If urine is used as the biological sampling fluid, urine should normally be collected over no less than three times the terminal elimination half-life. However, in line with the recommendations on plasma sampling, urine does not need to be collected for more than 72 h. If rate of excretion is to be determined, the collection intervals need to be as short as feasible during the absorption phase.

2.13.3 Sample fluids and their collection

Under normal circumstances blood should be the biological fluid sampled to measure the concentrations of the APIs. In most cases the APIs or its metabolites are measured in serum or plasma. If the APIs is excreted predominantly unchanged in the urine, urine can be sampled.

The volume of each sample must be measured at the study center, where possible immediately after collection, and included in the report. The number of samples should be sufficient to allow the estimation of pharmacokinetic parameters. However, in most cases the exclusive use of urine excretion data should be avoided as this does not allow estimation of the T_{max} and the maximum concentration.

In addition, the use of urinary excretion data as a surrogate for a plasma concentration may be acceptable in determining the extent of exposure where it is not possible to reliably measure the plasma concentration-time profile of parent compound.

However, the use of urinary data has to be carefully justified when used to estimate peak exposure. If a reliable plasma C_{max} can be determined, this should be combined with urinary data on the extent of exposure for assessing bioequivalence. When using urinary data, the applicant should present any available data supporting that urinary excretion will reflect plasma exposure.

Blood samples should be processed and stored under conditions that have been shown not to cause degradation of the analytes. This can be proven by analyzing duplicate quality control samples during the analytical period.

Quality control samples must be prepared in the fluid of interest (e.g. plasma), including concentrations at least at the low, middle and high segments of the calibration range.

The quality control samples must be stored with the study samples and analyzed with each set of study samples for each analytical run. The sample collection methodology must be specified in the study protocol.

2.14 Parameters to be assessed

2.14.1 For single-dose studies, the following parameters should be measured or calculated

- **AUC_{0-t} :** is the area under the plasma / serum / blood concentration–time curve from time zero to time t, where t is the last sampling time point with a measurable concentration of the APIs in the individual formulation tested. The method of calculating AUC-values should be specified. In general AUC should be calculated using the linear / log trapezoidal integration method. The exclusive use compartmental-based parameters is not recommended;
- **C_{max}:** is the maximum or peak concentration observed representing peak exposure of APIs (or metabolite)

in plasma, serum or whole blood.

- AUC_{0-t} and C_{max} are considered to be the most relevant parameters for assessment of bioequivalence.

In addition it is recommended that the following parameters be estimated:

• **$AUC_{0-\infty}$:** is the area under the plasma / serum / blood concentration–time curve from time zero to time infinity representing total exposure, where $AUC_{0-\infty} = AUC_{0-t} + C_{last}/k_{el}$; C_{last} is the last measurable drug concentration and k_{el} is the elimination rate constant calculated according to an appropriate method;

• **T_{max}:** is the time after administration of the drug at which C_{max} is observed.

For additional information, the following elimination parameters can be calculated:

• **$T_{1/2}$:** is the plasma, serum or whole blood half-life;

• **K_{el}:** is the elimination rate constant.

2.14.2 For steady-state studies the following parameters can be calculated

• **$AUC_{0-\tau}$:** is the area under the plasma / serum / blood concentration–time curve over one dosing interval (τ) at steady-state;

• **$C_{max,ss}$:** is the maximum plasma concentration at steady state;

• **$C_{min,ss}$:** is the concentration at the end of a dosing interval;

• **Peak-trough fluctuation:** is the percentage difference between C_{max} and $C_{min}/C_{average,ss}$;

• **$T_{max,ss}$:** Time until $C_{max,ss}$ is reached.

2.14.3 When urine samples are used

- Cumulative urinary excretion **(Ae0-t)** and maximum urinary excretion rate **(R_{max})** are employed instead of AUC_{0-t} and C_{max} respectively.

- Non-compartmental methods should be used for determination of pharmacokinetic parameters in bioequivalence studies. The use of compartmental methods for the estimation of parameters is not acceptable.

2.15 Parent compound or metabolites

Generally, evaluation of pharmacokinetic bioequivalence will be based upon the measured

concentrations of the parent drug released from the dosage form rather than the metabolite. The reason for this is that C_{max} of a parent compound is usually more sensitive to detect differences between formulations in absorption rate than C_{max} of a metabolite. It is important to state a priority in the study protocol which chemical entities (pro-drug, drug (APIs) or metabolite) will be analyzed in the samples.

In some situations it may be necessary to measure metabolite concentrations rather than those of the parent drug for instance:

- The measurement of concentrations of therapeutically active metabolite is acceptable if the substance studied is a pro-drug.
- Measurement of a metabolite may be preferred when concentrations of the parent drug are too low - and be quickly eliminated - to allow reliable analytical measurement in blood, plasma or serum for an adequate length of time, or when the parent compound is unstable in the biological matrix. This can only be considered if the applicant can adequately justify that the sensitivity of the analytical method for measurement of the parent compound cannot be improved and that it is not possible to reliably measure the parent compound after single dose administration taking into account also the option of using a higher single dose in the bioequivalence study.

In certain cases, data for both the parent compound and its active metabolite(s) may be required.

In addition, primary metabolite(s), formed directly from the parent compound, should be measured if they are both: (1) formed substantially through presystemic metabolism (first-pass, gut wall, or gut lumen metabolism) and (2) contribute significantly to the safety and efficacy of the product.

When measuring the active metabolites, wash-out period and sampling times may need to

be adjusted to enable adequate characterization of the pharmacokinetic profile of the metabolite, also the applicant should present any available data supporting the view that the metabolite exposure will reflect parent drug and that the metabolite formation is not saturated at therapeutic doses.

2.16 Measurement of individual enantiomers

A non-stereoselective assay is currently acceptable for most pharmacokinetic bioequivalence studies. However, it is recommended to use an achiral assay to measure the racemate.

In addition, it is only recommended to measure individual enantiomers in bioequivalence studies when all of the following conditions have been met: (1) the enantiomers exhibit different pharmacodynamic characteristics, (2) the enantiomers exhibit different pharmacokinetic characteristics, (3) primary efficacy and safety activity reside with the minor enantiomer, and (4) 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 where all of these conditions are met, it is recommended for applicants to apply bioequivalence analysis to the enantiomers separately.

If one enantiomer is pharmacologically active and the other is inactive or has a low contribution to activity, it is sufficient to demonstrate bioequivalence for the active enantiomer.

2.17 Endogenous Compounds

Endogenous compounds are drugs that are already present in the body either because the body produces them or they are present in the normal diet. Because these compounds are identical to the drug that is being administered, determining the amount of drug released from the dosage form and absorbed by each subject can be difficult. It is recommended that applicants measure and approximate the baseline endogenous levels in blood (plasma) or *urine* and subtract these levels from the total concentrations measured from each subject after the drug product has been administered. In this way, you can achieve an estimate of the actual drug availability from the

drug product. Depending on whether the endogenous compound is naturally produced by the body or is present in the diet, ***the recommended approaches for determining bioequivalence differ as follows:***

- ***When the body produces the compound,*** it is recommended to measure multiple baseline concentrations (2 – 3 samples) in the time period before administration of the study drug. In other cases, sampling at regular intervals throughout 1-2 day(s) prior to administration may be necessary in order to account for fluctuations in the endogenous baseline due to circadian rhythms and subtract the baseline in an appropriate manner consistent with the pharmacokinetic properties of the drug. Administration of supra-therapeutic doses can be considered in bioequivalence studies of endogenous drugs, provided that the dose is well tolerated, so that the additional concentrations over baseline provided by the treatment may be reliably determined. The exact method for baseline correction should be pre-specified and justified in the study protocol. In general, the standard subtractive baseline correction method, meaning either subtraction of the mean of individual endogenous pre-dose concentrations or subtraction of the individual endogenous predose AUC, is preferred. In rare cases where substantial increases over baseline endogenous levels are seen, baseline correction may not be needed.

When there is dietary intake of the compound:

- It is recommended to strictly control the intake both before and during the study. Subjects should be housed before the study and served standardized meals containing an amount of the compound similar to that in the meals to be served on the pharmacokinetic sampling day.

For both of the approaches above:

- It is recommended that you determine baseline concentrations for each dosing period that are period specific. If a baseline correction results in a negative plasma concentration value, the value should be set equal to 0 before calculating the baseline-corrected AUC. Pharmacokinetic and statistical analysis should be performed on both uncorrected and corrected data. Determination of bioequivalence should be based on the baseline-corrected data.

If a separation in exposure following administration of different doses of a particular endogenous

substance has not been previously established this should be demonstrated, either in a pilot study or as part of the pivotal bioequivalence study using different doses of the reference listed formulation, in order to ensure that the dose used for the bioequivalence comparison is sensitive to detect potential differences between formulations.

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

In cases where matrices without interference are not available, the following approaches can be used to calculate the concentration of the analyte in the study samples: 1) the surrogate matrix approach, 2) the surrogate analyte approach, 3) the background subtraction approach and 4) the standard addition approach., and it is validation to be considered according ICH guideline M10 on bioanalytical method validation and study sample analysis.

2.18 Analytical test methods

The bioanalytical part of bioequivalence studies should be performed in accordance with the principles of Good Laboratory Practice (GLP). However, human bioanalytical studies fall outside the scope of GLP.

All analytical test methods used to determine the active compound and / or its biotransformation product in the biological fluid must be well characterized, fully validated and documented.

The following are important recommendations for the conduct of analysis of biological samples in a pharmacokinetic study:

• The lower limit of quantitation should be 1/20 of C_{max} or lower, as pre-dose concentrations should be detectable at 5% of C_{max} or lower.

• Validation comprises pre-study and within-study phases. During the pre-study phase stability of the stock solution and spiked samples in the biological matrix, specificity, sensitivity,

accuracy, precision and reproducibility should be provided. Within-study validation proves the stability of samples collected during a clinical trial under storage conditions and confirms the accuracy and precision of the determinations.

• Validation must cover the intended use of the assay.

• The calibration range must be appropriate to the study samples. A calibration curve should be prepared in the same biological matrix as will be used for the samples in the intended study by spiking the matrix with known concentrations of the analyte. A calibration curve should consist of a blank sample, a zero sample, and 6-8 non-zero samples covering the expected range. Concentrations of standards should be chosen on the basis of the concentration range expected in a particular study.

• If an assay is to be used at different sites, it must be validated at each site, and cross-site comparability established.

• An assay which is not in regular use requires sufficient revalidation to show that it still performs according to the original validated test procedures. The revalidation study must be documented, usually as an appendix to the study report.

• Within a study, the use of two or more methods to assay samples in the same matrix over a similar calibration range is strongly discouraged.

• If different studies are to be compared and the samples from the different studies have been assayed by different methods, and the methods cover a similar concentration range and the same matrix, then the methods should be cross-validated.

• Spiked quality control samples at a minimum of three different concentrations in duplicate should be used for accepting or rejecting the analytical run.

• All the samples from one subject (all periods) should be analyzed in the same analytical run, if possible.

• Analysis of samples should be conducted without information on treatment.

• Validation procedures, methodology and acceptance criteria should be specified in the analytical protocol, and / or the SOPs.

• All experiments used to support claims or draw conclusions about the validity of the method should be described in a report (method validation report).

• Any modification of the method during the analysis of study samples will require adequate revalidation.

The results of study sample determination should be given in the analytical report together with calibration and quality control sample results, repeat analysis (if any), and a representative number of sample chromatograms.

• Reanalysis of study samples should be predefined in the study protocol (and / or SOP) before the actual start of the analysis of the samples.

• Normally reanalysis of subject samples because of a pharmacokinetic reason is not acceptable. This is especially important for bioequivalence studies, as this may bias the outcome of such a study.

2.19 Bio analytical method validation

2.19.1 Overview

The main objective of method validation is to demonstrate the reliability of a particular method for the determination of an analyte concentration in a specific biological matrix, such as blood, serum, plasma, urine, or saliva. Moreover, if an anticoagulant is used, validation should be performed using the same anticoagulant as for the study samples. Generally a full validation should be performed for each species and matrix concerned.

The main characteristics of a bioanalytical method that are essential to ensure the acceptability of the performance and the reliability of analytical results are: selectivity, lower limit of quantification, the response function and calibration range (calibration curve performance), accuracy, precision, matrix effects, stability of the analyte(s) in the biological

matrix and stability of the analyte(s) and of the internal standard in the stock and working solutions and in extracts under the entire period of storage and processing conditions.

Usually one analyte or drug has to be determined, but on occasions it may be appropriate to measure more than one analyte. This may involve two different drugs, but can also involve a parent drug with its metabolites, or the enantiomers or isomers of a drug. In these cases the principles of validation and analysis apply to all analytes of interest.

If system suitability is assessed, a specific SOP should be used. Apparatus conditioning and instrument performance should be determined using spiked samples independent of the study calibrators, QCs, or study samples. Data should be maintained with the study records.

2.19.2 Reference standards

During method validation and analysis of study samples, a blank biological matrix will be spiked with the analyte(s) of interest using solutions of reference standard(s) to prepare calibration standards, quality control samples and stability samples. In addition, suitable internal standard(s) (IS) can be added during sample processing in chromatographic methods. It is important that the quality of the reference standard and IS is ensured, as the quality (purity) may affect the outcome of the analysis, and therefore the outcome of the study data. Therefore the reference standards used during the validation and study sample analysis should be obtained from an authentic and traceable source.

If possible, the reference standard should be identical to the analyte. When this is not possible, an established chemical form (free base or acid, salt or ester) of known purity can be used. Three types of reference standards are usually used: (1) certified reference standards (e.g., USP compendial standards), (2) commercially-supplied reference standards obtained from a reputable commercial source, and/or (3) other materials of documented purity (certificate of analysis) custom-synthesized by an analytical laboratory or other noncommercial establishment. The source and lot number, expiration date, certificates of

analyses when available, and/or internally or externally generated evidence of identity and purity should be furnished for each reference and internal standard (IS) used. If the reference or internal standard expires, stock solutions made with this lot of standard should not be used unless purity is re-established.

2.19.3 Internal standards (IS)

Selection of IS is generally based on the following factors: (1) the physical and chemical properties (e.g., hydrophobicity, ionization properties) of the IS closely mimics the analyte during the analytical procedure, (2) purity of the IS is adequate, and (3) IS is stable during bioanalytical conduct. Since ISs are used to correct for variations in analyte response, variations in IS response are expected. While excessive variations in IS response may affect quantitation, a high variation does not necessarily equate to unreliable data. Therefore, assessment of the impact of IS variations on quantitation is vital. There is no consensus on what constitutes an “excessive” IS response that affects quantitation. However, it is commonly accepted that monitoring IS response variations during sample analysis is a good practice. It is recommended to monitor IS variations and establishing an objective, a priori criteria for abnormal IS variations. One of the common acceptance criteria for monitoring IS variations is setting a fixed percentage (e.g., ≤50 %) of mean IS response of spiked samples (i.e., calibrators and quality controls) within an analytical batch as an acceptable IS response range for the batch. Any sample with IS response outside the acceptable range in the batch will be flagged for reanalysis.

While the use of IS acceptance criteria based on IS response range of spiked samples is a good practice for reanalyzing samples with abnormal IS response, it has to be used with caution in certain situations. For example, when IS variations in unknown samples and spiked samples are similar, IS variations do not affect the accuracy of the calibrators and QCs. In such situations, the need for reanalysis for IS variations may be moot. Also, in cases where IS

variations in study samples are abnormally different from those in spiked samples, the IS acceptance criteria based on spiked samples may not be meaningful. In such cases, investigation should be conducted to confirm whether IS compensates for matrix effects. Abnormal variations in IS may occur for a number of reasons, including human errors (spiking twice or not spiking IS), imprecision of pipettes used to spike samples with IS (repeater pipettes), partial or complete blockage of autosampler needle. Trends or patterns in variations in IS response may need to be investigated. Trends or patterns in IS variation include, but not limited to, contamination of the orifice or rods of MS due to incomplete or inadequate sample cleanup, matrix effects due to coeluting components, improper IS selection, incomplete solubility of IS in stock solution or extraction solvent, or inadequate mixing of IS. Therefore, it is a good practice to evaluate IS variations across an analytical batch, and investigate any abnormal patterns IS response in terms of its impact on the quantitation of unknown samples.

**** Please, refer to Table 1 - Annex I at the end of this section for more details about some examples of abnormal IS response, reason for the response, and their impact on quantitation.***

2.19.4 Selectivity

The analytical method should be able to differentiate the analyte(s) of interest and IS from endogenous components in the matrix or other components in the sample. Selectivity should be proved using at least 6 individual sources of the appropriate blank matrix, which are individually analysed and evaluated for interference. Use of fewer sources is acceptable in case of rare matrices. Normally, absence of interfering components is accepted where the response is less than 20% of the lower limit of quantification for the analyte and 5% for the internal standard.

It may also be necessary to investigate the extent of any interference caused by metabolites

of the drug(s), interference from degradation products formed during sample preparation, and interference from possible co administered medications. Co-medications normally used in the subject population studied which may potentially interfere should be taken into account at the stage of method validation, or on a study specific and compound specific base. The possibility of back conversion of a metabolite into parent analyte during the successive steps of the analysis (including extraction procedures or in the MS source) should also be evaluated, when relevant (i.e. potentially unstable metabolites e.g. acidic metabolites to ester, unstable N-oxides or glucuronide metabolites, lactone-ring structures). The extent of back-conversion should be established and the impact on the study results discussed.

It is recognized that in some cases it is very difficult to obtain the metabolites of interest. Alternatively, back-conversion of a metabolite can be checked by applying incurred sample reanalysis. However, in this case potential back conversion during sample processing cannot be ruled out.

2.19.5 Carry over

Carry over should be addressed and minimized during method development. During validation carry over should be assessed by injecting blank samples after a high concentration sample or calibration standard at the upper limit of quantification. Carry over in the blank sample following the high concentration standard should not be greater than 20% of the lower limit of quantification (LLOQ); and 5% for the internal standard. If it appears that carry-over is unavoidable, study samples should not be randomized. Specific measures should be considered, tested during the validation and applied during the analysis of the study samples, so that it does not affect accuracy and precision. This could include the injection of blank samples after samples with an expected high concentration, before the analysis of the next study sample.

2.19.6 Lower limit of quantification

The lower limit of quantification (LLOQ) is the lowest concentration of analyte in a sample which can be quantified reliably, with an acceptable accuracy and precision i.e., sensitivity.

The LLOQ is considered being the lowest calibration standard. In addition, the analyte signal of the LLOQ sample should be at least 5 times the signal of a blank sample (in other words the analyte response at the LLOQ is at least five times the response compared to blank response). The LLOQ should be adapted to expected concentrations and to the aim of the study. As an example, for bioequivalence studies the LLOQ should be not higher than 5% of the C_{max}, while such a low LLOQ may be not necessary for exploratory pharmacokinetic studies. Peak response in blanks or zero standards greater than 20 % of LLOQ response is often referred to as interference and may affect accuracy and precision at the LLOQ.

The lowest standard on the calibration curve should be accepted as the LLOQ if the following conditions are met:

- The analyte response at the LLOQ should be at least five times the response compared to blank response;
- Analyte peak (response) should be identifiable, discrete, and reproducible, and the back-calculated concentration should have precision that does not exceed 20% of the CV and accuracy within 20% of the nominal concentration. The LLOQ should not be confused with the limit of detection (LOD) and/or the low QC sample;
- The LLOQ should be established using at least five samples and determining the CV and/or appropriate confidence interval should be determined.

2.19.7 Upper limit of quantification

The highest standard will define the ULOQ of an analytical method. Analyte peak (response) should be reproducible and the back-calculated concentration should have precision that does not exceed 15% of the CV and accuracy within 15% of the nominal concentration.

2.19.8 Calibration curve

The response of the instrument with regard to the concentration of analyte should be known, and should be evaluated over a specified concentration range. The calibration standards should be prepared in the same matrix as the matrix of the intended study samples by spiking

the blank matrix with known concentrations of the analyte. There should be one calibration curve for each analyte studied in the method validation and for each analytical run.

Ideally, before carrying out the validation of the analytical method it should be known what concentration range is expected. This range should be covered by the calibration curve range, defined by the LLOQ being the lowest calibration standard and the upper limit of quantification (ULOQ), being the highest calibration standard. The range should be established to allow adequate description of the pharmacokinetics of the analyte of interest.

A minimum of six calibration concentration levels i.e., non-zero samples (matrix samples processed with analyte and IS) should be used, in addition to the blank sample (processed matrix sample without analyte and without IS) and a zero sample (processed matrix with IS). Each calibration standard can be analysed in replicate.

A relationship which can simply and adequately describe the response of the instrument with regard to the concentration of analyte should be applied. The blank and zero samples should not be take into consideration to calculate the calibration curve parameters.

The calibration curve parameters should be reported (slope and intercept in case of linear fit). In addition, the back calculated concentrations of the calibration standards should be presented together with the calculated mean accuracy values. All the available (or acceptable) curves obtained during validation, with a minimum of 3 should be reported.

The back calculated concentrations of the calibration standards should be within ±15% of the nominal value, except for the LLOQ for which it should be within ±20%. At least 75% of the calibration standards, with a minimum of six calibration standard levels, must fulfil this criterion. In case replicates are used, the criteria (within ±15% or ±20% for LLOQ) should also be fulfilled for at least 50% of the calibration standards tested per concentration level. In case a calibration standard does not comply with these criteria, this calibration standard sample should be rejected, and the calibration curve without this calibration standard should be re-evaluated, including regression analysis. In case all replicates of the LLOQ or the ULOQ calibration standard are rejected then the batch should be rejected from the validation, the

possible source of the failure be determined and the method revised (if necessary). If the next validation batch also fails, then the method should be revised before restarting validation.

Acceptance/rejection criteria for spiked, matrix-based calibration standards and QCs should be based on the nominal (theoretical) concentration of analytes.

Although the calibration curve should preferably be prepared using freshly spiked samples, it is allowed to use previously prepared and stored calibration samples, if supported by appropriate stability data.

2.19.9 Quality Control Samples

- At least three concentrations of QCs in duplicate should be incorporated into each run as follows: one within three times the LLOQ (low QC), one in the midrange (middle QC), and one approaching the high end (high QC) of the range of the expected study concentrations.
- The QCs provide the basis of accepting or rejecting the run. At least 67% (e.g., at least four out of six) of the QCs concentration results should be within 15% of their respective nominal (theoretical) values. At least 50% of QCs at each level should be within 15% of their nominal concentrations. A confidence interval approach yielding comparable accuracy and precision in the run is an appropriate alternative.
- The minimum number of QCs should be at least 5% of the number of unknown samples or six total QCs, whichever is greater.
- It is recommended that calibration standards and QCs be prepared from separate stock solutions. However, standards and QCs can be prepared from the same spiking stock solution, provided the stability and accuracy of the stock solution have been verified. A single source of blank matrix may also be used, provided absence of matrix effects on extraction recovery and detection has been verified. At least one demonstration of precision and accuracy of calibrators and QCs prepared from separate stock solutions is expected.

2.19.10 Accuracy

The accuracy of an analytical method describes the closeness of the determined value obtained by the method to the nominal concentration of the analyte (expressed in percentage). Accuracy should be assessed on samples spiked with known amounts of the analyte, the quality control samples (QC samples). The QC samples should be spiked independently from the calibration standards, using separately prepared stock solutions, unless the nominal concentration(s) of the stock solutions have been established.

The QC samples are analysed against the calibration curve, and the obtained concentrations are compared with the nominal value. The accuracy should be reported as percent of the nominal value.

Accuracy should be evaluated for the values of the QC samples obtained within a single run (the within run accuracy) and in different runs (the between-run accuracy).

To enable evaluation of any trends over time within one run, it is recommended to demonstrate accuracy and precision of QC samples over at least one of the runs in a size equivalent to a prospective analytical run of study samples.

2.19.10.1 Within-run accuracy

Within-run accuracy should be determined by analysing in a single run a minimum of 5 samples per level at a minimum of 4 concentration levels which are covering the calibration curve range: the LLOQ, within three times the LLOQ (low QC), around 30 - 50% of the calibration curve range (medium QC), and at least at 75% of the upper calibration curve range (high QC). The mean concentration should be within 15% of the nominal values for the QC samples, except for the LLOQ which should be within 20% of the nominal value.

2.19.10.2 Between –run accuracy

For the validation of the between-run accuracy, LLOQ, low, medium and high QC samples from at least three runs analysed on at least two different days should be evaluated. The mean concentration should be within 15% of the nominal values for the QC samples, except

for the LLOQ which should be within 20% of the nominal value.

Reported method validation data and the determination of accuracy and precision should include all results obtained except those cases where errors are obvious and documented.

2.19.11 Precision

The precision of the analytical method describes the closeness of repeated individual measures of analyte. Precision is expressed as the coefficient of variation (CV). Precision should be demonstrated for the LLOQ, low, medium and high QC samples, within a single run and between different runs, i.e. using the same runs and data as for the demonstration of accuracy.

2.19.11.1 Within-run precision (intra-batch precision or within-run repeatability)

For the validation of the within-run precision, there should be a minimum of five samples per concentration level at LLOQ, low, medium and high QC samples in a single run. The within-run CV value should not exceed 15% for the QC samples, except for the LLOQ which should not exceed 20%.

2.19.11.2 Between –run precision (inter-batch precision or between-run repeatability)

For the validation of the between-run precision, LLOQ, low, medium and high QC samples from at least three runs analysed on at least two different days should be evaluated. The between-run CV value should not exceed 15% for the QC samples, except for the LLOQ which should not exceed 20%.

- Regarding precision calculation, the calculation should be performed using instrument response for the in-vitro dissolution studies and area ratios for in-vivo studies rather than the concentration.

2.19.12 Reproducibility

Reproducibility of the method is assessed by replicate measurements using the assay, including quality controls and possibly incurred samples. Reinjection reproducibility should be evaluated to determine if an analytical run could be reanalyzed in the case of instrument interruptions.

2.19.13 Recovery

Recovery pertains to the extraction efficiency of an analytical method within the limits of variability. Recovery of the analyte need not be 100%, but the extent of recovery of an analyte and of the internal standard should be consistent, precise, and reproducible. Recovery experiments should be performed by comparing the analytical results for extracted samples at three concentrations (low, medium, and high) with unextracted standards that represent 100% recovery. Alternatively, to avoid matrix effect, recovery is also measured by comparing analyte extracted from matrix against analyte spiked to extracted blank matrix.

2.19.14 Dilution integrity

Dilution of samples should not affect the accuracy and precision. If applicable, dilution integrity should

be demonstrated by spiking the matrix with an analyte concentration above the ULOQ and diluting this sample with blank matrix (at least five determinations per dilution factor). Accuracy and precision should be within the set criteria, i.e. within ±15%. Dilution integrity should cover the dilution applied to the study samples.

Evaluation of dilution integrity may be covered by partial validation. Use of another matrix may be acceptable, as long as it has been demonstrated that this does not affect precision and accuracy.

2.19.15 Matrix effect

Imprecision increased when the same method was validated with five different sources of plasma compared to a single source of plasma. Therefore, matrix effects may significantly

affect assay performance. Matrix effects should be investigated when using mass spectrometric methods, using at least 6 lots of blank matrix from individual donors. Pooled matrix should not be used.

For each analyte and the IS, the matrix factor (MF) should be calculated for each lot of matrix, by calculating the ratio of the peak area in the presence of matrix (measured by analysing blank matrix spiked after extraction with analyte), to the peak area in absence of matrix (pure solution of the analyte). The IS normalised MF should also be calculated by dividing the MF of the analyte by the MF of the IS. If matrix factor equals unity; it seems that there is no matrix effects, if it is less than unity; it is a result of ion suppression; and if it is more than unity; it is a result of ion enhancement or analyte loss in the absence of matrix during analysis.

The CV of the IS-normalised MF calculated from the 6 lots of matrix should not be greater than 15 %. This determination should be done at a low and at a high level of concentration (maximum of 3 times the LLOQ and close to the ULOQ).

If the matrix is difficult to obtain, less than 6 different lots of matrix may be used, but this should be justified. However, matrix effects should still be investigated.

Another procedure can be applied which is refer to ICH guideline M10 on bioanalytical method validation and study sample analysis ,for instance in the case of on-line sample preparation, the variability of the response from lot to lot should be assessed by-analysing at least 6 lots of matrix, spiked at a low and at a high level of concentration (maximum of 3 times the LLOQ and close to the ULOQ). The validation report should include the peak areas of the analyte and of the IS and the calculated concentration for each individual sample. the accuracy should be within ±15% of the nominal concentration. The overall CV calculated for the concentration should not be greater than 15 %.

In special cases the matrix effect may be evaluated in relevant patient populations or special populations (e.g., hepatically impaired or renally impaired) when available.

An additional evaluation of the matrix effect is recommended using hemolyzed or lipaemic matrix samples during method validation on a case-by-case basis, especially when these

conditions are expected to occur within the study.

- Recovery and matrix effect should be calculated for both drug & internal standard using detector response.

Minimization of Matrix Effects:

- Use IS of similar structure (preferably stable isotope –labeled).
- Avoid 'precipitate and shot' methods.
- Conduct sufficient sample cleanup; especially to remove phospholipids.
- Use new chromatographic methods (Ultra Performance LC, Rapid Resolution LC) to enhance separation.
- Use weak acid wash solution for on-line SPE negative ion methods to break up Na+/analyte ion pairs.

2.19.16 Stability

Evaluation of stability should be carried out to ensure that every step taken during sample preparation and sample analysis, as well as the storage conditions used do not affect the concentration of the analyte.

Stability should be ensured for every step in the analytical method, meaning that the conditions applied to the stability tests, such as sample matrix, anticoagulant, container materials, storage and analytical conditions should be similar to those used for the actual study samples. Reference to data published in the literature is not considered sufficient.

Drug stability in a biological fluid is a function of the storage conditions, the physicochemical properties of the drug, the matrix, and the container system. The stability of an analyte in a particular matrix and container system is relevant only to that matrix and container system and should not be extrapolated to other matrices and container systems.

All stability determinations should use a set of samples prepared from a freshly made stock solution of the analyte in the appropriate analyte-free, interference-free biological matrix. Stock solutions of the analyte for stability evaluation should be prepared in an appropriate solvent at known concentrations.

Stability of the analyte in the studied matrix is evaluated using low and high QC samples (blank matrix spiked with analyte at a concentration of a maximum of 3 times the LLOQ and close to the ULOQ) which are analysed immediately after preparation and after the applied storage conditions that are to be evaluated. The QC samples are analysed against a calibration curve, obtained from freshly spiked calibration standards, and the obtained concentrations are compared to the nominal concentrations. The mean concentration at each level should be within ±15% of the nominal concentration.

Stability of the stock and working solutions should be tested with an appropriate dilution, taking into consideration the linearity and measuring range of the detector.

Stability studies should investigate the different storage conditions over time periods that equal or exceed those applied to the actual study samples.

The following stability tests should be evaluated:

• Stability of the stock solution and working solutions of the analyte and internal standard to justify the duration of stock solution storage stability,

• Freeze and thaw stability of the analyte in the matrix from freezer storage conditions to room temperature or sample processing temperature,

• Short term stability of the analyte in matrix at room temperature or sample processing temperature (Bench top) to cover the laboratory handling conditions that are expected for study samples,

• Long term stability of the analyte in matrix stored in the freezer,

In addition the following tests should be performed if applicable:

• Stability of the processed sample at room temperature or under the storage conditions to be used during the study (dry extract or in the injection phase),

• On-instrument / autosampler stability of the processed sample at injector or autosampler temperature.

Regarding the freeze and thaw stability: During freeze/thaw stability evaluations, the freezing and thawing of stability samples should mimic the intended sample handling

conditions to be used during sample analysis. The QC samples are stored and frozen in the freezer at the intended temperature and thereafter thawed at room or processing temperature. After complete thawing, samples are refrozen again applying the same conditions. At each cycle, samples should be frozen for at least 12 hours before they are thawed. The number of cycles in the freeze-thaw stability should equal or exceed that of the freeze/thaw cycles of study samples. Stability should be assessed for a minimum of three freeze-thaw cycles.

Regarding long term stability of the analyte in matrix stored in the freezer: The QC samples should be stored in the freezer under the same storage conditions and at least for the same duration as the study samples (i.e, frozen at the intended storage temperature which should exceed the time between the date of first sample collection and the date of last sample analysis). For small molecules it is considered acceptable to apply a bracketing approach, i.e. in case stability has been proved for instance at -70°C and -20°C, it is not necessary to investigate the stability at temperatures in between. Study samples may be used in addition to QC samples, but the exclusive use of study samples is not considered sufficient as the nominal concentrations of those samples is not known. The results of the evaluation of long term stability should be available before the study report is issued.

Regarding the stability of stock and working solutions: It is not needed to study the stability at each concentration level of working solutions and a bracketing approach can be used. It is not needed to study the stability of stable-isotope labelled internal standards if it is demonstrated that no isotope exchange reactions occur under the same conditions as the stability of the analyte was demonstrated. In case of a multi-analyte study and specific for bioequivalence studies, attention should be paid to stability of the analytes in the matrix containing all the analytes.

Sufficient attention should be paid to the stability of the analyte in the sampled matrix directly after blood sampling of subjects and further preparation before storage, to ensure that the obtained concentrations by the analytical method reflect the concentrations of the analyte in

the subject at the moment of sampling. A demonstration of this stability may be needed on a case-by-case basis, depending on the structure of the analyte.

- % stability of samples should be calculated relative to the zero time samples (i.e, the first day of long-term stability testing) Calculated by dividing over fresh rather than nominal concentration and should be calculated for both drug & internal standard.

- It should be done starting from day one of period one (not from first day of analysis) until the end date of analysis.

**** Please, refer to Table 2 - Annex I at the end of this section for more details about some examples of sources of instability and approaches to overcome instability.***

2.19.17 Robustness

The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters.

If measurements are susceptible to variations in analytical conditions, the analytical conditions should be suitably controlled or a precautionary statement should be included in the procedure. One consequence of the evaluation of robustness should be that a series of system suitability parameters (e.g., resolution test) is established to ensure that the validity of the analytical procedure is maintained whenever used.

In the case of liquid chromatography, examples of typical variations are:

- Influence of variations of pH in a mobile phase;
- Influence of variations in mobile phase composition;
- Different columns (different lots and/or suppliers);
- Temperature;
- Flow rate.

2.19.18 Partial validation

In situations where minor changes are made to an analytical method that has already been

validated, a full validation may not be necessary, depending on the nature of the applied changes. Changes for which a partial validation may be needed include transfer of the bioanalytical method to another laboratory or analyst, change in analytical methodology (e.g., change in detection systems), change in equipment, calibration concentration range, limited sample volume, another matrix or species, change in anticoagulant, sample processing procedure, selectivity demonstration of an analyte in the presence of concomitant medications, and storage conditions etc. All modifications should be reported and the scope of revalidation or partial validation justified.

Partial validation can range from as little as the determination of the within-run precision and accuracy, to an almost full validation.

2.19.19 Cross validation

Where data are obtained from different methods within and across studies or when data are obtained within a study from different laboratories, applying the same method, comparison of those data is needed and a cross validation of the applied analytical methods should be carried out. Differences in sample preparation or the use of another analytical method may result in different outcomes between the study sites. Cross validation should be performed in advance of study samples being analysed if possible. For the cross validation, the same set of QC samples or study samples should be analysed by both analytical methods.

For QC samples, the obtained mean accuracy by the different method should be within 15% and may be wider, if justified. For study samples, the difference between the two values obtained should be within 20% of the mean for at least 67% of the repeats. The outcome of the cross validation is critical in determining whether the obtained data are reliable and whether they can be compared and used.

An example of cross validation would be a situation in which an original validated bioanalytical method serves as the reference, and the revised bioanalytical method is the comparator.

The comparisons should be done both ways. When sample analyses within a single study are

conducted at more than one site or more than one laboratory, cross-validation with spiked matrix standards and subject samples should be conducted at each site or laboratory to establish inter laboratory reliability.

2.20 Analysis of study samples

2.20.1 Sample extraction

Generally, prior to chromatography, sample clean-up is performed for method sensitivity. Proteins in biological matrices may bind to analyte of interest and can clog the chromatography columns. Blood contains intra- and extra-cellular proteins, plasma contains significant proteins, and urine and cerebrospinal fluids contain relatively less proteins but still require extraction to improve reliability. In addition to proteins, endogenous compounds such as phospholipids and fatty acids, and exogenous components in biological matrices can potentially affect separation and detection of the analyte of interest (e.g., foul high performance liquid chromatography (HPLC) columns and contaminate MS source). The purpose of sample clean-up is to extract out the analyte(s) of interest from biological matrices to minimize interference and maximize recovery. Consequently, sample clean-up reduces variability and inconsistencies during analysis. Different sample clean-up procedures are used depending on the choice of matrix, drug, chromatography, and detection systems. Broadly, sample clean-up procedures include, protein precipitation (PP), solid phase extraction (SPE), and liquid–liquid extraction (LLE).

In PP, miscible organic solvents (e.g., methanol or acetonitrile), often modified with buffer or acid and bases, are added to biological samples to denature proteins and consequently precipitate the samples. For example, if the analyte is highly protein bound, a volatile acid (e.g., formic acid) or base (ammonium hydroxide) is used to disrupt binding and increase analyte recovery. The precipitate is removed by centrifugation or filtration, and extract injected. Although PP is simple and fast, it does not necessarily yield clean extracts, as it may not remove endogenous components such as phospholipids, fatty acids, lipids.

More efficient sample clean-up may be obtained from LLE and SPE. In LLE, immiscible organic solvents (e.g., diethyl ether, ethyl acetate, methyl-tert-butyl ether (MTBE), hexane) are used to extract the analyte of interest by partitioning it into an organic layer. Therefore, LLE can mitigate or avoid matrix effects as ionized compounds, including salts or phospholipids, do not partition into the organic layer. The advantage of LLE is mainly its ease of use, and requires no special instrumentation. A major limitation of LLE is its applicability to polar compounds. To transfer an ionizable analyte to organic solvent it first needs to be converted to a nonionic form in an aqueous medium at an appropriate pH, followed by selection of a suitable solvent to efficiently and selectively extract the analyte. Usually multiple extractions are necessary and final re-suspension in an aqueous medium at the original pH is needed, resulting in reduction in recovery of the analyte. Also, in LLE, there is a tendency to form emulsions at the interface between liquid layers. Further, LLE may require large solvent volumes. These problems have been reported to be minimized with new versions of LLE, such as supported LLE. In supported LLE, the entire sample is adsorbed on a solid support (i.e., diatomaceous earth), and an organic solvent is passed through the solid support resulting in partition of the analyte of interest into the organic solvent. Recently, LLE has been scaled down, requiring relatively low volumes of sample (50–100 µL) and organic solvent (0.6–2 mL). Also, high throughput LLE versions using on-line extraction or 96-well plate arrangements are available. To further increase selectivity and clean-up, SPE is often employed. SPE can reduce sample volume, be easily automated, and used on-line with liquid chromatography separation. In SPE, the separation process is based on the affinity of the analyte to the stationary phase or sorbent. The sorbents are ion-exchange, normal phase, reverse phase or a combination to selectively retain the analyte of interest. The interfering matrix components either pass through unretained or are retained relatively longer than the analyte of interest. The choice of sorbent controls selectivity, affinity, and capacity depending on the physiochemical properties of the analyte, biological matrix, and interaction between sorbent and analyte. The SPE usually involves a wash step to remove undesired components, and an elution step to

extract the analyte of interest. Therefore, selection of the proper washing and elution solvents are important. It is reported that immunosorbents and molecularly imprinted polymer (MIP) sorbents can significantly increase selectivity of SPE. The drawbacks of SPE include, the time required for processing (manual SPE), expense, and lot-to-lot cartridge variability. Also, matrix effects have been reported to result from the sample pre-concentration step and the SPE procedure itself (i.e., from salts in buffers used). However, the advantages of SPE overshadow the drawbacks. SPE remains one of the most widely used extraction techniques for routine bioanalysis.

2.20.2 Analytical run

An analytical run consists of the blank sample (processed matrix sample without analyte and without IS) and a zero sample (processed matrix with IS), calibration standards at a minimum of 6 concentration levels, at least 3 levels of QC samples (low, medium and high) in duplicate (or at least 5 % of the number of study samples, whichever is higher), and study samples to be analysed. As indicated before the calibration standards and QC samples should have been spiked independently using separately prepared stock solutions, unless the nominal concentration(s) of the stock solutions have been established. All samples (calibration standards, QC samples, and study samples) should be processed and extracted as one single batch of samples in the order in which they intend to be submitted or analysed. A single batch is comprised of samples which are handled at the same time, i.e. subsequently processed without interruption in time and by the same analyst with the same reagents under homogeneous conditions. Analysing samples, which were prepared separately as several batches, in a single analytical run should be avoided. If such an approach cannot be avoided, for instance due to bench-top stability limitations, each batch of samples should include low, medium and high QC samples. Acceptance criteria should be pre-established in a Standard Operating Procedure (SOP) or in the study plan and should be defined for the whole analytical run and the separate batches in the run.

For bioequivalence studies it is advised to analyse all samples of one subject together in one analytical run to reduce the variability in outcome. The QC samples should be divided over the run in such a way that the accuracy and precision of the whole run is ensured.

Assays of all samples of an analyte in a biological matrix should be completed within the time period for which stability data are available. Extrapolation of concentrations in study samples either below the LLOQ or above the ULOQ of the standard curve is not recommended.

2.20.3 Acceptance criteria of an analytical run

Criteria for acceptance or rejection of an analytical run should be defined in the protocol, in the study plan or in a SOP. In case a whole run consist of more batches, acceptance criteria should be applied to the whole run and to the individual batches. The run can be acceptable, although a batch might have to be rejected, as criteria were not met.

The following acceptance criteria should apply for accuracy:

The back calculated concentrations of the calibration standards should be within ±15% of the nominal

value, except for the LLOQ for which it should be within ±20%. At least 75% of the calibration standards, with a minimum of six, must fulfil this criterion. If one of the calibration standards does not meet these criteria, this calibration standard should be rejected and the calibration curve without this calibration standard should be re-evaluated, and regression analysis performed.

If the rejected calibration standard is the LLOQ, the LLOQ for this analytical run is the next lowest acceptable calibration standard of the calibration curve. If the highest calibration standard is rejected, the ULOQ for this analytical run is the next acceptable lower calibration standard of the calibration curve. The revised calibration range must cover all QC samples (low, medium and high).

The accuracy values of the QC samples should be within ±15% of the nominal values. At least 67% of the QC samples and at least 50% at each concentration level should comply with this criterion.

In case these criteria are not fulfilled the

analytical run should be rejected, and the study samples re-extracted and analysed.

In the case of the simultaneous determination of several analytes, there should be one calibration curve for each analyte studied. If an analytical run is acceptable for one analyte but has to be rejected for another analyte, the data for the accepted analyte can be used, but the samples should be re-extracted and analysed for determination of the rejected analyte.

If replicate calibration standards are used and only one of the LLOQ or ULOQ standards fails, the calibration range is unchanged.

The overall (mean) accuracy and precision of the QC samples of all accepted runs should be calculated at each concentration level and reported in the analytical report. In case the overall mean accuracy and precision exceeds 15%, this should lead to additional investigations justifying this deviation. In the

case of bioequivalence trials it may result in the rejection of the data.

2.20.4 Calibration range

If a narrow range of analyte concentrations of the study samples is known or anticipated before the start of study sample analysis, it is recommended to either narrow the calibration curve range, adapt the concentrations of the QC samples, or add new QC samples at different concentration levels as appropriate, to adequately reflect the concentrations of the study samples.

If a narrow range of analysis values is unanticipated, but observed after the start of sample analysis, it is recommended that the analysis is stopped and either the standard calibration range narrowed, existing QC concentrations revised, or QC samples at additional concentrations are added to the original curve before continuing with study sample analysis. It is not necessary to reanalyse samples analysed before optimising the standard curve range or QC concentrations.

The same applies if it appears that a large number of the analyte concentrations of the study samples appear to be above the ULOQ. The calibration curve range should be extended, if

possible, and QC samples added or their concentrations modified.

At least 2 QC sample levels should fall within the range of concentrations measured in study samples. If the calibration curve range is changed, the bioanalytical method should be revalidated (partial validation) to verify the response function and to ensure accuracy and precision.

Concentrations in unknown samples should not be extrapolated below the LLOQ or above the ULOQ of the standard curve. Instead, the standard curve should be extended and revalidated, or samples with higher concentration should be diluted and reanalyzed. Concentrations below the LLOQ should be reported as zeros.

2.20.5 Reanalysis of study samples

Possible reasons for reanalysis of study samples and criteria to select the value to be reported should be predefined in the protocol, study plan or SOP, before the actual start of the analysis of the samples. The number of samples (and percentage of total number of samples) that have been reanalysed should be discussed in the study report.

The following are examples of reasons for study sample reanalysis:

• Rejection of an analytical run because the run did not fulfil the acceptance criteria with regard to accuracy of the calibration standards and/or the QC samples,

• Internal standard response significantly different from the response for the calibration standard and QC samples, if such criteria have been pre-defined in a SOP,

• Improper sample injection or malfunction of equipment,

• The obtained concentration is above the ULOQ or below the run's LLOQ, in runs where the lowest standard sample has been rejected from a calibration curve, resulting in a higher LLOQ compared with other runs,

• Identification of quantifiable analyte levels in pre-dose samples or placebo sample,

• Poor chromatography.

For bioequivalence studies, normally reanalysis of study samples because of a

pharmacokinetic reason is not acceptable, as this may affect and bias the outcome of such a study. In this case, reanalysis might be considered as part of laboratory investigations, to identify possible reasons for results considered as abnormal and to prevent the recurrence of similar problems in the future.

In case of reanalysis because of positive pre-dose samples or because of a pharmacokinetic reason, the reanalysed samples should be identified and the initial value, the reason for reanalysis, the values obtained in the reanalyses, the finally accepted value and a justification for the acceptance should be provided.

Re-injection of samples can be made in case of instrument failure if reinjection reproducibility and on-injector stability have been demonstrated during validation. Re-injection of a full analytical run or of individual calibration standard samples or QC samples, simply because the calibration or QCs failed, without any identified analytical cause, is not acceptable.

The safety of trial subjects should take precedence over any other aspect of the trial. Consequently, there may be other circumstances when it is necessary to re-extract and/or re-analyse specific study samples, for example where an unexpected or anomalous result is identified that may impact on patient safety.

2.20.6 Integration

Chromatogram integration and re-integration should be described in a SOP. Any deviation from this SOP should be discussed in the analytical report.

Chromatogram integration parameters and in case of re-integration, initial and the final integration data should be documented at the laboratory and should be available upon request.

2.20.7 Special cases

Typically, the calibration range validated pre-study should be used in the analytical batches. However, in some situations, at the start of analysis, the study sample concentration range may be narrower than the expected concentration range. Consequently, the validated

calibration range is too broad and QC concentrations may not be reflective of the study sample concentrations. In such instances, this guideline recommends: (1) to narrow the calibration curve and modify QC concentrations, or (2) retain the original standard curve but include additional QC or new QC concentrations to reflect the study sample concentrations. In either case, partial validation of the modifications is necessary. It is not necessary to reanalyze samples analyzed prior to modifying standard curve and/or QC concentrations as long as the partial validation is acceptable. Selection of samples for reanalysis and reporting of final values are recommended to be based on a priori, objective criteria. It is a good practice to restrict sample reanalysis to samples with assignable causes that will invalidate the data (e.g., poor chromatogram, instrument failure, documented processing errors, samples below LLOQ or above ULOQ). Reanalysis of possible outliers (including PK, suspected, and confirmatory repeats) is discouraged, and when necessary needs to be justified with appropriate pre-established criteria. It is not a good practice to re-inject failing analytical batches to bring them to acceptance. A high frequency of analytical batch failures needs to be investigated and resolved prior to continuing sample analysis. Also, following batch interruptions, the decision to continue analysis of the remaining samples or re-inject all the samples depends on the cause, duration, and resolution of the interruption. Generally, it is a good practice to have objective, pre-established criteria for analysis following batch interruption. Also, before re-injecting batches, it is important to establish re-injection reproducibility to determine whether an analytical batch can be reanalyzed. Integration of chromatograms must be objective and consistent. When re-integration of chromatograms is normally discouraged, however, when performed this guidline recommends that the rationale for the re-integration is clearly described and documented, and audit trails maintained. It is recommended that objective procedures are established that specify the situations when re-integration is necessary and how it needs to be performed. While modification of integration parameters may be necessary in some situations, it is a generally good practice to use the same integration parameters for all analytical batches on the same instrument for a given

study provided the integration is valid and consistent.

2.21 Requirement to perform incurred sample reanalysis (ISR)

Incurred sample reanalysis (ISR) is a necessary component of bioanalytical method validation and is intended to verify the reliability of the reported subject sample analyte concentrations. ISR is conducted by repeating the analysis of a subset of subject samples from a given study in separate runs on different days to critically support the precision and accuracy measurements established with spiked QCs; the original and repeat analysis is conducted using the same bioanalytical method procedures. ISR samples should be compared to freshly prepared calibrators.

The use of calibration standards and QC samples during validation may not mimic the actual study samples. Differences for instance in protein binding, back-conversion of known and unknown metabolites, sample inhomogeneity or concomitant medications, may affect the accuracy and precision of the analyte in such samples during processing and storage. It is therefore recommended to evaluate accuracy of incurred samples by reanalysis of study samples in separate runs at different days. The extent of testing depends on the analyte and the study samples, and should be based upon in-depth understanding of the analytical method and analyte.

However, as a guide, 10% of the samples should be reanalysed in case the number of samples is less than 1000 samples and 5% of the number of samples exceeding 1000 samples. Furthermore, it is advised to obtain samples around C_{max} and in the elimination phase. The percent difference between the initial concentration and the concentration measured during the repeat analysis should not be greater than 20% of their mean for at least 67 % of the repeats.

The following equation should be used for the calculations:

$$\% \text{ Difference} = \frac{(\text{Repeat Value} - \text{Initial Value})}{\text{Mean Value}} \times 100$$

Large differences between results may indicate analytical issues and should be investigated.

In case incurred sample analysis showed deviating results, this should be investigated, and adequate steps should be taken to minimize inaccuracy (and imprecision).

Different sources can be identified which might contribute to the failure of ISR. Some sources may be more likely to occur than other depending on the method, active substance, and analyst, however they cannot be excluded.

Sources of ISR failure may be:

• Execution, i.e. switched samples, instrument issues, scientist performance of method,

• Method, i.e. metabolite interferences, back conversion of metabolites, poor ruggedness, internal standard response,

• Samples, i.e. matrix effects, mislabelling, handling.

It is recognized that some of these sources are also likely to occur during validation, like switching samples and mislabelling. ISR failure and thus lack of the reliability of the study outcome can happen in each study and as such it is difficult to generalise it. Especially with pivotal studies it should be ensured that the results are reliable. However it is also understood that ISR is an additional confirmation of results next to a complete validation.

In compliance with this framework, the regulatory assessment requires the review of the bioanalytical method validation in any application against the current regulatory standards as set out in the guideline, including the requirement to address incurred sample reanalysis. If an element of the validation is missing, e.g. lack of incurred sample reanalysis, then this would need to be scientifically justified by the applicant. Any justification will need to be reviewed on a case-by-case basis considering the overall validation data, the study results, as well as

the reliance of the application on these data.

For the scientific justification of the lack of ISR the applicant should take all the following points into consideration:

- **Metabolite back conversion:**

The applicant should support that back conversion is not an issue for the drug compound or that the risk of back conversion on the outcome of the study results is low as for instance it is known that the drug compound is (almost) not metabolised. For drug compounds for which it is known that back conversion is an issue, i.e. clopidogrel, atorvastatin, ramipril, lack of ISR is considered not acceptable.

- **Other ISR data obtained in the same laboratory:**

ISR data obtained for the same analyte from other studies carried out in the same laboratory and with the same analytical method may be used as supportive data to justify the lack of ISR.

- **Data from repeat analysis:**

In most studies repeat analysis of study samples has to be carried out for different reasons. Repeat analysis can be considered as ISR in certain situations, however due to the nature of the reanalysis (for instance run acceptance criteria failure) those data are considered not reliable. The applicant should report the data of these reanalysis and take into account and discuss the reason for the reanalysis in the justification for supportive data.

In case of a multi analyte analysis, if the repeat analysis was due to run acceptance criteria failure for one of the analytes, but the other has passed, the results of the analyte(s) which passed can be used to infer ISR, if analysed.

- **The obtained pharmacokinetic data in the study:**

The applicant should compare the obtained pharmacokinetic data with data obtained previously or with reported data and should show that these are comparable.

- **90% confidence interval:**

As one element of such justification, if applicable, the applicant could also take into consideration the width of the 90% confidence interval and the ratio to possibly justify that a false positive outcome due to ISR problems has a low probability.

The last two bullet points need to be thoroughly discussed specifically for bioequivalence studies.

2.22 Statistical analysis

Statistical analysis of the bioequivalence study should demonstrate that a clinically significant difference in bioavailability between the generic product and the reference listed product unlikely. The statistical procedures should be specified in the protocol before the data collection starts. The statistical method for testing pharmacokinetic bioequivalence is based upon the determination of the 90% confidence interval around the ratio of the log-transformed population means (generic / reference listed) for the pharmacokinetic parameters under consideration and by carrying out two one-sided tests at the 5% level of significance. To establish pharmacokinetic bioequivalence, the calculated confidence interval should fall within a preset bioequivalence limit. 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 generic formulation is compared to the reference listed product or the reference listed product to the generic formulation).

All concentration-dependent pharmacokinetic parameters (e.g. AUC and C_{max}) should be log-transformed using either common logarithms to the base 10 or natural logarithms. The choice of common or natural logs should be consistent and should be stated in the study report. Logarithmically transformed, concentration-dependent pharmacokinetic parameters should be analyzed using analysis of variance (ANOVA). Usually, the ANOVA model includes the formulation, period, sequence or carry- over and subject factors.

Parametric methods, i.e. those based on normal distribution theory, are recommended for the

analysis of log-transformed bioequivalence measures. The general approach is to construct a 90% confidence interval for the quantity (μT−μR) and to reach a conclusion of pharmacokinetic equivalence if this confidence interval is within the stated limits. The nature of parametric confidence intervals means that this is equivalent to carrying out two one-sided tests of the hypothesis at the 5% level of significance. The antilogs of the confidence limits obtained constitute the 90% confidence interval for the ratio of the geometric means between the generic and reference products.

The same procedure should be used for analyzing parameters from steady state studies or cumulative urinary recovery, if required.

For T_{max} descriptive statistics should be given. If T_{max} is to be subjected to a statistical analysis this should be based on non-parametric methods (e.g.: Wilcoxon Test) and should be applied to untransformed data. A sufficient number of samples around predicted maximal concentrations should have been taken to improve the accuracy of the T_{max} estimate. For parameters describing the elimination phase ($T_{1/2}$) only descriptive statistics should be given.

Methods for identifying and handling of possible outlier data should be specified in the protocol. Medical or pharmacokinetic explanations for such observations should be sought and discussed. As outliers may be indicative of product failure, post hoc deletion of outlier values is generally discouraged. An approach to dealing with data containing outliers is to apply distribution-free (non-parametric), statistical methods. If the distribution of log-transformed data is not normal, non-parametric statistical methods can be considered. The justification of the intent to use non-parametric statistical methods should be included a priori in the protocol.It is only acceptable to use a validated software for statistical analysis.

2.22.1 General considerations

The statistical analyses should include all data for all subjects who provide evaluable data for the drug products being compared. Decisions made to exclude subjects from the BE analysis

population, e.g., due to incomplete sampling or protocol violation, should be documented at the end of the clinical blood sampling portion of the study and prior to subject sample analysis. A study will not be considered acceptable if there are fewer than 24 subjects with evaluable data for primary statistical analysis for a crossover design or for each treatment arm for a parallel design.

In studies with more than two treatment arms, e.g., a four-period study examining fasting and fed conditions or a three-period study including two comparator products or two test Products, the analysis for each comparison should be conducted excluding the data from the treatment arms that are not relevant for the comparison in question.

The model to be used for the statistical analysis should be pre-specified in the study protocol. The statistical analysis should take into account sources of variation that can be reasonably assumed to have an effect on the response variable. Post hoc and data-driven adjustments are not acceptable for the primary statistical analysis.

The report on the data analysis should be sufficiently detailed to enable the PK and the statistical analyses to be repeated, e.g., data on actual time of blood sampling after dose, drug concentrations, the values of the PK parameters for each subject in each period, and the randomisation scheme should be provided.

2.23 Acceptance ranges

2.23.1 Area under the curve-ratio

The 90% confidence interval for this measure of relative bioavailability should lie within a bioequivalence range of 0.80–1.25 (80 – 125%). If the therapeutic range is particularly narrow, the acceptance range may need to be reduced based on clinical justification. A larger acceptance range may be acceptable in exceptional cases if justified clinically.

2.23.2 C_{max}-ratio

In general, the acceptance limit 0.80–

1.25 (80 – 125%) should be applied to the C_{max}-ratio.

However, in certain cases a wider acceptance range may be acceptable for highly variable drug products. The range used must be defined prospectively and should be justified, taking into account safety and efficacy considerations. In specific cases of products with a narrow therapeutic range, the acceptance interval may need to be tightened.

2.23.3 T_{max}-difference

Statistical evaluation of T_{max} makes sense only if there is a clinically relevant claim for rapid onset of action or concerns about adverse effects. The non-parametric 90% confidence interval for this measure of relative bioavailability should lie within a clinically relevant range. There should be no apparent difference in median T_{max} and its variability between test and reference product.

For other pharmacokinetic parameters the same considerations as outlined above apply.

2.24 Reporting of results

The report of a bioequivalence study should give the complete documentation of its protocol, conduct and evaluation complying with good clinical practice rules. The responsible investigator(s) should sign their respective parts of the report. Names and affiliations of the responsible investigator(s), site of the study and period of its execution should be stated. The names, batch numbers and expiry dates of the pharmaceutical products used in the study as well as the composition(s) of the test product(s) should be given. Results of in vitro dissolution tests should be provided. In addition, the applicant should submit a signed statement confirming that the test product is identical to the pharmaceutical product which is submitted for registration.

The bioanalytical validation report should be attached. The bioanalytical report should include the data on calibrations and quality control samples. A representative number of

chromatograms or other raw data should be included covering the whole calibration range, quality control samples and specimens from the study.

All results should be presented clearly. All concentrations measured in each subject and the sampling time should be tabulated for each formulation. Tabulated results showing APIs concentration analysis according to analytical run (including runs excluded from further calculations, including all calibration standards and quality control samples from the respective run) should also be presented. The tabulated results should present the date of run, subject, study period, product administered (generic or reference listed) and time elapsed between drug application and blood sampling in a clear format. The procedure for calculating the parameters used (e.g. AUC) from the raw data should be stated. Any deletion of data should be justified. If results are calculated using pharmacokinetic models, the model and the computing procedure used should be justified.

Individual blood concentration / time curves should be plotted on a linear / linear and log / linear scale. All individual data and results should be given, including information on those subjects who dropped out. The drop-outs and / or withdrawn subjects should be reported and accounted for.

Results of all measured and calculated pharmacokinetic parameters should be tabulated for each subject–formulation combination together with descriptive statistics. The statistical report should be sufficiently detailed to enable the statistical analysis to be repeated if necessary.

If the statistical methods applied deviate from those specified in the study protocol, the reasons for the deviations should be stated.

2.24.1 Validation report

Depending on the level of detail of the information provided in the validation report, reference to the SOPs for relevant analysis specific

procedures may be sufficient. Otherwise these SOPs should be appended to the report. All source data should be available in its original format and available on request.

All measurements with the individual calculated concentrations have to be presented in the validation report. Any deviation from the validation protocol should be recorded.

The validation report should include at least the following information:

• Summary of the validation performances,

• Details of the applied analytical method and where appropriate, the source of the analytical method (references from literature and/or modifications in the procedure),

• Details of the assay procedure (analyte, IS, sample pre-treatment, extraction and analysis),

• Reference standards (origin, batch number, certificate of analysis, stability and storage conditions),

• Calibration standards and QC samples (matrix, anticoagulant if applicable, preparation, preparation dates, and storage conditions),

• Run acceptance criteria,

• Unexpected results obtained during validation with full justification of the action taken,

• Deviations from method and/or SOPs (description of deviations, impact on study, supportive data),

- Analysis:
  - Table of all analytical runs with analysis dates, whether passed or failed and the reason for the failure,
  - Table of calibration results of all accepted analytical runs, including calibration range, response function, back-calculated concentrations, and accuracy,
  - Table of QC results of all accepted analytical runs (within- and between-run precision and accuracy); values outside acceptance criteria should be clearly marked,
  - Stability data of stock solution, working solution, QC, covering the applied storage

conditions,

• Data on selectivity, LLOQ, carry-over, matrix effect if applicable, dilution integrity;

2.24.2 Analytical report

The analytical report should include a reference to the validation report(s) applicable to the analysis of the study samples. Furthermore, it should include a detailed description of the analysis of the study samples. If the analytical report provides detailed information, a reference to the analysis specific SOPs in the analytical report is sufficient. Otherwise, the SOPs should be appended to the analytical report. All source data should be available in its original format and available on request. Any deviation from the protocol, analytical procedure or SOPs should also be discussed in the analytical report.

The results of incurred sample reanalysis may be supplied either in the validation report, in the analytical report or in a stand-alone report.

For bioequivalence studies, all chromatograms from the runs which include 20% of the subjects, including the corresponding QC samples and calibration standards should be appended to the analytical study report. For other studies representative chromatograms should be appended to the report. Additional chromatograms should be available on request.

The analytical report should include at least the following information:

• Reference standards (origin, batch, certificate of analysis, stability, storage conditions),

• Calibration standards and QC samples (storage conditions),

• Run acceptance criteria (short description, reference to specific protocol or SOP),

• Assay procedure (short description),

• Sample tracking (dates of receipt and contents, sample conditions on receipt, storage location and conditions, if applicable),

• Study sample analysis:

- Content of the analytical run,

- Table identifying all analytical runs and study samples, with run dates and results,
- Table of calibration results of all (passed) analytical runs,
- Table of QC results of all (passed) analytical runs; values outside acceptance criteria should be clearly marked;

• Failed analytical runs (identity, assay date, reason for failure),

• Deviations from method and/or SOPs (description of deviations, impact on study, supportive data),

• Reassay, excluding reassay due to analytical reasons, such as failed run (table of sample identification, reason for re-assay, original and re-assay values).

ANNEX I

Table 1: Examples of abnormal IS response, reason for the response, and their impact on quantitation:

SN	Observations	Root cause identified	Effect on quantitation
1.	Zero or nearly doubled IS response.	Missed or double addition of IS.	Yes.
2.	Random and sharp drop in IS response.	Autosampler needle blockage.	Usually no, unless S/N is too low.
3.	Gradual decrease of IS responses.	Charging of mass spectrometer.	Not in this case, it usually depends on how well an IS follows an analyte.
4.	Random, sharp drop, and overall downward trend in IS response.	Autosampler needle blockage plus charging of mass spectrometer.	It depends, but batch should be reinjected.
5.	Low IS responses for most of the extracted samples.	Mixed usage of right and wrong caps in LLE.	It depends, but samples should be reassayed by using correct materials.
6.	High IS responses observed for incurred samples only (usually a whole subject).	Relatively less ion suppression in subject samples than in CS/QC.	It depends on how well an IS follows an analyte.
7.	High IS responses observed for incurred samples only (usually a whole subject).	Recovery variation plus relatively less ion suppression in subject	It depends on how well an IS follows an analyte.

		samples than in CS/QC.	
8.	Low IS responses for incurred samples only (usually a whole subject).	Transfer of salt-containing intermediate layer in LLE.	It depends, but samples should be reassayed.
9.	Less IS response variation with analogue IS than with deuterated IS.	Analogue IS did not follow analyte well.	Quantitation affected with analogue IS and it should be changed.
10.	Gradual increase of IS responses.	Insufficient mixing.	Not in this case, but should be evaluated case by case.
11.	Randomly scattered low IS responses for incurred samples only and not repeated during reanalysis.	Not conclusive, but speculated as due to ascorbic acid and different cycles of F/T.	Not in this case, but should be evaluated case by case.
12.	Deuterated IS not following the analyte and re-injection results not matching those of 1st injection.	Not conclusive, but speculated due to differential matrix effect between analyte and IS.	Yes in this case, but should be evaluated case by case.

Table 2: Examples of sources of instability and approaches to overcome instability:

SN	Causes of instability	Strategies to avoid instability
1.	Enzymatic hydrolysis.	Addition of enzyme inhibitors and/or freezing samples immediately after collection, or harvesting plasma at reduced temperature followed by immediate frozen storage.
2.	Hemolysis.	Depending on the analyte, testing the impact of different degrees of hydrolysis during method development. Factoring sample hemolysis during stability evaluations.
3.	Temperature.	Lowering temperature during sample collection, processing, storage, extraction, reconstitution and analysis.
4.	pH.	Controlling pH within the desired range during sample collection, processing, storage, extraction, reconstitution and analysis.
5.	Light.	For photo-sensitive compounds, protection from light during sample handling is necessary, e.g., wrapping tubes in foil, using amber glass vials, or sample processing under yellow light or UV-filtered light.
6.	Autooxidation.	Addition of antioxidants to samples, e.g., ascorbic acid, sodium metabisulfite and ethylenediaminetetraacetic acid (EDTA).
7.	Lactone/hydroxyl acid Interconversion.	Decreasing pH and sample processing temperature or time.
8.	Adsorption to container walls.	Using appropriate containers for sample collection, extraction, storage and analysis, e.g., silanized glass tubes. Addition of surfactants.
9.	In-source fragmentation/ transformation.	Selecting suitable analyte-specific MS tuning of ionization conditions, assuring adequate chromatographic separation.

SECTION 3

SPECIAL CONSIDERATIONS

3.1 Fixed-dose combination products

If the pharmacokinetic bioequivalence of fixed-dose combination (FDC) products is assessed by in vivo studies, the study design should follow the same general principles as described previously. The generic FDC product should be compared with the pharmaceutically equivalent reference listed FDC product. In certain cases (e.g. when no reference listed FDC product is available on the market) separate products administered in free combination can be used as a reference. Sampling times should be chosen to enable the pharmacokinetic parameters of all APIs to be adequately assessed. The bioanalytical method should be validated on respect to all compounds measured. Statistical analysis should be performed with pharmacokinetic data collected on all active ingredients; the 90% confidence intervals of test / reference ratio of all active ingredients should be within acceptance limits.

The conditions regarding proportional composition should be fulfilled for all active substances of fixed combinations. When considering the amount of each active substance in a fixed combination the other active substance(s) can be considered as excipients. In the case of bilayer tablets, each layer may be considered independently.

3.2 Highly variable drugs

A "highly variable API" has been defined as an API with an intra-subject variability of > 30% in terms of the ANOVA-CV. Proving the bioequivalence of finished pharmaceutical product containing highly variable APIs can be problematic because the higher the ANOVA-CV, the wider the 90% confidence interval. Thus large numbers of subjects must be enrolled in studies involving highly variable APIs to achieve adequate statistical power.

Several factors influence the sample size needed to meet the criteria for acceptable BE. First,

each one-sided test (in the two one-sided tests procedure) is carried out at the 5 % level of significance, corresponding to the 90 % CI. The 5 % level of significance represents the type I error rate (α), which is the probability of incorrectly deeming as bioequivalent two formulations whose population Geometric Mean Ratio (GMR) fails to meet the BE limits. The second factor influencing sample size is study power, defined as the likelihood or chance of correctly demonstrating BE when it, in fact, exists. A third factor influencing sample size is the test/reference BE measure ratios. If the true test/reference ratio differs from unity, the overall power to show BE is reduced at any given sample size, resulting in an increase in the number of study subjects needed. Thus, GMR between test and reference listed product is preferred to be near 1.00. Other factors influencing sample size include the study design and the expected within-subject variability.

The sources of within-subject variability include:

- Physiological factors affecting bioavailability such as regional pH in the gastrointestinal tract, bile and pancreatic secretions, luminal and mucosal enzymes, gastrointestinal motility, gastric emptying, small intestinal transit time, and colonic residence time.
- Inherent properties of the drug such as distribution, first-pass metabolism, systemic metabolism, and elimination.
- Physicochemical properties of drug substance such as solubility.
- Formulation factors such as drug release.
- Other factors such as food intake.

Calculation of within-subject variability:

$$CV_{intra}\ \% = 100 \cdot \sqrt{e^{MSE} - 1}$$

Where;

$$MSE = 2 \left(\frac{\Delta_{CL}}{\sqrt{(1/n_1 + 1/n_2)} \cdot t_{1-2.\alpha,\,(n1+n2)-2}} \right)^2$$

$$\Delta_{CL} = Ln\ PE - Ln\ CL_{lower} \quad or \quad \Delta_{CL} = Ln\ CL_{upper} - Ln\ PE$$

$$PE = \sqrt{CL_{lower} \cdot CL_{upper}}$$

Where; PE = Point estimate, CL_{lower} = Lower confidence limit, CL_{upper} = Upper confidence limit, ΔCL = Difference between one CL and the PE in log-scale, n_1 & n_2 = Number of subjects for test and reference, and MSE = Mean Square Error.

It is recommended to involve the scaling of bioequivalence acceptance criteria based on the intra-subject standard deviation in the relevant parameters for the reference listed product. Of the most common assessment parameters C_{max} is subject to the highest variability and hence is the parameter for which a modified approach is most needed.

For highly variable finished pharmaceutical products, it is recommended that a three-way partial replicate (where the reference listed product is administered twice) or a four-way fully replicated cross-over bioequivalence study be conducted and reference-scaled average bioequivalence be employed to widen the acceptance interval for the C_{max} parameter, if the intra-subject variability for C_{max} following replicate administrations of the reference listed product is > 30%. If this is the case the acceptance criteria for C_{max} can be widened to a maximum of 69.84–143.19%. The applicant should justify that the calculated intra-subject variability is a reliable estimate and that it is not the result of outliers. This approach adjusts the bioequivalence limits of highly variable drugs by scaling to the within-subject variability of the reference listed product in the study.

The extent of the widening of the acceptance interval for C_{max} is defined based upon the intra-subject variability seen in the bioequivalence study using scaled-average-bioequivalence according to $[U, L] = \exp [\pm K.s_{WR}]$, where U is the upper limit of the acceptance range, L is the lower limit of the acceptance range, k is the regulatory constant set to 0.760 and s_{WR} is the intra-subject standard deviation of the log-transformed values of C_{max} of the reference listed product. It is recommended to have a s_{WR} cutoff value of 0.294, at or above which reference scaling is permitted and below which the unscaled limits of 0.8 –1.25 are applied. The selection of 0.294

as the variation at which use of reference scaling of the limits is permissible is consistent with the general understanding that drugs are considered highly variable if the within-subject CV % observed in the study is > 30 %, and, as such, is determined by using the conversion formula of $S^2 = \ln(CV^2 + 1)$.

The following table gives examples of how different levels of variability lead to different acceptance limits using this methodology:

Intra-subject CV (%)	Lower limit	Upper limit
30	80.00	125.00
35	77.23	129.48
40	74.62	134.02
45	72.15	138.59
≥ 50	69.84	143.19

$$CV\ (\%) = \sqrt{(e^{S_{WR}^2}) - 1}$$

The geometric mean ratio (GMR) for C_{max} should lie within the conventional acceptance range 80.00–125.00%. The standard bioequivalence acceptance criterion for AUC should be maintained without scaling. If the intra-subject variability for C_{max}, following replicate administration of the reference listed product, is found to be < 30%, standard bioequivalence acceptance criteria should be applied to both AUC and C_{max} without scaling.

For multiple dose studies, a similar approach can be applied to the following parameters if the intra-subject variability for the parameter is found to be > 30%: C_{max}, C_{tau} and partial AUCs if required. The standard bioequivalence acceptance criterion will apply to AUCτ without scaling. The approach to be employed should be clearly defined prospectively in the study protocol.

3.3 Narrow therapeutic index drugs

Narrow Therapeutic Index (NTI) Drugs or Critical Dose Drugs are defined as drugs where small differences in dose or blood concentration may lead to serious therapeutic failures and/or

adverse drug reactions that are life-threatening or result in persistent or significant disability or incapacity and they should meet assayed potency specifications of 95.0% to 105.0% that allow interchangeability between reference listed ang generic drug products:

- There is little separation between therapeutic and toxic doses or associated blood/plasma concentrations.
- Subtherapeutic concentrations may lead to serious therapeutic failure and/or above-therapeutic concentrations may lead to serious adverse drug reactions in patients.
- Subject to therapeutic monitoring based on pharmacokinetic or pharmacodynamics measures.
- Possess low-to-moderate (i.e., no more than 30 %) within-subject variability.
- In clinical practice, doses are often adjusted in very small increments (less than 20 %).

List of examples of narrow therapeutic index drugs:

"Aminophylline, Aprindine, Carbamazepine, Clindamycin, Clonazepam, Clonidine, Colchicine, Cyclosporine, Digitoxin, Digoxin, Disopyramide, Dyphylline, Ethinyl Estradiol, Ethosuximide, Flecainide, Guanethidine, Isoetharine Mesylate, Isoprenaline, Isoproterenol, Levoxyine, Lithium Carbonate, Metaporterenol, Methotrexate, Minoxidil, Oxytriphylline, Phenobarbital, Phenytoin, Prazosin, Primidone, Procainamide, Quinidine Gluconate, Sirolimus, Sulfonylurea Antidiabetic Drugs Compounds, Tacrolimus, Theophylline Compounds, Valproate Sodium, Valproic Acid, Warfarin Sodium, Zonisamide, and Glybuzole".

The applicant may conduct a BE study that met one of the following approaches:

(1) A single-dose, two-way crossover or parallel study in healthy subjects should be done to demonstrate bioequivalence of NTI drugs. The acceptance interval for AUC of NTI drugs should be tightened to 90.00 –111.11 %. Where C_{max} is of particular importance for safety, efficacy, or drug level monitoring, the 90.00 –111.11 % acceptance interval should also be applied for C_{max}.

(2) A four-way, crossover, fully replicated study design may also be done and it is preferred

because such a study design will permit variability comparison in addition to the mean comparison. Both comparisons have to be considered when declaring bioequivalent. The baseline BE limits for NTI drugs is 90 –111 %, which would be scaled based on the within-subject variability of the reference listed product. When the reference variability is ≤ 10 %, the BE limits will be narrower than 90 –111 %. Conversely, when the reference variability is >10 %, the BE limits will be wider than 90 –111 %, but are capped at 80 – 125 %. To ensure that the BE limits for NTI drugs are never wider than those for conventional drugs, it is critical that every study pass the scaled average BE and the unscaled average BE limits of 80 –125 %. Because most NTI drugs have low within-subject variability, the BE limits for these drug products would almost always be tightened to less than 80–125 % accordingly. The four-way, crossover, fully replicated study design will also permit the comparison of within-subject variability in the test and reference listed products to confirm that their variances do not differ significantly. When test and reference listed products have unacceptably large differences in within-subject variability, it may still pass the reference-scaled BE limits, suggesting that the reference-scaled average bioequivalence approach alone is not adequate to ensure the similarity of test and reference listed products for NTI drugs. Thus an F-test should be done to evaluate whether the within-subject variability of test and reference listed products are comparable by calculating the 90 % confidence interval of the ratio of the within-subject standard deviation of the test to reference listed product with an appropriate upper limit of the 90 % confidence interval that should be ≤ 2.5.

3.4 Pharmacodynamic studies

Studies in healthy subjects or patients using pharmacodynamic (PD) measurements may be used for establishing equivalence between two pharmaceutical products. Pharmacodynamic bioequivalence studies may become necessary if quantitative analysis of the APIs and / or metabolite(s) in plasma or urine cannot be made with sufficient accuracy and sensitivity.

Pharmacodynamic bioequivalence studies may be appropriate for pharmaceutical products administered topically and for inhalation dosage forms. If pharmacodynamic studies are to be used they must be performed as rigorously as bioequivalence studies, and the principles of GCP must be followed.

Overall, PD endpoint studies are very useful to establish bioequivalence of drug products when pharmacokinetic (PK) endpoint and in vitro approaches are not applicable. An ideal PD endpoint for establishing bioequivalence needs to (1) be sensitive (steep dose–response curve); (2) be reproducible; (3) have low variability of PD response at baseline and following drug treatment; and (4) have adequate statistical power with feasible sample size. Besides the PD endpoint selection, the study design, pilot study, and study population are critical for the success of the study. This approach can be useful for some products such as orally inhaled formulations and topically applied dermatologic formulations.

The following requirements must be recognized when planning, conducting and assessing the results of a study intended to demonstrate equivalence by measuring pharmacodynamic drug responses:

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

• The methodology must be validated for precision, accuracy, reproducibility and specificity.

• Neither the test product nor the reference listed product should produce a maximal response in the course of the study, since it may be impossible to detect differences between formulations given in doses which give maximum or near-maximum effects. Investigation of dose–response relationships may be necessary part of the design.

• The response should be measured quantitatively, preferably under double-blind conditions, and be recordable by an instrument that produces and records the results of repeated measurements to provide a record of the pharmacodynamic events, which are substitutes for

measurements of plasma concentrations. Where such measurements are not possible, recordings on visual analogue scales may be used. Where the data are limited to qualitative (categorized) measurements appropriate special statistical analysis will be required.

• volunteers should be screened prior to the study to exclude non-responders. The criteria by which responders are distinguished from non-responders must be stated in the protocol.

• In instances where an important placebo effect can occur, comparison between pharmaceutical products can only be made by a priori consideration of the potential placebo effect in the study design. This may be achieved by adding a third phase with placebo treatment in the design of the study.

• The underlying pathology and natural history of the condition must be considered in the study design. There should be knowledge of the reproducibility of baseline conditions.

• A cross-over design can be used. Where this is not appropriate, a parallel group study design should be chosen.

• The selection basis for the generic and reference listed products should be the same as described under pharmacokinetic bioequivalence studies.

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

• The statistical considerations for the assessment of the outcome of the study are in principle the same as those outlined for the analysis of pharmacokinetic bioequivalence studies. However, a correction for the potential non-linearity of the relationship between the dose and the area under the effect–time curve should be performed on the basis of the outcome of the dose-ranging study.

• It should be noted that the acceptance range as applied for bioequivalence assessment may

not be appropriate and should be justified on a case-by-case basis and defined in the protocol.

• In some cases, when a drug substance produces its effects by local action in the gastrointestinal tract, it may be appropriate to determine bioequivalence using pharmacokinetic endpoints. In other cases, it may be appropriate to determine bioequivalence using clinical endpoints, pharmacodynamic endpoints and / or suitably designed and validated in vitro studies in addition to, or instead of, measuring drug plasma concentrations.

3.5 Clinical Bioequivalence Study

On conducting a clinical bioequivalence study the following should be defined in the protocol:

• The target parameters that usually represent relevant clinical end-points from which the onset, if applicable and relevant and intensity of the response are to be derived.

• The number of patients which will depend on the variability of the target parameters and the acceptance range and is usually much higher than the number of subjects needed in pharmacokinetic bioequivalence studies to achieve adequate statistical power.

• The size of the acceptance range has to be defined case by case, taking into consideration the specific clinical conditions. These include, among others, the natural course of the disease, the efficacy of available treatments and the chosen target parameter.

• The size of the acceptance range in clinical trials should be set individually according to the therapeutic class and indication(s).

• A one-sided confidence interval (for efficacy and/or safety) may be appropriate. The confidence intervals can be derived from either parametric or non-parametric methods.

• Where appropriate, a placebo leg should be included in the design.

• In some cases it is relevant to include safety end-points in the final comparative assessments.

• The selection basis for the generic and reference products should be the same for in vivo equivalence studies.

3.6 Non-linear pharmacokinetics

In case of non-linear pharmacokinetics (i.e. not proportional increase in AUC with increased dose) there may be a difference between different strengths in the sensitivity to detect potential differences between formulations. In the context of this guideline, pharmacokinetics is considered to be linear if the difference in dose-adjusted mean AUCs is no more than 25% when comparing the studied strength (or strength in the planned bioequivalence study) and the strength(s) for which a waiver is considered.

• **For drugs with non-linear pharmacokinetics characterised by a more than proportional increase in AUC with increasing dose over the therapeutic dose range,** the bioequivalence study should in general be conducted at the highest strength. As for drugs with linear pharmacokinetics a lower strength may be justified if the highest strength cannot be administered to healthy subjects for safety / tolerability reasons. Likewise a higher dose may be used in case of sensitivity problems of the analytical method.

• **For drugs with a less than proportional increase in AUC with increasing dose over the therapeutic dose range,** bioequivalence should in most cases be established both at the highest strength and at the lowest strength (or a strength in the linear range), i.e. in this situation two bioequivalence studies are needed. If the non-linearity is not caused by limited solubility but is due to e.g. saturation of uptake transporters and provided that conditions of the general requirements for waiving are fulfilled and the test and reference listed products do not contain any excipients that may affect gastrointestinal motility or transport proteins, it is sufficient to demonstrate bioequivalence at the lowest strength (or a strength in the linear range).

Selection of other strengths may be justified if there are analytical sensitivity problems preventing a study at the lowest strength or if the highest strength cannot be administered to healthy subjects for safety / tolerability reasons.

3.7 Bracketing approach

Where bioequivalence assessment at more than two strengths is needed, e.g. because of deviation from proportional composition, a bracketing approach may be used. In this situation it can be acceptable to conduct two bioequivalence studies, if the strengths selected represent the extremes, e.g. the highest and the lowest strength or the two strengths differing most in composition, so that any differences in composition in the remaining strengths is covered by the two conducted studies.

Where bioequivalence assessment is needed both in fasting and in fed state and at two strengths due to nonlinear absorption or deviation from proportional composition, it may be sufficient to assess bioequivalence in both fasting and fed state at only one of the strengths. Waiver of either the fasting or the fed study at the other strength(s) may be justified based on previous knowledge and/or pharmacokinetic data from the study conducted at the strength tested in both fasted and fed state. The condition selected (fasting or fed) to test the other strength(s) should be the one which is most sensitive to detect a difference between products.

3.8 Orodispersible tablets (ODT)

If the ODT is a generic / hybrid to an approved ODT reference listed medicinal product, ***the following recommendations regarding study design apply:***

• If the reference listed medicinal product can be taken with or without water, bioequivalence should be demonstrated without water as this condition best resembles the intended use of the formulation. This is especially important if the substance may be dissolved and partly absorbed in the oral cavity. If bioequivalence is demonstrated when taken without water, bioequivalence when taken with water can be assumed.

• If the reference listed medicinal product is taken only in one way (e.g. only with water), bioequivalence should be shown in this condition (in a conventional two-way crossover design).

• If the reference listed medicinal product is taken only in one way (e.g. only with water), and

the test product is intended for additional ways of administration (e.g. without water), the conventional and the new method should be compared with the reference listed in the conventional way of administration (3 treatment, 3 period, 6 sequence design).

In studies evaluating ODTs without water, it is recommended to wet the mouth by swallowing 20 ml of water directly before applying the ODT on the tongue. It is recommended not to allow fluid intake earlier than 1 hour after administration.

Other oral formulations such as orodispersible films, buccal tablets or films, sublingual tablets and chewable tablets may be handled in a similar way as for ODTs. Bioequivalence studies should be conducted according to the recommended use of the product.

3.9 Oral solutions

If the test product is an aqueous oral solution at time of administration and contains an active substance in the same concentration as an approved oral solution, bioequivalence studies may be waived. However if the excipients may affect gastrointestinal transit (e.g. sorbitol, mannitol, etc.), absorption (e.g. surfactants or excipients that may affect transport proteins), in vivo solubility (e.g. co-solvents) or in vivo stability of the active substance, a bioequivalence study should be conducted, unless the differences in the amounts of these excipients can be adequately justified by reference to other data.

3.10 Parenteral solutions

Bioequivalence studies are generally not required if the test product is to be administered as an aqueous intravenous solution containing the same active substance as the currently approved product.

However, if any excipients interact with the drug substance (e.g. complex formation), or otherwise affect the disposition of the drug substance, a bioequivalence study is required unless both products contain the same excipients in very similar quantity and it can be adequately justified that any difference in quantity does not affect the pharmacokinetics of the active substance.

In the case of other parenteral routes, e.g. intramuscular or subcutaneous, and when the test product is of the same type of solution (aqueous or oily), contains the same concentration of the same active substance and the same excipients in similar amounts as the medicinal product currently approved, bioequivalence studies are not required. Moreover, a bioequivalence study is not required for an aqueous parenteral solution with comparable excipients in similar amounts, if it can be demonstrated that the excipients have no impact on the viscosity.

3.11 Liposomal, micellar and emulsion dosage forms for intravenous use

• **Liposomal formulations:** Pharmacokinetic issues related to liposomal formulations for I.V. administration require special considerations which are not covered by the present guideline.

• **Emulsions:** Emulsions normally do not qualify for a biowaiver.

However, emulsion formulations may be considered eligible for a biowaiver where:

(a) The drug product is not designed to control release or disposition;

(b) The method and rate of administration is the same as the currently approved product.

In these cases, the composition should be qualitatively and quantitatively the same as the currently approved emulsion and satisfactory data should be provided to demonstrate very similar physicochemical characteristics, including size distribution of the dispersed lipid phase, and supported by other emulsion characteristics considered relevant e.g. surface properties, such as Zeta potential and rheological properties.

• **Lipids for intravenous parenteral nutrition:** They may be considered eligible for a biowaiver if satisfactory data are provided to demonstrate comparable physicochemical characteristics. Differences in composition may be justified taking into consideration the nature and the therapeutic purposes of such dosage forms.

• **Micelle forming formulations:** Micelle solutions for intravenous administration may be regarded as "complex" solutions and therefore normally do not qualify for a biowaiver.

However, micelle formulations may be considered eligible for a biowaiver where:

(a) Rapid disassembly of the micelle on dilution occurs and the drug product is not designed to control release or disposition;

(b) The method and rate of administration is the same as the currently approved product;

(c) The excipients do not affect the disposition of the drug substance.

In these cases, the composition of the micelle infusion, immediately before administration, should be qualitatively and quantitatively the same as that currently approved and satisfactory data should be provided to demonstrate similar physicochemical characteristics. For example, the critical micelle concentration, the solubilisation capacity of the formulation (such as Maximum Additive Concentration), free and bound active substance and micelle size.

This also applies in case of minor changes to the composition quantitatively or qualitatively, provided this does not include any change of amount or type of surfactants.

3.12 Modified release dosage forms with systemic action

- **Modified release oral dosage forms:** Bioequivalence studies are required under fast and fed states.

- **Modified release transdermal dosage forms:** Bioequivalence studies are required.

- **Modified release intramuscular or subcutaneous dosage forms:** For suspensions or complexes or any kind of matrix intended to delay or prolong the release of the active substance for I.M. or S.C. administration, demonstration of bioequivalence follows the rules for extra vascular modified release formulations, e.g. transdermal dosage forms as per corresponding guideline.

3.13 Locally acting locally applied products

For products for local use (after oral, nasal, pulmonary, ocular, dermal, rectal, vaginal,…etc. administration) intended to act at the site of application, recommendations can be found in other guidelines.

A waiver of the need to provide equivalence data may be acceptable in the case of solutions,

e.g. eye drops, nasal sprays or cutaneous solutions, if the test product is of the same type of solution (aqueous or oily), and contains the same concentration of the same active substance as the medicinal product currently approved. Minor differences in the excipient composition may be acceptable if the relevant pharmaceutical properties of the test product and reference listed product are identical or essentially similar.

Any qualitative or quantitative differences in excipients must be satisfactorily justified in relation to their influence on therapeutic equivalence. The method and means of administration should also be the same as the medicinal product currently approved, unless otherwise justified.

Whenever systemic exposure resulting from locally applied, locally acting medicinal products entails a risk of systemic adverse reactions, systemic exposure should be measured. It should be demonstrated that the systemic exposure is not higher for the test product than for the reference listed product, i.e. the upper limit of the 90% confidence interval should not exceed the upper bioequivalence acceptance limit 125.00.

3.14 Locally Acting Gastrointestinal Drugs

The function of locally acting gastrointestinal (GI) drug products is to deliver active ingredients directly to the site of action in the GI tract, which allows the intended therapeutic effect to occur without entering the systemic circulation. While local delivery is excellent from a therapeutic effect standpoint, it presents challenges when attempting to evaluate bioequivalence using standard techniques.

There is a strong possibility that systemic exposure may not be directly correlated to the local concentration of the drug in the GI tract. In order to confirm bioequivalence, a selection of BE methods are often used depending on considerations of various factors, such as mechanism of drug delivery, mechanism of drug release, systemic absorption of the drug, drug physiochemical properties, and study feasibility.

The following are some examples of locally acting GI drug products and respective BE methods:

Product category	Bioequivalence methods
Insoluble binding agents.	In vitro disintegration and binding assay.
High solubility immediate release dosage forms.	In vitro dissolution in addition to studies to show that any difference in formulation does not affect the safety and efficacy of drug product.
Low solubility immediate release dosage forms.	In vivo PK, in vivo PD, or clinical studies or combination of two methods.
Modified release dosage forms.	In vitro dissolution, in vivo PK or in vivo PD, or clinical studies, or combination of two methods.

3.15 Gases

If the product is a gas for inhalation, bioequivalence studies are not required.

3.16 Chewable Tablets

Applicants should administer chewable tablets according to the directions on the label. If the label states that the tablet must be chewed before swallowing, the product should be chewed when administered in bioequivalence studies. If the label gives the option of either chewing the product or swallowing it whole, the product should be swallowed whole, with (150-250) ml of water, when administered in bioequivalence studies. It is also recommended to conduct in vitro dissolution testing on intact, whole tablets of the chewable drug product.

According to ICH- M13A If the comparator product labelling states that the chewable tablets can be taken with or without water, the test and comparator products should be administered in the BE study without water, as this is considered to be the more discriminating scenario. BE of the test and comparator chewable tablet products taken with water can then be inferred.

3.17 Oral Suspension

For tablets, granules, and powders labelled as being only intended to be dispersed in a liquid before administration as an oral suspension, BE studies should be conducted according to the comparator product labelling.

SECTION 4

IN VITRO DISSOLUTION TESTING

4.1 Purposes for in vitro dissolution testing

4.1.1 Testing on product quality

• To get information on the test batches used in bioavailability/bioequivalence studies and pivotal clinical studies to support specifications for quality control.

• To be used as a tool in quality control to demonstrate consistency in manufacture.

• To get information on the reference listed product used in bioavailability/bioequivalence studies and pivotal clinical studies.

4.1.2 Bioequivalence surrogate inference

• To demonstrate in certain cases similarity between different formulations of an active substance and the reference listed medicinal product (biowaivers e.g., variations, formulation changes during development and generic medicinal products)

• To investigate batch to batch consistency of the products (test and reference listed) to be used as basis for the selection of appropriate batches for the in vivo study.

It isn't recommended using in vitro approaches for drug products that are intended to be systemically absorbed. Such approaches would be appropriate; however, in other circumstances (e.g., for drug products that bind bile acids in the gastrointestinal tract).

In vitro dissolution studies should be based on the generation of comparative dissolution profiles rather than a single-point dissolution test. The dissolution profile of the two products should be measured under the same test conditions.

4.2 The usual experimental conditions for in vitro dissolution testing

- **Apparatus:** paddle (USP Apparatus II) or basket (USP Apparatus I);

- **Volume of dissolution medium:** 900 ml or less;

- **Temperature of the dissolution medium:** 37±1 °C;

- **Agitation:** paddle apparatus (usually 50 – 75 rpm) & basket apparatus (usually 100 rpm) ;

- **Media:** pH 1.2 (usually 0.1 N HCl or Simulated Gastric Fluid "SGF" without enzymes), pH 4.5 (usually Acetate Buffer), and pH 6.8 (usually Phosphate Buffer or Simulated Intestinal Fluid "SIF" without enzymes); in addition to the most suitable medium which should be used based on FDA-Recommended Dissolution Methods or pharmacopeial requirements.

- **Sampling time points:**

- Based on FDA-Recommended Dissolution Methods & pharmacopeial requirements.
- If not stated, they should be sufficient to obtain meaningful dissolution profiles, and at least every 15 minutes. More frequent sampling during the period of greatest change in the dissolution profile is recommended, in case of immediate-release products, Sampling time at 15 minutes is necessary.
- Number of sampling time points should be sufficient to calculate similarity.
- For very rapidly & rapidly dissolving products, where complete dissolution is within 15 or 30 minutes, generation of an adequate profile by sampling at 5- or 10-minute intervals may be necessary.

The dissolution sampling times for both generic and reference product profiles should be the same e.g.:

- For example for immediate-release products 5, 10, 15, 20, 30, 45 and 60 minutes;

- For example for 12 hour extended-release products 1, 2, 4, 6, 8 and 12 hours;

- For example for 24 hour extended-release products 1, 2, 4, 6, 8, 16 and 24 hours.

- Other conditions: no surfactant should be added at pH 1.2, 4.5 or 6.8; in case of gelatin capsules or tablets with gelatin coatings the use of enzymes may be acceptable.

- Number of dosage units: A minimum of 12 dosage units of each product should be evaluated.

• At pH values where sink conditions may not be achievable for all strengths in vitro dissolution may differ between different strengths. However, the comparison with the respective strength of the reference listed medicinal product should then confirm that this finding is drug substance

rather than formulation related. In addition, the applicant could show similar profiles at the same dose (e.g. as a possibility two tablets of 5 mg versus one tablet of 10 mg could be compared).

Samples should be analyzed using a validated method of analysis.

- For delayed-release and enteric-coated products, it is recommended to perform in vitro dissolution testing based on FDA-Recommended Dissolution Methods or pharmacopeial requirements (if present), otherwise, it should be performed at two different pHs only (At acidic stage & buffer stage).

- With regard to stability, the validated dissolution test should appropriately reflect relevant changes in the drug product over time that are caused by temperature, humidity, photosensitivity, and other stresses. Physical and chemical data for the drug substance and dosage unit need to be determined before selecting the dissolution medium. Two key properties of the drug are the solubility and solution state stability of the drug as a function of the pH value. When selecting the composition of the medium, the influence of buffers, pH value, and surfactants on the solubility and stability of the drug need to be evaluated. The specificity should be extended to analysis in the presence of possible degradation products. Alternatively, the peak purity test results of the main peak should be presented. This is particularly required for in house developed assays.

4.3 In vitro dissolution tests complementary to bioequivalence studies

In case of performing the in vivo bioequivalence (biowaiver is not applicable), the complementary dissolution test required should abide to the usual experimental conditions mentioned above.

In the event that the results of comparative in vitro dissolution of the biobatches do not reflect bioequivalence as demonstrated in vivo the latter prevails. However, possible reasons for the discrepancy should be addressed and justified.

4.4 Similarity of dissolution profiles

- For Pharmacopoeial products should achieve % dissolved (Q) in USP.
- The dissolution profiles of the generic and reference listed products can be compared using a similarity factor (f_2).

The evaluation of the similarity factor is based on the following conditions:

• A minimum of three time points (zero excluded);

• The time points should be the same for the two formulations;

• Twelve individual values for every time point for each formulation;

• Not more than one mean value of > 85% dissolved for both of the formulations;

• To allow use of mean data, The relative standard deviation or the percent coefficient of variation of any product should be less than 20% for the first earlier time point and less than 10% from second to last time point.

• Early time points of the dissolution profile can be obtained as 5, 10 or 15 minutes for immediate release products and 2 hours for modified release products; except delayed release and enteric coated products; furthermore, early time points should not be omitted from calculation of similarity factor not only because there was no scientific reason to exclude it but because the amount released was considered relevant. The choice of early time points in a comparative dissolution profile test should be based on the relevance (mainly amount released and release controlling mechanism).

An f_2 value of 50 or greater (50–100) reflects sameness or equivalence of the two curves (less than 10% difference) and thus equivalence of the in vitro performance of the two products.

The similarity factor f_2 is to be computed using the equation:

$$f_2 = 50 \times \log \{[1 + (1/n)\Sigma_{t=1}^{n}(R_t - T_t)^2]^{-0.5} \times 100\}$$

Where R_t and T_t are the cumulative percentage of the drug dissolved at each of the selected, n time-points of the reference listed and generic (test) product respectively.

For immediate release formulations, comparison at 15 minutes is essential to know if complete dissolution is reached before gastric emptying.

Where more than 85% of the drug is dissolved within 15 minutes i.e., very rapidly dissolving, for both the test and the reference listed products in all media dissolution profiles may be accepted as similar without further mathematical evaluation.

In case more than 85% is not dissolved at 15 minutes but within 30 minutes i.e., similarly rapidly dissolving, at least three time points are required: the first time point before 15 minutes, the second one at 15 minutes and the third time point when the release is close to 85%.

Other appropriate statistical methods can also be used for comparison of dissolution profiles, provided that the same criterion is used for acceptance (maximum 10% difference between the profiles).

The difference "dissimilarity" factor (f_1) calculates the percent (%) difference between the two curves at each time point and is a measurement of the relative error between the two curves.

The difference "dissimilarity" factor (f_1) is to be computed using the equation:

$$f_1 = \{ [\Sigma_{t=1}^{n} |R_t - T_t|] / [\Sigma_{t=1}^{n} R_t] \} \times 100$$

- The difference "dissimilarity" factor (f_1) is required in additional to the similarity factor (f_2).

Model Independent Multivariate Confidence Region Procedure

In instances w.here within batch variation is more than 15% CV, a multivariate model independent procedure is more suitable for dissolution profile comparison. The following steps are suggested:

1. Determine the similarity limits in terms of multivariate statistical distance (MSD) based on interbatch differences in dissolution from reference(standard approved) batches.
2. Estimate the MSD between the test and reference mean dissolutions.
3. Estimate 90% confidence interval of true MSD between test and reference batches.
4. Compare the upper limit of the confidence interval with the similarity limit.

The test batch is considered similar to the reference batch if the upper limit of the confidence interval is less than or equal to the similarity limit.

4.5 Waiver of in vivo bioequivalence studies (Biowaiver)

4.5.1 Biowaivers based on the biopharmaceutics classification systems (BCS)

4.5.1.1 Overview

The BCS is a scientific framework for classifying drug substances based on their aqueous solubility and intestinal permeability. When combined with the dissolution of the drug product, the BCS takes into account three major factors that govern the rate and extent of drug absorption from IR solid oral dosage forms: (1) dissolution, (2) solubility, and (3) intestinal permeability.

According to the BCS, drug substances are classified as follows:

Class I: High Solubility - High Permeability (Absorption) Drugs

Class II: Low Solubility - High Permeability (Absorption) Drugs

Class III: High Solubility - Low Permeability (Absorption) Drugs

Class IV: Low Solubility - Low Permeability (Absorption) Drugs

Goals of the BCS:

• To improve the efficiency of drug development and the review process by recommending a strategy for identifying expendable clinical bioequivalence tests.

• To recommend a class of immediate-release (IR) solid oral dosage forms for which bioequivalence may be assessed based on in vitro dissolution tests.

• To recommend methods for classification according to dosage form dissolution, along with the solubility and permeability characteristics of the drug substance.

• Solubility

The pH-solubility profile of the drug substance should be determined and discussed. The drug substance is considered highly soluble if the highest single dose administered as immediate

release formulation(s) is completely dissolved in 250 ml of buffers within the range of pH 1 – 6.8 at 37±1 °C. This demonstration requires the investigation in at least three buffers within this range (preferably at pH 1.2, 4.5 and 6.8) and in addition at the pKa, if it is within the specified pH range. Replicate determinations at each pH condition may be necessary to achieve an unequivocal solubility classification (e.g. shake-flask method or other justified method). Solution pH should be verified prior and after addition of the drug substance to a buffer.

- **Absorption**

The demonstration of complete absorption in humans is preferred for BCS-based biowaiver applications. For this purpose complete absorption is considered to be established where measured extent of absorption is ≥ 85 %. Complete absorption is generally related to high permeability. Complete drug absorption should be justified based on reliable investigations in human. Data from absolute bioavailability or mass-balance studies could be used to support this claim.

4.5.1.2 BCS-Class I biowaivers are applicable for an immediate release drug product if:

a) The drug substance has been proven to exhibit high solubility and complete absorption; **and**

b) Either very rapid (> 85 % within 15 minutes) or similarly rapid (85 % within 30 minutes) in vitro dissolution characteristics of the test and reference listed products have been demonstrated considering specific requirements; **and**

c) Excipients are not suspect of having any relevant impact on bioavailability.

4.5.1.3 BCS-Class III biowaivers are also applicable for an immediate release drug product if:

a) The drug substance has been proven to exhibit high solubility and limited absorption where extent of absorption is less than 85%; **and**

b) very rapid (> 85 % within 15 minutes) in vitro dissolution of the test and reference listed

products have been demonstrated considering specific requirements; **and**

c) Excipients in both the test and reference listed products are qualitatively the same and quantitatively very similar. This is due to the concern that excipients can have a greater impact on absorption of low permeability drugs.

4.5.1.4 BCS-Class II Weak Acids biowaivers are also applicable for an immediate release drug product if:

a) The APIs has a dose: solubility ratio of 250ml or less at pH 6.8; **and**

b) The generic product is rapidly dissolving (no less than 85% in pH 6.8 in 30minutes) ; **and**

c) Its dissolution profile is similar to that of the reference listed product at pH 1.2, 4.5 and 6.8; **and**

d) The excipients should additionally be critically evaluated in terms of type and amounts, e.g. of surfactants, in the formulation.

- Furthur, if the C_{max} is critical to the therapeutic efficacy of the APIs, the risk of reaching an inappropriate biowaiver decision is existing and unacceptable. (i.e., If the time to maximum plasma concentration is critical to the intended use, biowaiving concept based on BCS does not apply, e.g, labeling claims of early or rapid onset of action "e.g., rapid analgesia, rescue medications, etc.").
- Biowaiver may be applicable when the active substance(s) in test and reference listed products are identical. Biowaiver may also be applicable if test and reference listed products contain different salts provided that both belong to BCS-class I. Biowaiver is not applicable when the test product contains a different ester, ether, isomer, mixture of isomers, complex or derivative of an active substance from that of the reference listed product, since these differences may lead to different bioavailabilities not deducible by means of experiments used in the BCS-based biowaiver concept.
- The drug substance should not belong to the group of "narrow therapeutic index" drugs or

drugs with non-linear pharmacokinetics and modified release formulations. In addition, it is not applicable for dosage forms intended for absorption in the oral cavity (e.g., sublingual or buccal formulations). For orodispersible formulations the BCS-based biowaiver approach may only be applicable when absorption in the oral cavity can be excluded.

- Generally, Excipients that might affect bioavailability, like e.g. sorbitol, mannitol, sodium lauryl sulfate, polysorbate 80 or other surfactants, should be identified as well as their possible impact on gastrointestinal motility, susceptibility of interactions with the drug substance (e.g. complexation), drug permeability, and interaction with membrane transporters or other processes affecting absorption should be qualitatively and quantitatively the same in the test product and the reference listed product.
- Permeability of prodrugs will generally depend on the mechanism and (anatomical) site of conversion to the drug substance. When the prodrug-to-drug conversion is shown to occur predominantly after intestinal membrane permeation, the permeability of the prodrug should be measured. When this conversion occurs prior to intestinal permeation, the permeability of the drug should be determined. Dissolution and pH-solubility data on both prodrug and drug can be relevant.

Fixed Dose Combinations

a. If all active components belong to BCS class I: BCS-based biowaivers are applicable for IR fixed dose combination products if all the drugs in the combination belong to BCS class I; provided there is no pharmacokinetic interaction between the components, and the excipients are not suspect of having any relevant impact on bioavailability. If there is a pharmacokinetic interaction, the excipients in both the test and reference listed products are qualitatively the same and quantitatively very similar. Otherwise, in vivo bioequivalence testing is required.

b. If all components of the combination belong to BCS class III or a combination of class I and III: BCS-based biowaivers are applicable for IR fixed dose combination products in this situation provided the excipients in both the test

and reference listed products are qualitatively the same and quantitatively very similar. Otherwise, in vivo bioequivalence testing is required.

4.5.2 Biowaivers based on dose-proportionality of formulations

A prerequisite for qualification for a biowaiver based on dose-proportionality of formulations is that the generic product at one strength has been shown in in vivo studies to be bioequivalent to the corresponding strength of the reference listed product.

The following general requirements must be met where a waiver for additional strength(s) is claimed:

(I) The pharmaceutical products are manufactured by the same manufacturing process,

(II) The qualitative composition of the different strengths is the same,

(III) The composition of the strengths are quantitatively proportional, i.e. the ratio between the amount of each excipient to the amount of active substance(s) is the same for all strengths (e.g. a tablet of 50 mg strength has all the active and inactive ingredients exactly half that of a tablet of 100 mg strength, and twice that of a tablet of 25 mg strength),

(For immediate release products coating components, capsule shell, color agents and flavors' are not required to follow this rule).

If there is some deviation from quantitatively proportional composition, condition (III) is still considered fulfilled if one of the following is met:

(1) Condition i) and ii) or i) and iii) below apply to the strength used in the bioequivalence study and the strength(s) for which a waiver is considered:

i. the amount of the active substance(s) is less than 5 % of the tablet core weight, the weight of the capsule content;

ii. The amounts of the different core excipients or capsule content are the same for the concerned strengths and only the amount of active substance is changed;

iii. The amount of a filler is changed to account for the change in amount of active substance. The amounts of other core excipients or capsule content should be the same for the concerned strengths.

(2) For a high potency APIs, where the amount of the APIs in the dosage form is relatively low (up to 10 mg per dosage unit):

a) The total weight of the dosage form remains nearly the same for all strengths (within ±10% of the total weight);
b) The same inactive ingredients are used for all strengths;
c) The change in any strength is obtained by altering the amount of the active ingredients only or with change in one or more of the inactive ingredients.

(IV) Appropriate in vitro dissolution data should confirm the adequacy of waiving additional in vivo bioequivalence testing. Comparative dissolution testing should be conducted on 12 dosage units each of all strengths of the test with each of all respective strengths of the reference listed products.

Biowaivers based on dose-proportionality of modified-release beaded capsules formulations can also be applied when different strengths have been achieved solely by means of adjusting the number of beads (i.e., different strengths of capsules having the same beads' composition qualitatively and quantitatively but differ only in their number).

4.5.3 Biowaivers for scale-up and post-approval changes

Following minor formulation or manufacturing changes after drug approval, in vitro dissolution testing may also be suitable to confirm similarity of product quality and performance characteristics

SECTION 5

ADDITIONAL GUIDANCE FOR ORGANIZATIONS PERFORMING IN VIVO BIOEQUIVALENCE STUDIES

The objective of this document is to provide guidance to organizations involved in the conduct and analysis of in vivo bioequivalence studies.

I- Organization and management

1. The CRO should have an organizational chart that lists the key positions and the names of responsible people. The organizational chart should be authorized (signed and dated).

2. There should be job descriptions for all personnel, including a description of the responsibilities of key personnel.

At a minimum, the CRO management should:

- Ensure that the principles of GCP and GLP, as appropriate, are complied with in the CRO;
- Ensure that a sufficient number of qualified personnel, appropriate facilities, equipment and materials are available for the timely and proper conduct of the study;
- Ensure the maintenance of a record of the qualifications, training, experience and job description for each professional and technical individual;
- Ensure that personnel clearly understand the functions they are to perform and, where necessary, provide training for these functions;
- Ensure that appropriate and technically valid SOPs are established and followed, and approve all original and revised SOPs and ensure the maintenance of a historical file of all SOPs;
- Ensure that there is a quality assurance (QA) programme with designated personnel and assure that the QA responsibility is being performed in accordance with the principles of GLP and GCP, as appropriate;
- Ensure that an individual is identified as responsible for the management of the archive(s),

and ensure that the documents transferred to the archives are kept under adequate conditions for the appropriate duration;

- Ensure that supplies meet requirements appropriate to their use in a study;
- Establish procedures to ensure that computerized systems are suitable for their intended purpose, and are validated, operated and maintained in accordance with the principles of GCP and GLP, as appropriate.

II- Computer systems

Hardware

1. There should be a sufficient number of computers to enable personnel to perform data entry and data handling, required calculations and compilation of reports.

2. Computers should have sufficient capacity and memory for the intended use.

3. There should be controlled access to the study-related information entered and stored in computers. The method of access control should be specified (e.g. password protection) and a list of people who have access to the database should be maintained.

Software

1. The software programs selected should be suitable for the intended use.

2. Software programs used, frequency of virus testing, storage of data and the making, archiving and keeping of back-ups should be specified in writing.

3. The programs used should be able to provide the required quality and management information, reliably and accurately. Necessary programmes for data management include word processing, data entry, databases, graphics, pharmacokinetics and statistical programmes.

4. There should be SOPs in place for usage of each software program that is used to perform key steps of a BE study.

5. There should be a system in place for the implementation of regular updates to key software programs (e.g. such as those

used for control and data processing of chromatographic and mass spectrometry systems) whenever required, following an appropriate risk assessment on the potential impact that it could have on current data and on qualification/validation status.

Networks

1. Networks, including the full client/server architecture and interfaces such as laboratory information management systems, when used, should be appropriately designed, qualified, managed and controlled.

2. Access to each component of the system by the different users at any given organization involved in the studies, should be appropriately defined, controlled and documented.

3. There should be a documented inventory of all computerized systems on the network. Any changes to the network, including the temporary addition or removal of systems from the network, should be documented.

Data management

1. Data entry includes transfer of the data from source data forms, case-report forms (CRF) and analytical data to the computerized system for pharmacokinetic and statistical analysis and reporting.

2. Data-entry procedures should be designed to prevent errors. The data-entry process should be specified in the standard operating procedure (SOP).

3. Data validation methodology (proofreading, double-data entry, electronic logical control) should be specified in writing.

4. Changes to data entered in the database should be made by authorized persons only. Changes should be specified and documented.

5. Electronic data should be backed up at regular intervals. The reliability and completeness of these back-ups should be verified – data should not be selected but comprehensively backed up.

All of the raw electronic data must be kept. This includes:

- All meta data associated to a computerized system and the equipment that is associated to it (which includes the audit trails for integration, for projects and for the entire instrument);
- Validation data and meta data in the form of their source electronic files.
- All electronic records obtained from HPLC and MS analysis (e.g. HPLC-MS/MS) are required to be retained, maintained and backed-up. It should be ensured that back-up data are exact and complete and that they are secure from alteration, inadvertent erasures or loss shall be maintained. The printed paper copy of the chromatogram would not be considered a “true, exact and complete copy” of the entire electronic raw data used to create that chromatogram. Printed chromatograms do not generally include, for example, the sample sequence, instrument method, processing method, integration settings or the full audit trail, of which all were used to create the chromatogram or are associated with its validity. Therefore there should be a higher emphasis on conservation of electronic data than paper data, as paper data is usually not considered the true source data, except in the case of paper logbooks where the original record was handwritten, for instance.

III- Archive facilities

1. An SOP should be in place for archiving.

2. Access to archive storage areas should be controlled and restricted to authorized personnel.

3. Study documentation including raw data and product samples is kept for at least five years in the archive and should be defined in the SOP.

4. All data, including both paper and electronic, should be easy to retrieve and traceable.

IV- Premises

1. Studies must be carried out under conditions which ensure adequate safety for the subjects.

2. The CRO should have sufficient space to accommodate the personnel and activities required to perform the studies.

3. The site must have adequate facilities, including laboratories. The facilities used for the clinical phase of the study should be well organized to allow the activities to be carried out in a logical order. Also, entry to the facility should be restricted and controlled.

4. The premises for the various laboratories should be designed to suit the operations to be carried out in them. Sufficient space should be available to avoid mix-ups, contamination and cross-contamination. There should be adequate and suitable storage space for samples, standards, instruments, equipment, solvents, reagents and records. There should be an alarm system and an adequate system to monitor the temperature of the critical stage and storage areas. If there is an automatic alarm system, it has to be tested regularly to ensure its functionality. Daily temperature records should be kept and all the alarm checks should be documented.

5. There should be access to telephone, e-mail to ensure good communication. The CRO should have the necessary office equipment (e.g. printer and copier) to perform the required activities.

6. The facilities should be maintained clean and should have adequate conditions of lighting, ventilation and, if required, environmental control. Floor, walls and working benches surfaces should facilitate the cleaning and decontamination.

7. Utilities such as water, air, gas and electricity should be adequate, stable and uninterrupted.

8. Laboratory premises should be designed to provide adequate protection to all employees and visitors, including inspectors or auditors, by ensuring their safety while handling or working in the presence of chemicals and biological samples. Improper working conditions can negatively impact on the quality of the work performed and of the data generated.

9. Premises should have suitable systems in place to dispose of waste, to treat fumes and to

protect the environment in conformance to local or national regulation.

The following general rules for safe working in accordance with national regulations and SOPs normally include the following requirements:

- Safety data sheets should be available to staff before testing is carried out; staff working in the laboratory should be familiar with and knowledgeable of the material safety data sheets for the chemicals and solvents that they are handling;
- Smoking, eating and drinking in the laboratory should be prohibited;
- Staff should be familiar with the use of fire-fighting equipment, including fire extinguishers, fire blankets and gas masks;
- Staff should wear laboratory coats or other protective clothing, including eye protection;
- Special care should be taken, as appropriate, in handling, for example, highly potent, infectious or volatile substances;
- Highly toxic and/or genotoxic samples should be handled in a specially designed facility to avoid the risk of contamination;
- All containers of chemicals should be fully labelled and include prominent warnings (e.g. “poison”, “flammable”, “radioactive”) whenever appropriate;
- Adequate insulation and spark-proofing should be provided for electrical wiring and equipment, including refrigerators;
- Rules on safe handling of cylinders of compressed gases should be observed and staff should be familiar with the relevant colour identification codes;
- Staff should be aware of the need to avoid working alone in the laboratory;
- First-aid materials should be provided and staff instructed in first-aid techniques, emergency care and the use of antidotes:
- Containers containing volatile organic solvents, such as mobile phases or liquid/liquid extraction solvents, should be closed with an appropriate seal;
- Volatile organic chemicals should be

handled under certified fume-hoods or air extractors and safety and eye showers should be available in the laboratory.

V- Clinical phase

1. There should be sufficient space to accommodate the study subjects.

2. Where appropriate, beds should be available for the volunteers. The necessity for beds and facilities for overnight stays depends on the type of study and the drug under investigation and should be specified in the study protocol.

3. Facilities for changing and storing clothes and for washing and toilet purposes should be easily accessible and appropriate for the number of users.

4. The study site should have facilities which should be separate areas where appropriate as specified in " The Egyptian Licensing Requirements For Bioequivalence Centers".

5. Provisions should be made for the urgent transportation of subjects to a hospital or clinic equipped for the emergency care of subjects, if required by their condition.

6. Access to key documents, such as the randomization list, should be restricted to only certain specific members of personnel. Such documents should be password-secured (if electronic) or kept under lock and key (if distributed as a hardcopy) and their distribution should be documented.

VI- Personnel

1. There should be a sufficient number of qualified and appropriately trained personnel for the activities performed. At all stages during the study, including at night, there should be a sufficient number of appropriately qualified and trained personnel to ensure that the rights, safety and well-being of the subjects are maintained, and to take care of the subjects in emergency situations.

2. Current curriculum vitae and training records should be kept for personnel.

3. The personnel responsible for the planning and conduct of the study should have appropriate qualifications and sufficient knowledge.

4. Records of training and assessment of knowledge of GCP and GLP should be maintained.

5. The delegation of significant study-related duties should be documented in writing.

6. There should be adequate measures in place to protect personnel from accidental contamination (e.g. from accidental needle pricks) while obtaining blood samples from subjects or while handling the samples that are derived from blood products (e.g. plasma and its extracts) or while handling or disposing of infectious waste.

VII- Quality assurance

1. The CRO should have an appropriate quality assurance (QA) system.

2. The QA system and the person(s) responsible for QA should operate independently of those involved in the conduct or monitoring of the study.

The QA unit should be responsible for:

- Verifying all activities undertaken during the study;
- Ensuring that the QA systems, including SOPs of the CRO, are followed, reviewed and updated;
- Checking all the study data for reliability and traceability;
- Planning and performing self-inspections (internal audits) at regular and defined intervals in accordance with an SOP, and following up on any corrective action as required;
- Ensuring that contract facilities, such as analytical laboratories, adhere to good practices for quality control laboratories. This would include auditing of such facilities, and following up on any corrective action as required;
- Verifying that the study report accurately and completely reflects the data of the study.
- Promptly reporting audit findings in writing to management, to the investigator and to the study director, as applicable.

3. The CRO should allow the sponsor to monitor the studies and to perform audits of the clinical and analytical study and the sites.

4. The laboratory should have a QA unit which should be independent from the person(s) responsible for analytical work and which should ensure that the analytical method in use is validated and current.

5. Both retrospective and in-process (e.g. in bioanalysis, as the samples and standards are being prepared and tested) QA verifications should be performed.

6. The quality management system should include root cause analysis, tracking for trends and ensuring all aspects of data integrity.

VIII- Ethics

Independent ethics committee

- Studies must be approved by an independent ethics committee (IEC) before a study is conducted. This committee must be independent from the sponsor and CRO. The discussions, recommendations and decisions of the IEC meetings should be documented in detailed minutes of the meeting. The IEC should be given sufficient time for reviewing protocols, informed consent forms (ICFs) and related documentation.

Informed consent

- Information for study volunteers should be given in a language and on a level of complexity appropriate and understandable to the subject, both orally and in writing.
- Informed consent must always be given by the subject and documented in writing before the start of any study-related activities, in accordance with GCP.
- The information must make clear that participation is voluntary and that the subject has the right to withdraw from the study on his or her own initiative at any time, without having to give a reason (compensation should be paid). If subjects who withdraw from the study offer their reasons for doing so, those reasons should be included in the study records.
- The subject must have access to information about insurance, and other procedures for

- compensation or treatment should he or she be injured or disabled as a result of participating in the study.
- The volunteers/subjects should be given opportunity to discuss their concerns with a physician regarding potential side effects or reactions from the use of the investigational products before participation in the trial. They should also be given the opportunity and sufficient time to discuss their concerns with their participation in the trial with individuals outside of the CRO, such as friends and family members, if they wish.
- If the ICF is available in several languages (e.g. in English and in Arabic), it should be ensured that all versions of the form contain the same information.

IX- Monitoring

1. The monitor should be qualified. The main responsibility of the monitor for a bioequivalence study is to ensure that the study is conducted in accordance with the protocol, GCP, GLP and applicable ethical and regulatory requirements. This includes provision of guidance on correct procedures for completion of CRFs and verification of the accuracy of data obtained.

2. In exceptional cases, the sponsor can delegate the monitoring function to the CRO. In such cases the CRO should be able to arrange for the monitoring of the study according to regulatory requirements.

3. The frequency of monitoring visits should be agreed to between the CRO and the sponsor. However, a pre- and post-study visit as well as a monitoring visit during the conduct of the study are usually performed. The monitor should prepare a written report after each site visit.

4 Separate SOPs (with checklists for the monitor) for the initiation visit, routine monitoring visits and a closing visit are recommended.

X- Investigators

1. The principal investigator should have the overall responsibility for the clinical conduct of

the study, including clinical aspects of study design, administration of the products under investigation, contacts with The Egyptian Drug Authority and the ethics committee, and for signing the protocol and the final study report.

2. The investigator should have appropriate qualifications, be suitably trained and have experience in the conduct of bioequivalence studies.

3. The medically qualified investigator should be responsible for the integrity, health and welfare of the subjects during the study, and the accurate documentation of all study-related clinical data.

XI- Receiving, storage and handling of investigational drug products

1. CROs should document all the information concerning the receipt, storage, handling and accountability of test and reference listed products at all stages of the study. CROs must keep records of the shipment, delivery, receipt, storage (including storage conditions), dispensing, administration, return and/or destruction of any remaining test product. Details of the drug product used should include dosage form and strength, lot number, expiry date and other coding that identifies the specific characteristics of the product tested.

2. A suitable location within the CRO, a local drug store, should assume responsibility for storage, delivery, return and record-keeping of both test and reference listed products.

3. Drug products should be stored under appropriate conditions as specified in the official drug information provided by the sponsor.

4. All study medication should be kept in a securely locked area accessible only to authorized persons.

5. Measures during labeling taken to ensure that the randomization list is followed and to avoid possible mistakes should be documented.

6. The investigator should follow the protocol requirements, randomization scheme and where required, use blinding. The investigator should ensure that the products' use is documented in such a way as to ensure correct dosage. This documentation should confirm

that each subject did receive the product dispensed for him or her and state the identity, including the dosage, of the product received.

Labelling should be performed in accordance with the following requirements:

- The printing step should be done in a manner that reduces potential risks of mislabelling and should be done in accordance with a SOP;
- Each label should include the following information:
  - Name of the sponsor,
  - Study number,
  - Batch number,
  - Subject identification number (to which the product is destined to be given to),
  - Period,
  - Active ingredient and dosage,
  - The storage conditions,
  - Expiry date (month/year) or retest date,
  - Identification of the product (test or reference).
- Compliance of all labels with the randomization list should be verified once printed, prior to labelling of the containers;
- Labelling should be done on the container, not on the lid, to ensure that the information is not lost once the lid is removed;
- The system used for labelling and documenting the administration of the product should make it possible to verify that each subject indeed received the product dispensed for him, for instance, by using labels with a tear-off portion.
- The empty containers should be labelled separately for the test and the reference listed products and should remain adequately segregated and placed in a secure area under lock and key, to ensure absence of risk of any potential mix ups, until the dispensing stage;

- label reconciliation should be performed;
- Appropriate, detailed records should be maintained for each of the above steps.

Dispensing/packaging should be performed in accordance with the following requirements:

- The surface area on to which the product will be handled should be thoroughly cleaned prior to bringing bottles of the product in the area. Any product container (full or empty), lone dosage formulations, labelling materials contaminants/dirt/debris should be removed from the area;
- Test and reference listed products should be handled using an appropriate instrument, such as a spatula or spoon, as opposed to gloved hands;
- Tablets should be distributed in each container in accordance with the randomization list either for the comparator or for the test product. Both products should never be handled at the same time. This also applies to the labelled containers;
- Drug accountability and dispensing records should be maintained at all times. Each activity should be documented at the time it is performed. This includes records of doses dispensed and returned or destroyed, records of cleaning and clearance of the area prior to dispensing, record of verification of adequate cleaning and clearance of the area, record of verification by a second person of each step;

Dosing should be performed in accordance with the following requirements:

- Dosing should be performed in accordance with a SOP;
- It should be performed under the supervision of the investigator or of qualified staff to whom this task has been explicitly delegated in writing;
- Whenever possible, just prior to dosing, a check should be performed of container contents matching the information on the label;
- The exact time of dosing should be documented;
- In order to ensure that the subject has swallowed the product, a mouth check should be

- performed by looking under the tongue, under the lips, in the corners of the mouth and between gums and cheeks, using a tongue depressor or a spatula and a flashlight, in the case of solid oral dosage forms. For other types of dosage forms verification of adequate administration should be performed by other suitable means. It should be documented;
- If more than one dosage unit is administered this should be clearly documented;
- Dosing can be documented directly in the case report forms. If retranscribed in the case of report forms from other documents the original documents should be retained;

XII- Case-report forms

1. CRFs should be used to record data on each subject during the course of the study.

2. The CRO should have a standardized format for CRFs; this should be adapted for each study protocol in accordance with the requirements for the particular study.

3. The required data to be collected on each volunteer should be specified in the study protocol. A sample CRF should be appended to the protocol.

4. CRFs should be used to guarantee preservation, retention and retrieval of information on volunteers. CRFs should reflect the actual results obtained during the study and allow easy access for verification, audit and inspection of the data.

5. A subject file should be kept for each subject to record his or her participation in successive studies and to record any information that could be useful for subsequent studies.

XIII-Volunteers – recruitment methods

1. Informed consent of potential subjects should be obtained for any screening procedures required to determine eligibility for the study, in addition to informed consent for participation in the research portion of the study.

2. Criteria for selection of subjects (inclusion and exclusion criteria) and recruitment procedures should be described in the study protocol.

XIV- Dietary considerations

1. Fasting and meals should be adequately controlled during the study days, as food intake can significantly affect the absorption of drugs. Standardized meals, snacks and drinks should be planned and provided to study subjects in accordance with the study protocol.
2. Records should be maintained of the timing and duration of meals, and amount of food and fluids consumed.

XV- Safety, adverse events and reporting of adverse events

1. Appropriate study planning includes adequate evaluation of any risk to the subjects. The study should be planned, organized, performed and monitored so that the safety profile will be acceptable to all concerned, including to the volunteers.
2. First-aid emergency equipment and appropriate rescue medication should be available at the study site and adequate facilities for the proper care of subjects who require emergency or other medical care.
3. The investigator should be responsible for medical decisions in case of adverse events and for notifying the Egyptian Drug Authority, the sponsor and, when applicable, the ethics committee, without delay.
4. The CRO should have the appropriate forms for the registration and reporting of adverse events, which should be provided to the investigator. The forms can be part of the CRF. If required, the relevant sponsor's forms may be used.

XVI- Sample collection, storage and handling of biological material

1. The specification of the samples (serum, plasma or urine), sampling method, volume and number of samples should be stated in the study protocol and the information provided to the volunteer. In the case of plasma samples the anticoagulant to be used should be specified in the protocol.
2. There should be documented procedures for the collection, preparation, transport and storage of samples.

3. Actual sampling times and deviations from the pre-specified sampling times should be recorded.

4. Labelling of collected samples should be clear to ensure correct identification and traceability of each sample.

5. The conditions for the storage of samples depend on the drug under investigation. However, all storage conditions (e.g. temperature in the freezer) should be specified in the study protocol, controlled, monitored and recorded throughout the storage period and during transportation. Procedures should be in place to ensure sample integrity in case of system failures.

6. Records of the storage and retrieval of samples should be maintained.

8. Handling and destruction or disposal of biological materials should be followed as specified in " The Egyptian Licensing Requirements For Bioequivalence Centers".

XVII- Bioanalytical data (laboratory phase)

1. The laboratory in which analysis performed in should be established with quality assurance systems.

Premises and equipment

- The laboratory should have sufficient space and infrastructure to perform the required analysis. Separate areas for specified activities should be provided to prevent possible contamination and mix-ups of samples during preparation and analysis.
- Utilities such as water, air, gas and electricity should be adequate, stable and uninterrupted.
- Analytical equipment and instruments should be appropriately calibrated, qualified and maintained, and methods used should be described and validated.
- There should be SOPs for the operation, use, calibration and preventive maintenance of equipment. Records should be maintained.

- Items of equipment used during the course of the study should be identified to allow verification that they have been appropriately qualified and calibrated and to ensure traceability.

2. Validation requirements for the analytical method should be described in the protocol. There should be separate SOPs for analytical method validation.

3. Data to support the stability of the samples under the stated conditions and period of storage should be provided in the study report.

4. Chemicals, reagents, solvents and solutions should be labelled to indicate identity, purity concentration (if appropriate), expiry date and specific storage instructions. Information concerning source, preparation date and stability should be available.

XVIII- Documentation

1. All original analytical raw data (e.g. calculations, chromatograms, etc.) should be documented in a manner that will ensure traceability with respect to the sample number, equipment used, date and time of analysis and the name(s) of the technician(s). In the case of raw data presented as paper chromatograms, these should be printed at an appropriate scale, allowing the visual verification of the peak shape and integration.

2. Each data point should be traceable to a specific sample, and information given should include, e.g. sample number, time of collection of the sample, time of centrifugation (if applicable), time when the sample was placed in the freezer (if applicable) and time of sample analysis, to enable the investigators to determine whether any aberrant results might have been due to sample mishandling.

3. The laboratory should have suitable coding techniques and methods to perform blinded analysis when relevant.

SECTION 6

Regulatory Guidelines for Centers performing Bioavailability and Bioequivalence Studies

First: The organizing rules of conducting bioavailability, bioequivalence and Comparative in-vitro studies in the centers:

1. A sample withdraw report for bioavailability or bioequivalence studies or Comparative in-vitro studies shall be provided. The report must indicate the name of the bioavailability and bioequivalence center, provided that it must be issued by the inspector in the Central Administration of Operations in Egyptian Drug Authority.

2. The center must have a sufficient quantity of (Test & Reference Products) to conduct the study (bioavailability study, bioequivalence study or Comparative in-vitro studies in the centers) before start of study.

3. In case of conducting the bioequivalence studies for (Modified Release Products), the study must be conducted in (Fasting & Fed States)provided that in case of conducting two bioequivalence studies as follows: "Two Separate Two-Way Cross-Over" the two bioequivalence studies in the fasting and fed states shall be sent in the form of two separate files and the part of "Bioanalytical Method and validation & Invitro Part" only once in one of the two files.

4. With regard to conducting Comparative in-vitro studies in the centers (As a Biowaiver or a Complementary for In - vivo studies)

The study must be conducted as follows:

-At three different pHs (1.2, 4.5 & 6.8) in addition to the most suitable medium, provided that the most suitable medium shall be chosen according to the full method indicated on the website of the American Food and Drug Administration (FDA) or the United States Pharmacopeia (USP)… etc. except for the studies conducted on the (Delayed Release or Enteric Coated Products), where the study shall only conducted according to the reference

method stipulated on the website of (FDA) or (USP)… etc. In case of lack to the most suitable medium, the study shall be conducted as follows:

-At two pHs (at acidic stage & buffer stage).

5. The Department of Protocols Evaluation and Following-up of the Centers of bioavailability and Bioequivalence - Unit of Evaluation of Bioavailability and Bioequivalence Studies of Human Products in Egyptian Drug Authority shall be notified of the date in which the Bioavailability or bioequivalence studies are conducted as follows:

-The Center shall notify the Department of Protocols Evaluation and Following-up of the Centers of bioavailability and Bioequivalence - Unit of Evaluation of Availability and Bioequivalence Studies of Human Products in Egyptian Drug Authority of the date of conducting each study separately and before a sufficient time; not less than three working days (from Sunday to Thursday from 8 am to 3 pm) and not more than two weeks before the study initiating, via the department's e-mail address:

Hdr.bioequivalence@edaegypt.gov.eg

Taking into account that the center must commit to the following:

1. Submitting the following data of the bioavailability or bioequivalence study:

-Test Product Name & Dosage Form:

-Active Ingredients(s):

-Manufacturer & License Holder:

-Dates of Study Phases: --/--/----

-Study Status: New Study or Repeated Study.

-Study Type: Fasting or Fed Study.

-Study Design.

2. Submitting the following documents of the product for which the bioavailability or bioequivalence study will be conducted:

A- In case of conducting studies on registered pharmaceutical products (on a production

batchs or a pilot batchs according to the withdrawal report issued by the e Central Administration of Factory Inspection in Egyptian Drug Authority) according to different registration status, a copy of the latest registration notification of the product under study shall be sent accompanied by a copy of the composition form approved by Egyptian Drug Authority, provided that the registration notification shall be valid and indicating the decision of the Bioavailability and Bioequivalence Studies Evaluation Committee regarding the type of study required for the product. In case of the company and/or center have submitted a petition to adjudicated on the position of the product in terms of the required study or any matter related to the method of conducting the study and the petition has been presented to the Bioavailability and Bioequivalence Studies Evaluation Committee, a copy of the Committee decision regarding this petition shall be sent.

B- In case of conducting studies on pharmaceutical products under registration (on a pilot batch according to the withdrawal report issued by central administration of inspection on pharmaceutical institutions in Egyptian Drug Authority), a copy of the approval of the Specialized Scientific Committee for Evaluating Stability Studies shall be sent by the company. That copy shall be accompanied by a copy of the composition form approved by the Specialized Scientific Committee for Evaluating Stability Studies. In the case of submitting products for registration under the system (425) of 2015, a copy of the composition form signed by the inspector of central administration of inspection on pharmaceutical institutions in Egyptian Drug Authority shall be sent in addition to a copy of the decision of the Bioavailability and Bioequivalence Studies Evaluation Committee regarding the type of study required for the product under study submitted by the company owning the product. In case of the company and/or center have submitted a petition to adjudicate on the position of the product in terms of any matter relating to the method of conducting the study and the petition has been presented to the Bioavailability and Bioequivalence Studies Evaluation Committee, a copy of the decision of the said Committee regarding this petition shall be sent.

C- In both aforementioned cases (A, B), a copy of the samples withdraw report of the studies of bioequivalence of the products under study, issued by central administration of inspection on pharmaceutical institutions in Egyptian Drug Authority, shall also be sent, provided that report copy shall indicate the following: (the trade name, concentration and pharmaceutical form of the product, manufacturer name, type of batch (initial production batch, production batch, pilot batch) batch number, manufacturing date and expiration date, type of the required study, name of the bioavailability and equivalence center that will conduct the study, taking into account that the report shall state that the samples were withdrawn after changing the excipient materials or adding a source of the raw material, provided that the new source shall be indicated... "if any").

3. the names list of the names of volunteers participating in the study in the form of a Word Document or Excel Sheet, shall be sent as follows:

SN The quadruple volunteer's name in Arabic The volunteer's ID No. consisting of "14 numbers"

1. All of the volunteers data must be correct and on the responsibility of the center and a copy of the volunteers' ID No. shall be attached, noting that only forty five volunteers are approved for studies performed by cross over design & fifty five volunteers are approved for studies performed by replicate design by the Department of Protocols Evaluation and Following-up of the Centers of Bioavailability and Bioequivalence - Unit of Evaluation of bioavailability and Bioequivalence Studies of Human Products in Egyptian Drug Authority to participate in the study. The names of the remaining volunteers will not be considered after approving the maximum limit except for some cases that require involving a larger number of the volunteers. The center is allowed to complete the list of volunteer names until the maximum limit is reached within one working day before initiating the study conduct.

4. The Center shall be committed to provide a list of the volunteers names actually participating in the study, the "Attendance Sheet for Volunteers", after each phase of the

study. The list shall be submitted in the form of a Word Document or Excel Sheet also as a scanned document after signing it by the volunteers and center officials, as follows:

SN The quadruple volunteer's name in Arabic The volunteer's ID No. consisting of "14 numbers"

5. The volunteers participating in the study shall be among the volunteers who have previously been approved by the Department of Protocols Evaluation and Following-up of the Centers of bioavailability and Bioequivalence - Unit of Evaluation of bioavailability and Bioequivalence Studies of Human Products in Egyptian Drug Authority. Any volunteer is not allowed to participate in Bioavailability studies or bioequivalence studies except after obtaining an approval by the Department of Protocols Evaluation and Following-up of the Centers of bioavailability and Bioequivalence - Unit of Evaluation of bioavailability and Bioequivalence Studies of Human Products in Egyptian Drug Authority.

6. The period between the participation of the same volunteer in two consecutive bioequivalence studies is two months starting from the date of the last (Phase) of the previous study.

7. Every volunteer shall have an ID card during the attendance period inside the center. Any volunteer has no ID card is not permitted to participate in bioavailability or bioequivalence studies.

8. The volunteers shall be selected in accordance with Selection Criteria mentioned in the Egyptian rules regulating the conduct of bioavailability and bioequivalence studies.

(6) In case of conducting a bioavailability or bioequivalence study on more than one stage, the following shall be adhered to:

A. The study shall be conducted on no more than two stages.

B. The stages of study shall be indicated in the study protocol.

C. When conducting the study on two stages (Stage I & Stage II), the washout period between the two stages must not exceed two weeks, provided that the washout period between the

study (Phases) shall be fixed.

D. The results of the (Stage I) must not significantly statistically different from the results of the (Stage II).

E. The center shall notify the Department of Protocols Evaluation and Following-up of the Centers of bioavailability and Bioequivalence - Unit of Evaluation of bioavailability and Bioequivalence Studies of Human Products in Egyptian Drug Authority of the date of conducting each (stage) in sufficient time; not less than three working days and not more than two weeks before initiating the relevant stage of the study.

(7) In the case of occurring sudden changes (postponement or cancellation of the bioavailability or bioequivalence study), the following must be adhered to:

A. The Department of Protocols Evaluation and Following-up of the Centers of bioavailability and Bioequivalence & Unit of Evaluation of bioavailability and Bioequivalence Studies of Human Products in Egyptian Drug Authority shall be notified of postponement or cancellation of the study no later than the morning of the study day, via the e-mail of the Unit of Evaluation of bioavailability and Bioequivalence Studies of Human Products or by telephone in case of necessity.

B. When setting a new date for the study, the center shall notify the Department of Protocols Evaluation and Following-up of the Centers of bioavailability and Bioequivalence - Unit of Evaluation of bioavailability and Bioequivalence Studies of Human Products in Egyptian Drug Authority of the new study date in sufficient time; not less than three working days and not more than two weeks before initiating the study, provided that the new set date for the study shall be within two weeks of the previously specified date for conducting the postponed or canceled study which formerly sent to Section of Protocols Evaluation and Following-up of the Centers of Availability and Bioequivalence & Unit of Evaluation of Availability and Bioequivalence Studies of Human Products in Egyptian Drug Authority.

C. In case of the study postponing exceeded two weeks from the date specified for conducting

the postponed or canceled study, the approved list of participating volunteers in the study sent by the center shall be cancelled, then those volunteers will be allowed to participate in any other study after obtaining an approval by the Department of Protocols Evaluation and Following-up of the Centers of bioavailability and Bioequivalence - Unit of Evaluation of bioavailability and Bioequivalence Studies of Human Products in Egyptian Drug Authority. The aforementioned rules relevant to notifying the Department of Protocols Evaluation and Following-up of the Centers of Availability and Bioequivalence - Unit of Evaluation of bioavailability and Bioequivalence Studies of Human Products in Egyptian Drug Authority of the new date of conducting the bioequivalence studies shall be applied.

(8) In case of conducting a (pilot study), the aforementioned rules and requirements shall be adhered to after obtaining approval for pilot study performance from BE unit

(9) The Department of Protocols Evaluation and Following-up of the Centers of Bioavailability and Bioequivalence - Unit of Evaluation of Bioavailability and Bioequivalence Studies of Human Products in Egyptian Drug Authority shall be notified of conducting Comparative in-vitro as follows:

-The Center shall notify Department of Protocols Evaluation and Following-up of the Centers of Bioavailability and Bioequivalence - Unit of Evaluation of Bioavailability and Bioequivalence Studies of Human Products in Egyptian Drug Authority of the date of conducting each study separately and in sufficient time, before initiating the study, via the department's e-mail, provided that the center shall be committed to send the product data for which the Comparative in-vitro will be conducted as follows:

-Test Product Name & Dosage Form,

-Active Ingredients(s), and

-Manufacturer & License Holder.

Noting that the center may not initiate the study until fulfilling all of the product documents for which the study will be conducted, as aforementioned indicated.

-In case of postponing or cancellation of the study, the Section of Protocols Evaluation and Following-up of the Centers of Availability and Bioequivalence & Unit of Evaluation of Availability and Bioequivalence Studies of Human Products in Egyptian Drug Authority shall be notified of that postponing or cancellation in sufficient time, via the e-mail of the Section of Protocols Evaluation and Following-up of the Centers of Availability and Bioequivalence & Unit of Evaluation of Availability and Bioequivalence Studies of Human Products.

(10) The Section of Protocols Evaluation and Following-up of the Centers of Availability and Bioequivalence & Unit of Evaluation of Availability and Bioequivalence Studies of Human Products in Egyptian Drug Authority shall be notified of the date of analyzing of the samples withdrawn for bioavailability and bioequivalence studies (Analysis) as follows:

-The Center shall notify the Department of Protocols Evaluation and Following-up of the Centers of Availability and Bioequivalence - Unit of Evaluation of Availability and Bioequivalence Studies of Human Products in Egyptian Drug Authority of the date of analyzing of the samples withdrawn for bioavailability and bioequivalence studies (Analysis), within a period not less than two working days, via the unit's e-mail, provided that the center must be committed to send the data of this study as follows:

-Test Production Name & Dosage Form,

-Active Ingredient (s), and

-Manufacturer & License Holder.

Second: The regulating rules of the work in the centers conducting bioavailability and bioequivalence studies:

(1) The organizational structure members in the center must be as follows:

A. The center manager, the technical manager and the quality assurance manager must attend during conducting of the studies at all stages.

B. The analysis manager must attend during analysis of studies.

C. The physician and responsible person for collecting samples must be present during period

of the volunteers' attendance at the center.

D. In the emergency cases only, the center manager, technical manager, quality assurance manager or analysis manager may delegate a member of the center's organizational structure to carry out the work on his behalf. One of the aforementioned managers also may delegate a member outside the organizational structure based on a written authorization, provided that the delegated person shall be at the same scientific level, and the number of authorizations should not exceed one authorization per day & two per month, otherwise the study must be postponed to another date "in certain cases if need authorizations exceed two per month center must return to BE Unit to decide.

E. The center manager, the technical manager, the quality assurance manager and the analysis manager are permitted to hold only one position for each, and none of them may hold another position in addition to his original work.

F. When making any change in the center's organizational structure, the center shall review the regulating rules of licensing the centers conducting the bioavailability and bioequivalence studies with regard to the organizational structure, then the department of Protocols Evaluation and Following-up of the Centers of bioavailability and Bioequivalence - Unit of Evaluation of bioavailability and Bioequivalence Studies of Human Products in Egyptian Drug Authority shall be notified of any changes. The new members' documents shall be entirely submitted, which include: the appointment contract, CV, obtained certificates, updated organizational structure and an acknowledgment on the center's stationary to work full-time at the center, before starting the work at the center.

(2) An acknowledgment of the volunteers' participating in bioavailability or bioequivalence studies (Consent Form):

A. All of the participating volunteers shall be notified of the entire important information in the internal leaflet of the product under study, which includes: side effects, contraindications, drug-drug interactions, warnings… etc., as well as they shall be informed of all steps of

conducting the study and the volunteers' rights.

B. The acknowledgement of the volunteer's participation in bioavailability or bioequivalence studies shall be written in Arabic stating the study data.

C. Each volunteer must have his own acknowledgement, containing the aforementioned data.

D. The volunteer shall sign the acknowledgement before (not during or after) conducting the study.

E. The responsible members of the center shall sign the acknowledgements.

(3) Health status report of the volunteers participating in bioavailability or bioequivalence studies (Case Report):

A. Each volunteer must have his own health status report and the selection of volunteers must be in accordance with the aforementioned Selection Criteria, including (Demographic Data).

B. The case report shall be written before (not during or after) conducting the study.

C. The report must be signed by the center's physician.

D. The results of all tests conducted on volunteers shall be written by the center's physician.

E. The full (Medical History) of each volunteer shall be presented.

F. The results of the pregnancy test shall be written for the volunteers confirming that they are not breast feeding.

G. (Drugs of Abuse Test) results of the volunteers shall be written.

(4) Laboratory analyzes of the volunteers participating in the study (Laboratory Data).

A. Each volunteer must have his / her own Laboratory test results.

B. All of the analyzes results must be written on the original stationary of the laboratory contracting with the center conducting the analyzes, provided that they shall be signed and stamped by the laboratory officer and state the (Reference Ranges) of each test.

C. All of the analyzes must be conducted no more than three months before starting the study except in certain cases center will be notified prior to study.

4. The analyses must include the following:

A. Complete Blood Picture and Blood Group.

B. Complete Urine Analysis Report.

C. Biochemical Data:

- Fasting Blood Sugar

- Kidney Function:

(Serum Urea & Serum Creatinine).

- Liver Function:

(Serum GPT "ALT" & Serum GOT "AST").

- Lipid Profile:

(Total Cholesterol & HDL & LDL & Triglycerides).

D. Serology:

(HIV & HCV).

E. The (kits) of testing (drugs of abuse), (blood kits) for (pregnancy) testing, as well as the (kits) of testing hepatitis "C" (HCV) shall be available, provided that some volunteers shall be tested randomly in the presence of a representative of the Department of Protocols Evaluation and Following-up of the Centers of Bioavailability and Bioequivalence - Unit of Evaluation of Bioavailability and Bioequivalence Studies of Human Products in Egyptian Drug Authority

F. Durning performance of bioequivalence studies for certain pharmaceutical products it require specific lab. test, center will be notified prior to study to perform them.

(5) Study Protocol:

The study protocol shall be fully prepared, dated and signed by the center's officials and representatives of the company owning of the product before initiating the study, provided that it shall include the following data:

- Protocol Approval (signed & dated).
- Test & Reference Products Data:

(The reference product used should be the innovator, and a copy of the website mentioning

the used innovator should be attached, otherwise, there must be an approval of the Committee of the Evaluation of Bioequivalence Studies to use this reference product).

•Half Lives of Analytes to be measured:

(Calculation of washout period & sampling time intervals should depend on the higher range limit of the half-life of API (s) or its metabolite whenever needed).

- Washout period.
- Date of Volunteers Screening.
- Randomization Plan.
- Dates of Phase I & Phase II.
- Study design & Protocol Illustration and Justification.
- Time and Frequency of Sampling.
- Dosage Form Administration.
- Inclusion & Exclusion Criteria.
- Subjects' Disposition.
- Procedures to Minimize Risk.
- Type of Obtained Biological Samples.
- Storage Conditions of Biological Samples.
- Data Analysis (Pharmacokinetic & Statistical Analysis).
- Template of Informed Consent Form.
- Template of Case Report.
- Protocol Deviation & Justification (To be fulfilled if present).
- Complementary In-Vitro Dissolution Testing Methodology.

(6) Approval of the study protocol by the Scientific Research Ethics Committee (Ethics Committee and/or IRB Approval):

-The approval of the Scientific Research Ethics Committee must include the product name on which the study was conducted, provided that it shall be dated and signed by all members of

the Committee before initiating of the study.

(7) Records of samples withdrawals for volunteers participating in the study (Log Book i.e., Sample Time Interval sheets)

A. All of the samples collected for all volunteers participating in the study shall be recorded in samples withdrawal records.

B. The (phase) and (stage) of the study shall be determined in each record.

C. The records must be dated and signed by the person responsible for samples withdrawals.

D. All volunteers participating in the study must sign after each withdraw.

E. Each withdraw must contain the (Real Time) and the time specified in the protocol (Sample Time Interval) for each withdraw from each volunteer.

(8) The labels of the samples tubes withdrawn from volunteers participating in the study:

A. The name or code of the bioavailability or bioequivalence study shall be written.

B. The volunteer number shall be written.

C. The (phase) and (stage) of the study shall be written .

D. (Sample Time Interval) shall be written.

E. These tubes shall be kept in an organized and tidy manner in the (Deep Freezer -80°C).

(9) Measuring the vital signs of volunteers participating in the study (Vital Signs Sheet):

A. The (Vital signs) of the volunteers participating in the study shall be measured for each (Phase) at each (stage) of the study, within specific time before blood sample withdrawal

B. They shall be written, signed and dated by the center's physician.

C. The following must be measured:

- Blood Pressure.
- Pulse rate.
- Temperature.

In addition to conducting an examination of all volunteers, including the following:

- Chest, Abdomen Examination… etc.

(10) Recording the side effects in case of emerging on the volunteers participating in the study (Side Effects / Adverse: Reactions Sheet):

A. The side effects emerging on the volunteers participating in the study shall be monitored and recorded for all study phases in addition to establishing a plan to treat the volunteer.

B. The side effects shall be written, signed and dated by the center's physician.

C. In case of emerging side effects on a volunteer that affect the study (for example: vomiting), the study shall be stopped for that volunteer and he shall be excluded from the study.

D. In case of emerging any serious side effects, an emergency plan shall be followed to rescue the volunteer quickly.

(11) Plan of random selection of the participating volunteers (Randomization Plan Sheet):

A. It is necessary to write a plan of random selection of the participating volunteers (randomization plan sheet) for all volunteers participating in the study.

B. The study name and code must be indicated.

C. It must be signed and dated by the center officials.

Third: General rules:

1) The bioequivalence studies are not allowed to be conducted before attendance of all volunteers according to the number mentioned in the study protocol based on the (Sample Size) calculations. In case of uncompleted number, the center can conduct (Add-on Study), provided that the rules organizing this procedure shall be followed, knowing that the latter case is not allowed in case of (Replicate Design) studies. In the event of violation, the Specialized Scientific Committee for Evaluating Bioavailability and Bioequivalence Studies study shall not accept to evaluate the study.

2) The number of beds shall match the number of volunteers to participate in the study.

3) The center shall be committed to inform the Department of Protocols Evaluation and Following-up of the Centers of Bioavailability and Bioequivalence - Unit of Evaluation of Bioavailability and Bioequivalence Studies of Human Products of the names of volunteers

having positive results for (HCV, HIV & Drugs of abuse).

4) The Center shall be committed to destroy the samples (Biological Samples: Plasma, Blood, Urine… etc.) of the studies that have been approved by the Bioavailability and Bioequivalence Studies Evaluation Committee, within a week of the study's approval letter being issued to the Center and in case of violation the necessary measures shall be taken against the center.

5) The center shall be obligated to inform the Department of Protocols Evaluation and Following-up of the Centers of Bioavailability and Bioequivalence - Unit of Evaluation of bioavailability and Bioequivalence Studies of Human Products in Egyptian Drug Authority of the places in which the biological samples (Plasma, Plasma, Blood, Urine… etc.) are stored inside the center and any change in the method and locations of preservation as well as the method used to destroy the samples in details.

6) The center is committed to prepare and print the entire file of the (Bio-analytical Method Validation Report) along with (Representative Chromatograms) before starting to analyze the Biological Samples: Plasma, Blood, Urine… etc.)

7) The center shall be committed to conduct a Complementary Comparative In-Vitro study, before starting the blood sample withdrawal of the study.

8) All samples (blood- urine) withdrawn from volunteers must be analyzed inside center site only and it is not allowed to be analyzed in other site

9) Center must ensure that only samples related to studies performed in center are present

10) The samples withdrawn from all of the volunteers participating in the bioavailability or bioequivalence studies must be kept in the (Deep Freezer -80°C) until beginning of the analysis procedure. For the (Retained Samples) after the analysis, they must be kept in the (Deep freezer -80°C or -20°C), to be re-analyzed, when necessary, provided that proving the validity of the stability of those samples based on the stability study conducted at the center until obtaining the approval of the study by the Bioavailability and Bioequivalence Studies Evaluation Committee.

11) A full copy of the final study file (Study Report) for bioequivalence or Comparative In-vitro Dissolution Study must be kept, that include all of documents related to each study (Raw Data) (accompanied with all of the documents related to the generic product on which the study was conducted, as well as all the external packages of the generic and reference products on which the study was conducted) in an appropriate manner for a period of not less than five years after the approval date of the study by the Bioavailability and Bioequivalence Studies Evaluation Committee, so that it can be obtained upon request.

12) In case of conducting a bioequivalence or Comparative In-vitro Dissolution Study and the generic product appear to be not equivalent to the reference product, the Center shall send an informing report thereon to the Department of Protocols Evaluation and Following-up of the Centers of bioavailability and Bioequivalence - Unit of Evaluation of bioavailability and Bioequivalence Studies of Human Products in Egyptian Drug Authority.

13) In case of postponing or cancelling study center must inform BE unit.

14) The center is committed to implementing an integrated data integrity system at all study stages.

15) There must be written, reviewed, and stamped SOPS for every procedure carried out within the center.

16) The center's (LC/HPLC) equipment must contain original software versions approved by the manufacturers and updated periodically as needed.

17) It is necessary to adhere to the principle that all electronic data must be managed using password-protected systems that define access privileges for each user according to their job, as well as an audit trail system to track any data modification, and backup copies must be saved periodically to ensure that they are not lost or modified without authorization.

18) All equipment must be calibrated and maintained periodically according to contracts with the relevant authorities, with a general plan prepared for this purpose, in accordance with ISO 17025, or these authorities must be accredited by the National Accreditation Council. Calibration certificates from the official agent of the equipment may also be accepted.

SECTION 7

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28. TRS 1052 - Annex 6: WHO Biowaiver List: proposal to waive in vivo bioequivalence requirements for WHO Model List of Essential Medicines immediate-release, solid oral dosage forms 26 April 2024.
29. *the 75th WMA General Assembly, Helsinki, Finland, October 2024:* https://www.wma.net/policies-post/wma-declaration-of-helsinki
30. ICH Topic E 3 International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use - Structure and Content of Clinical Study Reports.
31. ICH-M 10- International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use - Bioanalytical Method Validation and Study Sample Analysis.
32. ICH M 13A - International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use - Bioequivalence for Immediate release Solid Oral Dosage Forms.

SECTION 7

Bioequivalence Summary Tables

Table 1 Submission Summary

Drug Product Name	
Strength(s)	
Applicant Name	
Address	
Point of Contact **Name** **Address** **Telephone Number** **Fax Number**	

Table 2 Summary of Bioavailability Studies

Study Ref. No.	Study Objective	Study Design	Treatments (Dose, Dosage Form, Route) Product ID	Subjects (No. (M/F) Type Age: mean (Range)	Mean Parameters (+/-SD)					
					C_{max} (units/mL)	t_{max} (hr)	AUC_{0-t} (units)	$AUC_{0-\infty}$ (units)	$t_{1/2}$ (hr)	k_{el} (hr-1)
Study #	Fasting study title	Randomized single-dose crossover	Test product strength Tab./Cap./Susp. p.o. (Batch #)	#completing (#M#F) Healthy subjects or patients mean age (range)	M (%CV)	Median (Range)	M (%CV)	M (%CV)	M (%CV)	M (%CV)
			Reference product strength Tab./Cap./Susp. p.o.		M (%CV)	Median (Range)	M (%CV)	M (%CV)	M (%CV)	M (%CV)

Study Ref. No.	Study Objective	Study Design	Treatments (Dose, Dosage Form, Route) Product ID	Subjects (No. (M/F) Type Age: mean (Range)	Mean Parameters (+/-SD)					
					C_{max} (units/mL)	t_{max} (hr)	AUC_{0-t} (units)	$AUC_{0-\infty}$ (units)	$t_{1/2}$ (hr)	k_{el} (hr-1)
Study #	Fed study title	Randomized single-dose crossover	Test product strength Tab./Cap./Susp. p.o. (Batch #) Reference product strength Tab./Cap./Susp. p.o. (Batch #)	#completing (#M#F) Healthy subjects or patients mean age (range)	M (%CV) M (%CV)	Median (Range) Median (Range)	M (%CV) M (%CV)	M (%CV) M (%CV)	M (%CV) M (%CV)	M (%CV M (%CV)

Table 3 Statistical Summary of the Comparative Bioavailability Data

Drug Dose(# x mg) Least Squares Geometric Means, Ratio of Means, and 90% Confidence Intervals				
Fasted Bioequivalence Study (Study No.)				
Parameter	**Test**	**Reference**	**Ratio**	**90% C.I.**
AUC $_{0-t}$				
AUC$_{0-\infty}$				
C$_{max}$				
Fed Bioequivalence Study (Study No.)				
Parameter	**Test**	**Reference**	**Ratio**	**90% C.I.**
AUC $_{0-t}$				
AUC$_{0-\infty}$				
C$_{max}$				

Table 4 Statistical Results Program Format

Subject	Period	Treatment	SEQUENCE	C_{MAX}	AUC_{0-T}	AUC_{0-INF}

Table 5Method Validation

Information Requested	Data
Bioanalytical method validation report location	Provide the volume(s) and page(s)
Analyte	Provide the name(s) of the analyte(s)
Internal standard (IS)	Identify the internal standard used
Method description	Brief description of extraction method; analytical method
Limit of quantitation	LOQ, units
Average recovery of drug (%)	%
Average recovery of IS (%)	%
Standard curve concentration (units/mL)	Standard curve range and appropriate concentration units
QC concentrations (units/mL)	List all the concentrations used
QC Intraday precision range (%)	Range or per QC
QC Intraday accuracy range (%)	Range or per QC
QC Interday precision range (%)	Range or per QC
QC Interday accuracy range (%)	Range or per QC
Bench-top stability (hrs)	hours at room temperature
Information Requested	**Data**
Stock stability (days)	days at 4°C
Processed stability (hrs)	hours at room temperature; hours at 4°C

Freeze-thaw stability (days)	# cycles
Long-term storage stability (days)	17 days at -20°C (or other)
Dilution integrity	Concentration diluted X-fold
Selectivity	No interfering peaks noted in blank plasma samples

* Please include table for each analyte.

* Please submit all Method Validation SOPs.

Table 6 Summary of In Vitro Dissolution Studies

Dissolution Conditions	**Apparatus:**	
	Speed of Rotation:	
	Medium:	
	Volume:	
	Temperature:	
Firm's Proposed Specification		
Dissolution Testing Site		

Study Ref No.	**Testing Date**	**Product ID /Batch No. (Test-Manufacture Date) (Reference-Expiration Date)**	**Dosage Strength & Form**	**No. of Dosage units**		**Collection Times (minutes or hours)**					
Study Report#:		Test Product	mg Tablet	12	Mean						
					Range						
					%CV						
Study Report#:		Reference Product	mg Tablet	12	Mean						
					Range						
					%CV						

* Provide dissolution data for all strengths (test and reference).

Table 7 Formulation Data

Ingredient	Amount (mg) / Tablet		Amount (%) / Tablet	
	Strength 1	Strength 2	Strength 1	Strength 2
Cores				
Coating				
T			**100.00**	**100.00**

* Please include the formulation of all strengths.

Table 8 Demographic Profile of Subjects Completing the Bioequivalence Study

Stu			
		Treatment Groups	
		Test Product N=	Reference Product N=
Age (years)	Mean±SD Range		
Age Groups	18-35 35-55	N(%) N(%)	N(%) N(%)
Sex	Male Female	N(%) N(%)	N(%) N(%)
BMI*	Mean±SD Range		
Other Factors			

* BMI: Body mass index.

* Please provide a separate table for each Bioequivalence Study.

Table 9 Incidence of Adverse Events in Individual

Studies Body System / Adverse Event	Reported Incidence by Treatment Groups*	
	Fasted/Fed Bioequivalence Study** Study No.	
	Test	Re
Body as a whole		
Cardiovascular		
Gastrointestinal		
Other organ sys.		
Total		

*Expressed as number and percentage

**Provide separate table for each Bioequivalence Study

Table 10 Reanalysis of Study Samples

Study No.								
Reason why assay was repeated	**Number of samples reanalyzed**				**Number of recalculated values used after reanalysis**			
	Actual number		**% of total assays**		**Actual number**		**% of total assays**	
	T	**R**	**T**	**R**	**T**	**R**	**T**	**R**
Pharmacokinetic*								
Reason A (e.g. below LOQ)								
Reason B								
Reason C								
Etc.								
Total								

*If no repeats were performed for pharmacokinetic reasons, insert"0.0."

* Please provide a separate table for each analyte measured for each in-vivo study

Table 11 Study Information

Study Number	
Study Title	
Clinical Site (Name, Address, Phone)	
Principle Investigator	
Dosing Dates	
Analytical site (Name, Address, Phone)	
Analysis Dates	
Analytical Director	
Storage Period of Biostudy Samples (no. of days from the first day of sample collection to the last day of sample analysis)	

*Please provide separate table for each Bioequivalence Study.

Table 12 Product Information

Product	Test	Reference
Treatment ID		
Product Name		
Active Ingredient(s)		
Molecular formula		
Dosage form		
Strength		
Dose Administered		
Route of Administration		
Manufacturer		
Batch/Lot No.		
Batch Size		N/A
Manufacture Date		N/A
Expiration Date	N/A	

Product	Test	Reference
Storage conditions		
Quantitative formulation	*(to be attached)*	*(If available)*
Potency		
Content Uniformity (mean,%CV)		N/A

Table 13 Dropout Information and reasons

Study No.				
Subject No.	**Reason for dropout/replacement***	**Period**	**Replaced?**	**Replaced with**

* Please provide a separate table for each Bioequivalence Study

Table 14 Dropout Information

Study No.		
Type	**Subject's (Test)**	**Subject's (Ref.)**

* Please provide a separate table for each Bioequivalence Study

Table 15 Summary of Standard Curve and QC Data for Bioequivalence Sample Analysis

Bioequivalence Study No. Analyte Name							
Parameter	**Standard Curve Samples**						
Concentration (ng, mcg/ml)							
Inter day Precision (%CV)							
Inter day Accuracy (%Actual)							
Linearity	**(Range of R^2 values)**						
Linearity Range (ng, mcg/ml)							
Sensitivity/LOQ (ng, mcg/ml)							
Bioequivalence Study No. Analyte Name							
Parameter	**Quality Control Samples**						
Concentration (ng, mcg/ml)							
Inter day Precision (%CV)							
Inter day Accuracy (%Actual)							

*If applicable, please provide separate tables for the parent drug and metabolite(s)

Table 16 SOP's Dealing with Bioanalytical Repeats of study samples

SOP No.	Effective Date of SOP	SOP Title

Table 17 Composition of Meal Used in Fed Bioequivalence Study

Composition of Meal Used in Fed Bioequivalence Study		
Composition	**Percent of total Kcal**	**Kcal**
Fat		
Carbohydrate		
Protein		
Total		

History Table

Version No.	Issue date	Summary of Changes
Version 1	28/05/2009 (1st Draft) 07/06/2009 (Adoption for feedback) 14/01/2010 (Date of Issue) 14/06/2015 (2nd Draft) 07/10/2015 (Adoption for feedback) 26/05/2016 (Date of Issue)	---------
Version 2	23/02/2017	- Updated for Publication on EDA website - Formatting adjustment
Version 3	17/07/2023	- Clarification of study design - Clarification of comparative in-vitro studies sampling
Version 4	06/2026	- Updated References - Implementation of ICH -M10 & ICH - M13A Guidelines -Addition of section 6: Updated Regulatory Guidelines for Centers Performing Bioavailability and Bioequivalence Studies (Version 1: issued 2015)