SOUTHERN AFRICAN DEVELOPMENT COMMUNITY

APPLICATION FOR REGISTRATION
OF A MEDICINE

GUIDELINE ON BIOAVAILABILITY / BIOEQUIVALENCE

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ABBREVIATIONS

Ae∞	amount excreted at infinity
Aet	amount excreted at time t
ANOVA	Analysis of variance
API	Active Pharmaceutical Ingredient
$AUC_{0-\infty}$	area under the plasma/serum/blood concentration-time curve from time zero to time infinity
AUC_{0-t}	area under the plasma/serum/blood concentration-time curve from time zero to time t where t is the last time point with measurable concentration.
AUC_{τ}	AUC during a dosage interval at steady state
BA/BE	Bioavailability/Bioequivalence
BCS	Biopharmaceutics Classification System
BMI	Body Mass Index
BP	British Pharmacopoeia
Cav	average plasma concentration
Cmax	maximum plasma concentration
Cmax (ss)	maximum plasma concentration at steady-state
Cmin	minimum plasma concentration
Cmin (ss)	minimum plasma concentration at steady-state
CoA	certificate of analysis
CRO	Contract Research Organization
EMA	European Medicines Agency
EP	European Pharmacopoeia
FDC	Fixed Dose Combinations
FPP	Finished Pharmaceutical Product
GCP	Good Clinical Practices
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
IVIVC	In vitro in vivo correlation
Kel	elimination rate constant
LOQ	Limit of quantification
MRA	Medicines Regulatory Authority
MRT	mean residence time
MS	Member State
ODT	Orodispersible tablet
QA	Quality Assurance
SD/RSD	Standard deviation
SOP	Standard Operating Procedure
tmax	time to C_{max}
USA	United States of America
US FDA	United States Food and Drug Administration
USP	United States Pharmacopoeia
WHO	World Health Organization

2. GLOSSARY

Active Pharmaceutical Ingredient (API) or drug substance

A substance or compound that is intended to be used in the manufacture of a pharmaceutical product as a therapeutically active ingredient.

Bioavailability

Bioavailability refers to the rate and extent to which the API, or its active moiety, is absorbed from a pharmaceutical product and becomes available at the site of action. Reliable measurements of medicine concentrations at the site(s) of action are usually not possible. The substance in the general circulation, however, is considered to be in equilibrium with the substance at the site(s) of action.

Bioavailability can be therefore 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. Based on pharmacokinetic and clinical considerations it is generally accepted that in the same participant an essentially similar plasma concentration time course will result in an essentially similar concentration time course at the site(s) of action.

It may be useful to distinguish between the "absolute bioavailability" of a given dosage form as compared with that (100 %) following intravenous administration (e.g. oral solution vs. iv.), and the "relative bioavailability" as compared with another form administered by the same or another non-intravenous route (e.g. tablets vs. oral solution).

Bioequivalence

Two pharmaceutical products are bioequivalent if they are pharmaceutically equivalent or pharmaceutical alternatives, and their bioavailabilities, in terms of peak (Cmax and Tmax) and total exposure (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.

Biopharmaceutics Classification System (BCS)

The BCS is a scientific framework for classifying medicinal substances based on their aqueous solubility and intestinal permeability. When combined with the dissolution of the drug product and critical examination of the excipients of the drug product, the BCS takes into account the major factors that govern the rate and extent of medicine absorption (exposure) from immediate release (IR) solid oral dosage forms: excipient composition, dissolution, solubility, and intestinal permeability.

Biowaiver

The term biowaiver is applied to a regulatory medicine approval process when the dossier (application) is approved based on evidence of equivalence other than through bioequivalence study (in vivo equivalence testing).

Comparable Dosage Form

A comparable dosage forms refers to different formulations of the same product given by the same route (e.g. capsules and tablets)

Dosage form

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

Equivalence requirements

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

Equivalence test

In vivo and/or in vitro approaches that determines the equivalence between the generic product and the reference product

Fixed-dose combination (FDC)

A formulation 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.

Generic Pharmaceutical Product

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

Innovator product

Generally, the innovator pharmaceutical product is that which was first authorised for marketing, on the basis of documentation of quality, safety and efficacy.

Interchangeable pharmaceutical product

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

In vitro equivalence dissolution test

An in vitro equivalence test is a dissolution test that includes comparison of the dissolution profile between the test (e.g. generic) product and the reference (e.g. innovator) product, typically in at least three media: pH 1.2, pH 4.5 and pH 6.8.

In vitro quality control dissolution test

A dissolution test procedure identified in the pharmacopoeia, generally a one-time point dissolution test for immediate-release products and a three – or more time points dissolution test for modified-release products.

Pharmaceutical alternatives

Medicinal products are pharmaceutical alternatives if they contain the same molar amount of the same active moiety but differ in chemical form (e.g. salt, ester) of that moiety or in the dosage form (e.g. tables versus capsules) or strength. 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 product.

Pharmaceutical Dosage Form

A pharmaceutical dosage form is a pharmaceutical product formulated to produce a specific physical form (e.g. tablet, capsule, solution) suitable for administration to human and animal participants.

Pharmaceutical Equivalence

Pharmaceutical products are pharmaceutically equivalent if they contain the same amount of the same API(s) in the same dosage form, if they meet the same or comparable standards and if they are to be administered by the same route.

Pharmaceutical equivalence does not necessarily imply bioequivalence as differences in the excipients and/or the manufacturing process and some other variables can lead to changes in dissolution and/or absorption.

Pharmaceutical Product

Any preparation for human or veterinary use containing one or more APIs, with or without pharmaceutical excipients or additives, that is intended to modify or explore physiological systems or pathological states for the benefit of the recipient.

Proportionally Similar Dosage Forms/Products

Pharmaceutical products are considered proportionally similar in the following cases:

- When all APIs and inactive pharmaceutical ingredients (IPIs) are in exactly the same proportion between different strengths (e.g. a 100 mg strength tablet has all API and IPIs exactly half of a 200 mg strength tablet and twice that of a 50 mg strength tablet).

- When the active and inactive ingredients are not in exactly the same proportion but the ratios of IPIs to the total mass of the dosage form are within the limits defined by the Post-registration amendment guideline.

- When the pharmaceutical products contain high potency APIs and these products are of different strengths but are of similar mass.

The difference in API content between strengths may be compensated for by mass changes in one or more of the IPIs provided that the total mass of the pharmaceutical product remains within 10 % of the mass of the pharmaceutical product on which the bioequivalence study was performed. In addition, the same IPIs should be used for all strengths, provided that the changes remain within the limits defined by the Post-registration amendment guideline.

Therapeutic Equivalence/Substitutable

Two pharmaceutical products are therapeutically equivalent/substitutable if they are pharmaceutically equivalent 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 labelling. This can be demonstrated by appropriate bioequivalence studies, such as pharmacokinetic, pharmacodynamics, clinical or in vitro studies.

Reference product

A reference product is a pharmaceutical product with which the new product is intended to be interchangeable in clinical practice. The reference product will normally be the innovator product for which efficacy, safety and quality have been established. Where the innovator product is not available, the applicant should consult the medicines regulatory authority for suitable reference product.

3. INTRODUCTION

Adequate evidence/proof of efficacy and safety for all generic (multisource)[1]products in the form of appropriate *in vivo* bioequivalence studies should be submitted with each application for the registration of a medicine.

To exert an optimal therapeutic action, an active moiety should be delivered to its site of action in an effective concentration for the desired period. To allow reliable prediction of the therapeutic effect, the characteristics of the dosage form containing the active pharmaceutical ingredient (API) or drug substance[2], should be well defined.

Comparison of the therapeutic performances of two pharmaceutical products containing the same API is a critical means of assessing the possibility of using either the innovator, or a generic pharmaceutical product. Assuming that in the same participant a similar plasma drug concentration time course will result in similar drug concentrations at the site of action and thus in a similar effect, pharmacokinetic data instead of therapeutic results may be used to establish bioequivalence.

The objectives of this guideline are to:
- a) Define when bioavailability or bioequivalence data will be required in order to prove safety and efficacy.
- b) Provide guidance on the design and conduct of studies and the evaluation of data.
- c) Provide guidance when in vitro instead of in vivo data may be used.
- d) Provide guidance when suitably validated pharmacodynamic or clinical methods can be used to demonstrate bioequivalence.

For pharmaceutical products, where the active ingredient is not to be delivered into the general circulation, the common systemic bioavailability approach cannot be applied. Under these conditions availability (local) may be assessed by quantitative measurements, which appropriately reflect the presence of the active ingredient at the site of action.

Direct demonstration of therapeutic equivalence through a comparative clinical trial is rarely a practical choice, as these trials tend to be insensitive to formulation differences and usually require a very large number of patients. Further, such studies in humans can be financially daunting, are often unnecessary and may be unethical. For these reasons the science of bioequivalence testing has been developed over the last 50 years.

According to the tenets of this science, therapeutic equivalence can be assured when the generic product is both pharmaceutically equivalent/alternative and bioequivalent. Assuming that, in the same participant, an essentially similar plasma concentration-time course will result in essentially similar concentrations at the site(s) of action and thus an essentially similar therapeutic outcome, pharmacokinetic data may be used instead of therapeutic results. Further, in selected cases, in vitro comparison of the dissolution profiles of the generic product with those of the reference product may be sufficient to provide an indication of equivalence.

It should be noted that the concept of interchangeability includes the equivalence of the dosage form as well as of the indications and instructions for use. Alternative approaches to the principles and practices described in this document may be acceptable provided they are supported by adequate scientific justification. These guidelines are based on the principles and requirements from the United States (US) Food and Drug Administration (FDA) (1), The European Medicines Agency (EMA) (2) and the World Health Organization (WHO) (3) adapted to suit the SADC region.

4. SCOPE

This guideline represents the current thinking on this subject. It does not create or confer any rights for or on any person and does not operate to bind the SADC medicine regulatory authorities (MRA) or the public. An alternative approach may be used if such approach satisfies the requirements of the applicable statutes and regulations in the member states.

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[1]Although the term generic and multisource can be used interchangeable, throughout this document, the term generic is used or preferred.
[2]The term active pharmaceutical ingredient or drug substance is used interchangeable in this document. Applicant should consult the respective SADC Member States, in terms of the preferred term to use in the submissions to Member States.

The guideline addresses how to meet the bioavailability (BA) and bioequivalence (BE) requirements as they apply to dosage forms intended for oral administration. It is also generally applicable to non-orally administered medicine products where reliance on systemic exposure measures is suitable to document BA and BE (e.g., transdermal delivery systems and certain rectal and nasal medicine products). It should be useful for applicants planning to conduct BA and BE studies during the investigational period for a new medicine application, BE studies intended for submission in generic medicine applications, and BE studies conducted in the post-approval period for certain changes in both new medicine and generic medicine applications.

5. DOCUMENTATION OF EQUIVALENCE FOR MARKETING AUTHORIZATION

Generic pharmaceutical products must be shown, either directly or indirectly, to be therapeutically equivalent to the reference product if they are to be considered interchangeable. Suitable test methods to assess equivalence are:

- comparative pharmacokinetic studies in humans, in which the active pharmaceutical ingredient (API) 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 measures, such as AUC and Cmax that are reflective of the systemic exposure;
- comparative pharmacodynamic studies in humans;
- comparative clinical trials;
- comparative in vitro tests.

The applicability of each of these four methods is discussed below. Detailed information is provided on conducting an assessment of equivalence studies using pharmacokinetic measurements and in vitro methods, which are currently the methods most often used to document equivalence for most orally administered pharmaceutical products for systemic exposure.

Acceptance of any test procedure in the documentation of equivalence between two pharmaceutical products by a medicines regulatory authority (MRA) depends on many factors, including the characteristics of the API and the pharmaceutical product. Where an API produces measurable concentrations in an accessible biological fluid such as plasma, comparative pharmacokinetic studies can be performed. This type of study is considered to be the gold standard in equivalence testing; however, where appropriate, in vitro testing and biopharmaceutics classification system (BCS) -based biowaivers for immediate-release pharmaceutical products can also assure equivalence between the generic product and the reference product (see sections 16 and 19). Where an API does not produce measurable concentrations in an accessible biological fluid and a BCS-based biowaiver is not an option, comparative pharmacodynamics studies may be an alternative method for documenting equivalence. Further, in certain cases when it is not possible to assess equivalence through other methods, comparative clinical trials may be considered appropriate. The criteria that indicate when equivalence studies are necessary are discussed section 6 and 16 of this guideline.

6. IN VIVO EQUIVALENCE STUDIES IN HUMANS

General considerations

Provisions for studies in humans

Pharmacokinetic, pharmacodynamic and clinical studies are clinical trials and should therefore be carried out in accordance with the provision and prerequisites for a clinical trial, as outlined in the WHO guidelines for good clinical practice (GCP) for trials on pharmaceutical products (4). Additional guidance for organizations performing in vivo bioequivalence studies is available from WHO (5).

All research involving human participants should be conducted in accordance with the ethical principles contained in the current version of the Declaration of Helsinki, including respect for persons, beneficence ("maximize benefits and minimize harms and wrongs") and non-maleficence ("do no harm").

As defined by the current revision of the International Ethical Guidelines for Biomedical Research Involving Human Participants issued by the Council for International Organizations of Medical Sciences (CIOMS), or laws and regulations of the country in which the research is conducted, whichever represents the greater protection for participants.

Justification of human bioequivalence studies

Most pharmacokinetic and pharmacodynamic equivalence studies are non-therapeutic studies in which no direct clinical benefit accrues to the participant. It is important for anyone preparing a trial of a medicinal product in humans that the specific aims, problems and risks or benefits of the proposed human study be thoroughly considered and that the chosen design be scientifically sound and ethically justified. It is assumed that people involved in the planning of a study are familiar with pharmacokinetic theories underlying bioavailability and bioequivalence studies. The overall design of the bioequivalence study should be based on the knowledge of the pharmacokinetics, pharmacodynamics and therapeutics of the API. Information about manufacturing procedures and data from tests performed on the product batch to be used in the study should establish that the product under investigation is of suitable quality.

Selection of investigators

The investigator(s) should have the appropriate expertise, qualifications and competence to undertake the proposed study. Prior to the trial the investigator(s) and the sponsor should draw up an agreement on the protocol, monitoring, auditing, standard operating procedures (SOP) and the allocation of trial-related responsibilities. The identity and duties of the individuals responsible for the study and safety of the participants participating in the study must be specified. The logistics and premises of the trial site should comply with requirements for the safe and efficient conduct of the trial.

Study protocol

A bioequivalence study should be carried out in accordance with a protocol agreed upon and signed by the investigator and the sponsor. The protocol and its attachments and/or appendices 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 trial participants 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 appended as amendments, except when necessary to eliminate an apparent immediate hazard or danger to a trial participant.

The protocol and attachments/appendices should be scientifically and ethically appraised by one or, if required by local laws and regulations, more review bodies (e.g. institutional review board, peer review committee, ethics committee, MRA), constituted appropriately for these purposes and independent of the investigator(s) and sponsor.

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

7. PHARMACOKINETIC COMPARATIVE BIOAVAILABILITY (BIOEQUIV ALENCE) STUDIES IN HUMANS

A bioequivalence study is basically a comparative bioavailability study designed to establish whether or not there is equivalence between test and reference products. Pharmacokinetic bioequivalence studies on products designed to deliver the API for systemic exposure serve two purposes:

- as a surrogate for clinical evidence of the safety and efficacy of the generic product;

- as an in vivo measure of pharmaceutical quality.

The design of the study should maximize the sensitivity to detect the difference between products, minimize the variability that is not caused by formulation effects and eliminate bias as far as possible. Test conditions should reduce variability within and between participants. In the following sections, requirements for the design and conduct of bioavailability or bioequivalence studies are formulated.

7.1. Design, conduct and evaluation of bioequivalence studies

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 enantio-selective analysis and the possibility of waiver for additional strengths (see sections 10, 11 and 12)

7.2. Study design

The study should be designed in such a way that the formulation effect can be distinguished from other effects.

7.3. Standard design

If the number of formulations to be compared is two, a balanced two-period, two-sequence crossover design is considered to be the design of choice. The treatment periods should be separated by a wash out period sufficient to ensure that drug concentrations are below the lower limit of bioanalytical quantification in all participants at the beginning of the second period. Normally at least 5 elimination half-lives are necessary to achieve this. The sampling schedule should be planned to provide an adequate estimation of C_{max} and to cover the plasma drug concentration time curve long enough to provide a reliable estimate of the extent of absorption. This is generally achieved if the AUC derived from measurements is at least 80 % of the AUC extrapolated to infinity.

If a reliable estimate of terminal half-life is necessary, it should be obtained by collecting at least three to four samples above the LOQ during the terminal log linear phase.

For long half-life drugs (> 24 hours) the study should cover a minimum of 72 hours unless 80 % is recovered before 72 hours.

7.4. Alternative design

Under certain circumstances, provided the study design and the statistical analyses are scientifically sound, alternatively well-established designs such as parallel designs for very long half-life substances, could be considered such as parallel design for substance with very long half-life and replicate designs e.g. for substances with highly variable pharmacokinetic characteristics (see section 14.4.5)

A single-dose, cross-over pharmacokinetic bioequivalence study of an orally-administered product with a long elimination half-life is preferred, provided an adequate wash-out period between administrations of the treatments is possible. The interval between study days should be long enough to permit elimination of essentially the entire 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 approximately six weeks. If the crossover study is problematic due to a very long elimination half-life, a pharmacokinetic bioequivalence study with a parallel design may be more appropriate. A parallel design may also be necessary when comparing some depot formulations.

For both crossover and parallel-design studies, sample collection time should be adequate to ensure completion of GI transit (approximately 2–3 days) of the pharmaceutical product and absorption of the API. Blood sampling should be conducted for up to 72 hours following administration but sampling beyond this time is not generally necessary.

The number of participants should be derived from statistical calculations but generally more participants are needed for a parallel study design than for a crossover study design.

In general, single dose studies will suffice, but there are situations in which steady-state studies may be required in which case the steady-state study design should be motivated. For example conduct of a multiple dose study in patients is acceptable if a single dose study cannot be conducted in healthy volunteers due to tolerability reasons, and a single dose study is not feasible in patients.

In the rare situation where problems of sensitivity of the analytical method preclude sufficiently precise plasma concentration measurements after single dose administration and where the

concentrations at steady state are sufficiently high to be reliably measured, a multiple dose study may be acceptable as an alternative to the single dose study. However, given that a multiple dose study is less sensitive in detecting differences in Cmax, 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 (see also section 10). 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.

Multiple dose studies may also be applicable for extended-release dosage forms with a tendency to accumulation (in addition to single-dose studies).

In steady-state studies, the washout period of the previous treatment can overlap with the build-up of the second treatment, provided the build-up period is sufficiently long (at least 5 times the terminal half-life). Appropriate dosage administration and sampling should be carried out to document for the attainment of a steady state.

8. PARTICIPANTS

8.1. Number of participants

The number of participants required for a sound pharmacokinetic bioequivalence study is determined by:
- 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 (i.e. at least 80% power);
- the mean deviation from the reference 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 participants to be recruited for the study should be estimated by considering the standards that must be met using an appropriate method (see, for example, Julious 2004 *(6)*). In addition, an extra number of participants should be recruited, dosed and their samples analysed based on the expected rate of dropouts and/or withdrawals, which depends on the safety and tolerability profile of the medicine. The number of participants recruited should always be justified by the sample-size calculation provided in the study protocol. A minimum of 12 participants is required.

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 as an alternative to conducting a pilot study.

Refer to section 14.3 for more information.

8.2. Dropouts and withdrawals

Sponsors should select a sufficient number of study participants to allow for possible dropouts or withdrawals. Because replacement of participants during the study could complicate the statistical model and analysis, dropouts generally should not be replaced. Reasons for withdrawal (e.g. adverse medicine reaction or personal reasons) must be reported. If a participant is withdrawn due to an adverse event after receiving at least one dose of the study medicine, the participant's plasma/serum concentration data should be provided.

The concentration-time profiles of participants who exhibit pre-dose concentrations higher than 5% of the corresponding Cmax should be excluded from the statistical analysis. The concentration-time profiles of participants who exhibit pre-dose concentrations equal to or less than 5% of the corresponding Cmax should be included in the statistical analysis without correction.

8.3. Outliers

Extreme values can have a significant impact on bioequivalence study data because of the relatively small number of participants typically involved; however, it is rarely acceptable to exclude data. Potential reasons for excluding outlying data and the procedure to be following when assessing potential outliers should be included in the study protocol. All data should be treated in an equivalent fashion so any procedures should be applied to all data. Exclusion of data for statistical or pharmacokinetic reasons alone is not acceptable.

Retesting of participants identified as outliers is not recommended.

8.4. Selection of Participants

The participant population for bioequivalence studies should be selected with the aim to minimise variability and permit detection of differences between pharmaceutical products. Therefore, the studies should normally be performed with healthy volunteers, unless the drug carries safety concerns that make this unethical. This model, in vivo healthy volunteers, is regarded as adequate in most instances to detect formulation differences and to allow extrapolation of the results to populations for which the reference medicinal product is approved (the elderly, children, patients with renal or liver impairment, etc.).

The inclusion/exclusion criteria should be clearly stated in the protocol.

In general, participants should exhibit the following characteristics:

a) **Sex:** Participants may be selected from either sex. However, the risk to women of childbearing potential should be considered on an individual basis and, if necessary, they should be warned of any possible dangers to the foetus if they should become pregnant. The investigators should ensure that female volunteers are not pregnant or likely to become pregnant during the study. Confirmation should be obtained by urine tests just before administration of the first and last doses of the product under study.
b) **Age:** Participants should be between 18 and 55 years of age.
c) **Mass:** Participants should have a body mass within the normal range according to accepted normal values for the Body Mass Index (BMI = mass in kg divided by height in meters squared, i.e. kg/m^2), preferably 18.5 and 30kg/m2, or within 15 % of the ideal body mass, or any other recognised reference.
d) **Informed Consent**: All participants participating in the study should be capable of giving informed consent.
e) **Medical Screening:** Participants should be screened for suitability by means of clinical laboratory tests, an extensive review of medical history, and a comprehensive medical examination. Depending on the drug's therapeutic class and safety profile, special medical investigations may have to be carried out before, during and after the completion of the study.
f) **Smoking/Drug and Alcohol Abuse:** Participants should preferably be non-smokers and without a history of alcohol or drug abuse. If moderate smokers are included they should be identified as such and the possible influences of their inclusion on the study results should be discussed in the protocol.

In parallel design studies, the treatment groups should be comparable in all known variables that may affect the pharmacokinetics of the active substance (e.g. age, body weight, sex, ethnic origin, smoking status, extensive/poor metabolic status). This is an essential pre-requisite to give validity to the results from such studies. 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.

8.5. Inclusion of Patients

If the API under investigation is known to have adverse effects and the pharmacological effects or risks are considered unacceptable for healthy volunteers, it may be necessary to use patients instead, under suitable precautions and supervision. In this case the applicant should justify the use of patients instead of healthy volunteers.

8.6. Genetic Phenotyping

Phenotyping for metabolizing activity can be of importance for studies with high- clearance medicines that are metabolized by enzymes that are subject to genetic polymorphism, e.g. propranolol. In such cases slow metabolizers will have a higher bioavailability of the parent medicine, while the bioavailability of possible active metabolites will be lower. Phenotyping of participants can be considered for studies of medicines 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 between the two groups of participants.

Phenotyping could also be important for safety reasons, determination of sampling times and washout periods in crossover design studies.

8.7. Monitoring the health of participants during the study

In keeping with GCP (4), the health of volunteers should be monitored during the study 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 medicine-induced is to be judged by the investigator.

Health monitoring before, during and after the study must be carried out under the supervision of a qualified medical practitioner licensed in the jurisdiction in which the study is conducted.

9. STUDY PRODUCTS

9.1. Reference Product

The innovator pharmaceutical product is usually the most logical reference product for a generic pharmaceutical product because its quality, safety and efficacy should have been well assessed and documented in premarketing studies and postmarketing monitoring schemes. Typically this will mean employing the innovator product available on the local market when studying generic products for national approval. There will be situations, however, where this is not feasible. Detailed guidance for the selection of reference products for use in national applications is provided in a recent, updated reference guidance (7).

Briefly, in principle, a national MRA has the following options for selection of a reference product, listed in order of preference:

- An innovator product, which is imported from a country with stringent regulatory authority where it has been approved on the basis of clinical data demonstrating safety and efficacy and is currently registered and marketed in that country.
- the WHO-recommended reference product (7) imported from a country considered by SADC to have a stringent regulatory authority such ICH or ICH associated countries;
- the innovator product for which quality, safety and efficacy has been established if this product has been granted a national marketing authorization ("nationally authorized innovator") and imported from a country with a stringent regulatory authority where it may /may not be currently registered and / or marketed;
- in the case that no innovator/reference product can be identified according to the above, the choice of the reference must be made carefully and must be comprehensively justified by the applicant. In this case, the most important selection criteria are
  - extensive documented use in clinical trials reported in peer-reviewed scientific journals, long and unproblematic period of post-market surveillance and approved and imported from a country by stringent regulatory authority.

Additionally, these reference products must conform to all appropriate compendial quality standards.
It is important to note that a product that has been approved based on comparison with a non-domestic reference product may or may not be interchangeable with currently marketed domestic products.

Reference products should be purchased from a well-regulated market with stringent regulatory authority. The applicant should justify the choice of reference product. The country of origin of the reference product should be reported together with lot number and expiry date, as well as results of pharmaceutical analysis. Further in order to prove the origin of the reference product the applicant must present all of the following documents:

1) Copy of the reference product labelling (snapshot of the carton label). The name of the product, name and address of the manufacturer, batch number, and expiry date should be clearly visible on the labelling.
2) Copy of the dated invoice from the distributor or company from which the reference product was purchased. The address of the distributor must be clearly visible on the invoice.
3) Documentation verifying the method of shipment and storage conditions (temperature data log) of the reference product from the time of purchase to the time of study initiation.
4) A signed statement certifying the authenticity of the above documents and that the reference product was purchased from the specified national market. The company executive responsible for the application should sign the certification.

The selection of the reference product used in a bioequivalence study should be based on assay content and dissolution data and is the responsibility of the applicant. Unless otherwise justified, the assayed content of the batch used as test product should not differ more than 5% from that of the batch used as reference product determined with the test procedure proposed for routine quality testing of the test product.

9.2. Test product

The test product used in the study should be representative of the product to be marketed and this should be discussed and justified by the applicant. 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 GMP regulations.

For example, for oral solid forms for systemic action:

a) The test product should usually originate from a batch of at least 1/10 of production scale or 100,000 units, whichever is greater, unless otherwise justified. In case of a production batch smaller than 100,000 units, a full production batch will be required

b) The production of batches used should provide a high level of assurance that the product and process will be feasible on an industrial scale.

c) The characterisation and specification of critical quality attributes of the drug product, such as dissolution, should be established from the test batch, i.e. the clinical batch for which bioequivalence has been demonstrated.

d) Samples of the product from additional pilot and / or full-scale production batches, submitted to support the application, should be compared with those of the bioequivalence study test batch, and should show similar in vitro dissolution profiles when employing suitable dissolution test conditions (see **APPENDIX 1 – DISSOLUTION REQUIREMENTS**).

Comparative dissolution profile testing should be undertaken on the first three production batches.

The results should be provided at the MRA's request or if the dissolution profiles are not similar together with proposed action to be taken.

For other immediate release pharmaceutical forms for systemic action, justification of the representative nature of the test batch should be similarly established.

It is recommended that potency and in vitro dissolution characteristics of the generic and the reference pharmaceutical products should be ascertained prior to performance of an equivalence study. Content

of the API(s) of the reference product should be close to the label claim and the difference between two products being compared should not be more than ± 5%. If, because of the lack of availability of different batches of the reference product, it is not possible to study batches with potencies within ± 5%, potency correction may be required on the statistical results from the bioequivalence study.

9.3. 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 for single products. The generic FDC product should be compared with the pharmaceutically equivalent reference FDC product. In certain cases (e.g. when no reference FDC product is available on the market) separate products administered in free combination can be used as a reference (3). Sampling times should be chosen to enable the pharmacokinetic parameters of all APIs to be adequately assessed. The bioanalytical method should be validated with respect to all compounds measured in the presence of the other compounds. Statistical analyses 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.

9.4. Retention samples

A sufficient number of retention samples of both test and reference products used in the bioequivalence study, should be kept for one year in excess of the accepted shelf-life, or two years after completion of the trial or until approval, whichever is longer, in order to allow re-testing if required by SADC MS.

9.5. Sample handling

A complete audit trail of procurement, storage, transport and use of both the test and reference products should be recorded.

10. STRENGTH TO BE INVESTIGATED

If several strengths of a test product are applied for, it may be sufficient to establish bioequivalence at only one or two strengths, depending on the proportionality in composition between the different strengths and other product related issues described below. The strength(s) to evaluate depends on the linearity in pharmacokinetics of the active substance.

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. In order to assess linearity, the applicant should consider all data available in the public domain with regard to the dose proportionality and review the data critically. Assessment of linearity will consider whether differences in dose-adjusted AUC meet a criterion of ± 25%.

If bioequivalence has been demonstrated at the strength(s) that are most sensitive to detect a potential difference between products, in vivo bioequivalence studies for the other strength(s) can be waived.

10.1. General biowaiver criteria

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

a) the pharmaceutical products are manufactured by the same manufacturing process,

b) the qualitative composition of the different strengths is the same,

c) 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 (for immediate release products coating components, capsule shell, colour agents and flavours are not required to follow this rule),

If there is some deviation from quantitatively proportional composition, condition c is still considered

fulfilled if 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

d) appropriate in vitro dissolution data should confirm the adequacy of waiving additional in vivo bioequivalence testing (see section 19).

10.2.   Linear pharmacokinetics

For products where all the above conditions a) to d) are fulfilled, it is sufficient to establish bioequivalence with only one strength.

The bioequivalence study should in general be conducted at the highest strength. For products with linear pharmacokinetics and where the drug substance is highly soluble (see Appendix III), selection of a lower strength than the highest is also acceptable. Selection of a lower strength may also be justified if the highest strength cannot be administered to healthy volunteers for safety/tolerability reasons. Further, if problems of sensitivity of the analytical method preclude sufficiently precise plasma concentration measurements after single dose administration of the highest strength, a higher dose may be selected (preferably using multiple tablets of the highest strength). The selected dose may be higher than the highest therapeutic dose provided that this single dose is well tolerated in healthy volunteers and that there are no absorption or solubility limitations at this dose.

10.3.   Non-linear pharmacokinetics

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 volunteers for safety/tolerability reasons. Likewise a higher dose may be used in case of sensitivity problems of the analytical method in line with the recommendations given for products with linear pharmacokinetics above.

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 a) to d) above are fulfilled and the test and reference 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 volunteers for safety/tolerability reasons.

10.4.   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.

10.5. Fixed combinations

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.

10.6. Modified Release Products

10.6.1. Beaded Capsules - Lower Strength

For extended release beaded capsules where the strength differs only in the number of beads containing the active ingredient, a single-dose, fasting BE study should be carried out on the highest strength. A biowaiver for the lower strength based on dissolution studies can be requested.

Dissolution profiles in support of a biowaiver should be generated for each strength using the recommended dissolution test methods described in Appendix I.

10.6.2. Tablets – Lower strength

 For extended release tablets when the drug product is:
a)   in the same dosage form but in a different strength, and
b)   is proportionally similar in its active and inactive ingredients, and
c)   has the same drug release mechanism,

An *in vivo* BE determination of one or more lower strengths may be waived based on dissolution testing as previously described. Dissolution profiles should be generated on all the strengths of the test and the reference products.

11. STUDY CONDUCT

11.1. Standardisation Of The Study Conditions

The test conditions should be standardised in order to minimise the variability of all factors involved, except that of the products being tested. Therefore, standardisation of the diet, fluid intake and exercise is recommended.

Dosing: The time of day for ingestion of doses should be specified.

Fluid Intake at Dosing: As fluid intake may profoundly influence the gastric transit of orally administered dosage forms, the volume of fluid administered at the time of dosing should be constant (usually 150 -200 ml).

 Food and Fluid Intake: In fasted studies, participants should fast for at least 8 hours prior to administration of the products, unless otherwise justified. It is recommended that water is allowed as desired except for one hour before and one hour after drug administration and no food is allowed for at least 4 hours post-dose. Meals taken after dosing should be standardised in regard to composition and time of administration during an adequate period of time (e.g. 12 hours).

 In case the study is to be performed during fed conditions, the timing of administration of the drug product in relation to food intake is recommended to be according to the SmPC of the originator product. If no specific recommendation is given in the originator SmPC, it is recommended that participants should start the meal 30 minutes prior to administration of the drug product and eat this meal within 30 minutes.

There are situations when the investigational products should be administered following consumption of a meal (under fed conditions). These situations are described below.

Immediate-release formulations

Fasted-state studies are generally preferred. However, when the product is known to cause GI disturbances if given to participants in the fasted state, or if the labelling of the reference product restricts administration to participants in the fed state, then the fed-state pharmacokinetic bioequivalence study becomes the preferred approach.

Typically a meal meeting the composition recommendations identified in section 11.2 should be employed in fed-state studies. The exact composition of the meal may depend on local diet and customs. For studies conducted with immediate-release products, there may be situations where it is appropriate to employ a pre-dose meal with different caloric/fat content. The regulatory authority for which the study is being conducted should be consulted prior to employing a meal other than the high fat, high-calorie meal described in section 11.2.

The test meal should be consumed within a 30-minute interval prior to administration of the medicine product.

Modified-release formulations

In addition to a study conducted under fasted conditions, food-effect studies are necessary for all generic modified-release formulations to ensure that the interaction between the varying conditions in the GI tract and the product formulations does not differentially impact the performance of the generic and reference products. The presence of food can affect product performance both by influencing the release of the API from the formulation and by causing physiological changes in the GI tract. A significant concern with regard to modified-release products is the possibility that food may trigger a sudden and abrupt release of the API leading to "dose dumping".

In these cases the objective is to select a meal that will challenge the robustness of the new generic formulation to prandial effects on bioavailability. To achieve this a meal that will provide a maximal perturbation to the GI tract relative to the fasted state should be employed. A high-fat (approximately 50% of total caloric content of the meal), high- calorie (approximately 800 to 1000 calories) test meal for fed BE studies is recommended.

This test meal should derive approximately 150, 250 and 500–600 calories from protein, carbohydrate and fat, respectively. One example of a high-fat, high-calorie test meal is the following breakfast: two eggs fried in butter, two strips of bacon, two slices of toast with butter, 120 grams of hash browns and 240 millilitres of whole milk. The caloric breakdown of the test meal should be provided in the study report.

The test meal should be consumed within a 30-minute interval prior to administration of the medicine product.

Concomitant Medication: Participants should not take other medicines for a suitable period prior to, and during, the study and should abstain from food and drinks, which may interact with circulatory, gastrointestinal, liver or renal function (e.g. alcoholic or xanthine-containing beverages or certain fruit juices). In case concomitant medication is unavoidable and a participant is administered other drugs, for instance to treat adverse events like headache, the use 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 participants 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 originator 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 to be administered concomitantly.

Posture and Physical Activity: As the bioavailability of an active moiety from a dosage form can be dependent upon gastrointestinal transit times and regional blood flows, posture and physical activity may need to be standardised.

Bioequivalence studies of endogenous substances: In bioequivalence studies of endogenous

substances, factors that may influence the endogenous baseline levels should be controlled if possible (e.g. strict control of dietary intake).

11.2. Fasting or 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. For products where the SmPC recommends intake of the reference medicinal product on an empty stomach or irrespective of food intake, the bioequivalence study should hence be conducted under fasting conditions. For products where the SmPC recommends intake of the reference medicinal product only in fed state, the bioequivalence study should generally be conducted under fed conditions.

However, for products with specific formulation characteristics (e.g. microemulsions, solid dispersions), bioequivalence studies performed under both fasted and fed conditions are required unless the product must be taken only in the fasted state or only in the fed state.

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

In studies performed under fed conditions, the composition of the meal is recommended to be according to the SmPC of the originator product. If no specific recommendation is given in the originator SmPC, the meal should be a high-fat (approximately 50 percent of total caloric content of the meal) and high-calorie (approximately 800 to 1000 kcal) meal. This test meal should derive approximately 150, 250, and 500-600 kcal from protein, carbohydrate, and fat, respectively. The composition of the meal should be described with regard to protein, carbohydrate and fat content (specified in grams, calories and relative caloric content (%)).

11.3. SAMPLE COLLECTION AND SAMPLING TIMES

Under normal circumstances, blood should be the biological fluid sampled to measure the concentrations of the drug. In most cases the drug may be measured in serum or plasma. However, in some cases, whole blood may be more appropriate for analysis.

11.3.1. When blood is collected:

a) A sufficient number of samples to adequately describe the plasma concentration-time profile should be collected. The sampling schedule should also cover the plasma concentration time curve long enough to provide a reliable estimate of the extent of exposure which is achieved if AUC (0-t) covers at least 80% of AUC (0-I). This period is approximately three terminal half-lives of the drug. However, it is not necessary to sample for more than 72 hours. The exact duration of sample collection depends on the nature of the API and the input function from the administered dosage form.

b) Sampling points should include a pre-dose sample, at least 1–2 points before Cmax, 2 points around Cmax and 3–4 points during the elimination phase. Consequently at least seven sampling points will be necessary for estimation of the required pharmacokinetic parameters. For most medicines the number of samples necessary will be higher to compensate for between-participant differences in absorption and elimination rate and thus enable accurate determination of the maximum concentration of the API in the blood (Cmax) and terminal elimination rate constant in all participants.

c) The sampling schedule should include frequent sampling around predicted tmax to provide a reliable estimate of peak exposure. In particular, the sampling schedule should be planned to avoid Cmax being the first point of a concentration time curve.

d) At least three to four samples above LOQ should be obtained during the terminal log linear phase to estimate K_{el} by linear regression analysis.

e) AUC truncated at 72 h (AUC (0-72h)) may be used as an alternative to AUC (0-t) for comparison of extent of exposure as the absorption phase has been covered by 72 h for immediate release formulations. A sampling period longer than 72 h is therefore not considered necessary for any immediate release formulation irrespective of the half-life of the

drug.

f) The actual clock time when samples are collected, as well as the elapsed time relative to drug administration, should be recorded.

If drug concentrations in blood are too low to be detected and a substantial amount (> 40 %) of the drug is eliminated unchanged in the urine, then urine may serve as the biological fluid to be sampled.

In multiple-dose studies, the pre-dose sample should be taken immediately before (within 5 minutes) dosing and the last sample should be taken within 10 minutes of the nominal time for the dosage interval to ensure an accurate determination of AUC (0-τ).

For endogenous substances, the sampling schedule should allow characterisation of the endogenous baseline profile for each participant in each period. Often, a baseline is determined from 2-3 samples taken before the drug products are administered. 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 (see section 12).

Blood samples should be processed and stored under conditions that have been shown not to cause degradation of the analytes. These conditions should be included in the analytical validation report (see section 13).

The sample collection methodology must be specified in the study protocol.

11.3.2. When urine is collected:

a) The volume of each sample should be measured immediately after collection and included in the report.

b) Urine should be collected over an extended period and generally no less than three times the terminal elimination half-life, so that the amount excreted to infinity (Ae_∞) can be estimated. 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 (see also section 12).

c) Sufficient samples should be obtained to permit an estimate of the rate and extent of renal excretion. For a 24-hour study, sampling times of 0 to 2, 2 to 4, 4 to 8, 8 to 12, and 12 to 24 hours post-dose are usually appropriate.

d) The actual clock time when samples are collected, as well as the elapsed time relative to drug administration, should be recorded.

12. CHARACTERISTICS TO BE INVESTIGATED

12.1. Pharmacokinetic parameters

Sampling points should be chosen such that the plasma concentration *versus* time profiles can be defined adequately, thereby allowing accurate estimation of relevant parameters.

The following bioavailability parameters are to be estimated:

a) AUC_t, AUC_∞, C_{max}, t_{max} for plasma concentration *versus* time profiles. In studies with a sampling period of 72 h, and where the concentration at 72 h is quantifiable, AUC (0-I) and residual area do not need to be reported; it is sufficient to report AUC truncated at 72h, AUC (0-72h). Additional parameters that may be reported include the terminal rate constant, λz, and t1/2. AUC0-t and Cmax are considered to be the most relevant paramaters for assessment of bioequivalence.

b) AUC (0-τ), Cmax,ss, Cmin,ss, tmax, ss, fluctuation (% PTF) and swing (% Swing) for bioequivalence studies for immediate release formulations conducted at steady state.

c) Ae (0-t) and, if applicable, Rmax should be determined when using urinary data,

d) Any other justifiable characteristics

e) The method of estimating AUC-values should be specified.

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.

As delivery mechanisms of pharmaceutical products become more complex, e.g. products with an immediate-release and modified-release component, additional parameters such as partial AUC measures may be necessary to ensure the bioequivalence of two products.

12.2.   Parent compound or metabolites

General recommendations

In most cases evaluation of bioavailability and bioequivalence will be based upon measured concentrations of the parent compound (i.e. the API) where the shape of, and the area under, the plasma concentration *versus* time curves are generally used to assess the rate and extent of absorption. The reason for this is that Cmax of a parent compound is usually more sensitive to detect differences between formulations in absorption rate than Cmax of a metabolite.

Inactive pro-drugs

Also for inactive prodrugs, demonstration of bioequivalence for parent compound is recommended. The active metabolite does not need to be measured. However, some pro-drugs may have low plasma concentrations and be quickly eliminated resulting in difficulties in demonstrating bioequivalence for parent compound. In this situation it is acceptable to demonstrate bioequivalence for the main active metabolite without measurement of parent compound. In the context of this guideline, a parent compound can be considered to be an inactive pro-drug if it has no or very low contribution to clinical efficacy.

Use of metabolite data as surrogate for active parent compound

In some situations, however, measurements of an active or inactive metabolite may be necessary instead of the parent compound. Instances where this may be necessary are as follows:

a) If the concentration of the API is too low to be accurately measured 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 (see also section 10). Due to recent developments in bioanalytical methodology it is unusual that parent drug cannot be measured accurately and precisely. Hence, the use of a metabolite as a surrogate for active parent compound is acceptable only in exceptional cases.
b) If the parent compound is unstable in the biological matrix.
c) If the half-life of the parent compound is too short, thus, giving rise to significant variability.

Justification for not measuring the parent compound should be submitted by the applicant and bioequivalence determinations based on metabolites should be justified in each case.

12.3.   Use of urinary data

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. Sampling points should be chosen so that the cumulative urinary excretion profiles can be defined adequately so as to allow accurate estimation of relevant parameters.

However, the use of urinary data has to be carefully justified when used to estimate peak exposure. If reliable plasma Cmax 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.

12.4.   Endogenous substances

If the substance being studied is endogenous, the calculation of pharmacokinetic parameters should be performed using baseline correction so that the calculated pharmacokinetic parameters refer to

the additional concentrations provided by the treatment. 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. 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 formulation, in order to ensure that the dose used for the bioequivalence comparison is sensitive to detect potential differences between formulations.

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 pre- dose AUC, is preferred. In rare cases where substantial increases over baseline endogenous levels are seen, baseline correction may not be needed.

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

12.5.   **Chirality**

Measurement of individual enantiomers in BE studies is recommended only when all of the following conditions are met, otherwise measurement of the racemate using an achiral assay is recommended.

a) The enantiomers exhibit different pharmacodynamic and/or different pharmacokinetic characteristics;

b) Primary efficacy/safety activity is primarily due to the minor enantiomer;

c) Non-linear absorption is present (as expressed by a change in the enantiomer concentration ratio with change in the administration rate of the medicine) for at least one of the enantiomers.

The individual enantiomers should also be measured if the above conditions are fulfilled or are unknown. 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.

13. BIOANALYSIS

The bioanalytical part of bioequivalence trials should be conducted according to the applicable principles of Good Laboratory Practice (GLP) and cGMP.

Bioanalytical methods used to determine the active moiety and/or its metabolic product(s) in plasma, serum, blood or urine, or any other suitable matrix, should be well characterised, and fully validated and documented to yield reliable results that can be satisfactorily interpreted. Within study validation should be performed using Quality control samples in each analytical run.

The principles and procedures for bioanalytical method validation and analysis of study samples described in an up-to-date guidance from EMA or the USFDA should be employed.

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

In general:
- The analytical method should be able to differentiate the analyte(s) of interest, and if employed, the internal standard (IS) from endogenous components in the matrix or other components in the sample;
- The lower limit of quantification (LLOQ), being the lowest concentration of analyte in a sample, should be estimated to prove that the analyte at this concentration could be quantified reliably, with an acceptable accuracy and precision. The lower limit of quantitation should be 1/20 of Cmax or lower, as pre-dose concentrations should be detectable at 5% of Cmax or lower (see section 14.2 Carry-

over effects).

- The response of the instrument with regard to the concentration of analytes should be known and should be evaluated over a specified concentration range. A calibration curve should be generated for each analyte in each analytical run, and it should be used to calculate the concentration of the analyte in the unknown samples in the run. The calibration curve should be prepared in the same matrix as the matrix of the intended participant samples by spiking the blank 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;
- Within-run and between-run accuracy and precision should be assessed on samples spiked with known amounts of the analyte, the quality control (QC) samples, at a minimum of three different concentrations;
- Matrix effects should be investigated when using mass spectrometric methods;
- Stability of the analyte in the stock solution and in the matrix should be proven covering every step taken during sample preparation and sample analysis, as well as the storage conditions used;
- When more than one analyte is present in participant samples, the stability of the analytes in the matrix should be demonstrated in the presence of the other analytes;
- In case 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 changes implemented. A partial validation may be acceptable;
- A cross-validation is needed in cases where data are obtained from different method within and across studies or when data are obtained with a study from different laboratories, applying the same method;
- Analysis of participant samples should normally be carried our after full validation of the analytical method. Before the start of the analysis of the participant samples the performance of the bioanalytical method should have been verified;
- Reasons for reanalysis, reinjection and reintegration 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, reinjection or reintegration of participant 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.
- It is recommended to evaluate accuracy of incurred samples by reanalysis of participant samples in separate runs different delays;
- It is recommended that all the samples from one participant (all periods) should be analysed 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 SOP. All experiments used to support claims of draw conclusions about the validity of the method should be described in the method validation report. The main objective of method validation is to demonstrate the reliability of a particular method for the quantitative determination of an analyte(s) in a specific biological matrix. Validation should, therefore, address the following characteristics of the assay:

a) Stability of stock solutions.
b) Stability of the analyte(s) in the biological matrix under processing conditions and during the entire period of storage.
c) Specificity.
d) Accuracy.
e) Precision.
f) Limits of detection and quantification.
g) Response function and calibration range (calibration curve performance).
h) Robustness and ruggedness.
i) Matrix effects

All procedures should be performed according to pre-established Standard Operating Procedures (SOPs).

All relevant procedures and formulae, used to validate the bioanalytical method, should be submitted and discussed.

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

14. DATA ANALYSIS

The primary concern of bioequivalence assessment is to quantify the difference in bioavailability between the test and reference products, and to demonstrate that any clinically important difference is unlikely. In bioequivalence studies, the pharmacokinetic parameters should in general not be adjusted for differences in assayed content of the test and reference batch. However, in exceptional cases where a reference batch with an assay content differing less than 5% from test product cannot be found (see section 9) content correction could be accepted. If content correction is to be used, this should be pre-specified in the protocol and justified by inclusion of the results from the assay of the test and reference products in the protocol.

Participant accountability

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

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

Reasons for exclusion

Unbiased assessment of results from randomised studies requires that all participants 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 participant from the statistical analysis must be made before bioanalysis.

In principle any reason for exclusion is valid provided it is specified in the protocol and the decision to exclude is made before bioanalysis. However the exclusion of data should be avoided, as the power of the study will be reduced and a minimum of 12 evaluable participants is required.

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

The permitted reasons for exclusion must be pre-specified in the protocol. If one of these events occurs it should be noted in the case report forms (CRF) as the study is being conducted. Exclusion of participants based on these pre-specified criteria should be clearly described and listed in the study report.

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

2) Participants with non-zero baseline concentrations > 5% of Cmax. Such data should be excluded from bioequivalence calculation (see carry-over effects below).

The above can, for immediate release formulations, be the result of participant non-compliance and an insufficient wash-out period, respectively, and should as far as possible be avoided by mouth check of participants after intake of study medication to ensure the participants have swallowed the study medication and by designing the study with a sufficient wash-out period. The samples from participants excluded from the statistical analysis should still be assayed and the results listed (see Presentation of data below).

As stated in section 11, AUC (0-t) should cover at least 80% of AUC (0-I). Participants should not be excluded from the statistical analysis if AUC (0-t) covers less than 80% of AUC (0-I), 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 (0-72h) is used instead of AUC (0-t).

14.1.   Statistical **Analysis**

The statistical method for testing relative bioavailability (i.e. average bioequivalence) is based upon the 90 % confidence interval for the ratio of the population geometric means (Test/Reference) for the parameters under consideration. This method is equivalent to two one-sided tests with the null hypothesis of bioinequivalence at the 5% significance level.

All concentration-dependent pharmacokinetic parameters (e.g. AUC and Cmax) 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. Pharmacokinetic parameters derived from measures of concentration, e.g. AUC_t, AUC_∞ and C_{max} should be analysed using analysis of variance (ANOVA). Data for these parameters should be transformed prior to analysis using a logarithmic transformation. A confidence interval for the difference between formulations on the log-transformed scale is obtained from the ANOVA model. This confidence interval is then back-transformed to obtain the desired confidence interval for the ratio on the original scale. A non-parametric analysis is not acceptable.

The precise model to be used for the analysis should be pre-specified in the protocol. The statistical analysis should take into account sources of variation that can be reasonably assumed to have an effect on the response variable. The terms to be used in the ANOVA model are usually sequence, participant within sequence, period and formulation. Fixed effects, rather than random effects, should be used for all terms.

The general approach is to construct a 90% confidence interval for the quantity uT-uR and to reach a conclusion of pharmacokinetic equivalence if this confidence interval is within the stated limits. The nature of parametric confidence intervals means this is equivalent to carrying out two one-sided tests of the hypothesis at the 5% level of significance (8,9). The antilogs of the confidence limits obtained constitute the 90% confidence interval for the ratio of the geometric means between the generic and the reference products.

The same procedure should be sued for analysing parameters from steady-state trials or cumulative urinary recovery, if required.

If appropriate to the evaluation, the analysis technique for t_{max} should be non-parametric and should be applied to untransformed data.

In addition to the appropriate 90 % confidence intervals, summary statistics such as geometric and arithmetic means, SD and % RSD, as well as ranges for pharmacokinetic parameters (minimum and maximum), should be provided.

14.2.   Carry-over effects

A test for carry-over is not considered relevant and no decisions regarding the analysis (e.g. analysis of the first period only) should be made on the basis of such a test. The potential for carry-over can be directly addressed by examination of the pre-treatment plasma concentrations in period 2 (and beyond if applicable).

If there are any participants for whom the pre-dose concentration is greater than 5 percent of the Cmax value for the participant in that period, the statistical analysis should be performed with the data from that participant for that period excluded. In a 2-period trial this will result in the participant being removed from the analysis. The trial will no longer be considered acceptable if these exclusions result in fewer than 12 participants being evaluable. This approach does not apply to endogenous drugs.

14.3. Two-stage 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 such that an accurate estimate of the variability can be determined in the first stage of the study. The number of participants employed in the first stage is generally based on the most likely intra-subject variance estimate with some added participants to protect against 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 participants as determined, based on the variance estimates 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 employ a two-stage design, adjustments must be made to protect the overall Type I error rate and maintain it at 5%. In order 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.t is recommended that the same alpha for both stages be employed which gives an alpha of 0.0294 for this case (10), 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.

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 crossover and parallel study designs.

14.4. **Acceptance Range for Pharmacokinetic Parameters**

The pharmacokinetic parameters to be tested, the procedure for testing and the acceptance ranges, should be stated beforehand in the protocol. Outlying data should be reported and appropriate explanation should be given.

14.4.1. **Single-Dose Studies**

In studies to determine bioequivalence after a single dose, the parameters to be analysed are AUC (0-t), or, when relevant, AUC (0-72h), and Cmax. In single-dose studies designed to determine average bioequivalence, acceptance criteria for the main bioequivalence parameters are as follows:

AUC$_t$ – ratio

The 90 % confidence interval for the test/reference ratio should lie within the acceptance interval of 80.00 – 125.00 %.

Cmax- ratio

The 90 % confidence interval for the test/reference ratio should lie within an acceptance interval of 80.00 – 125.00% calculated using log-transformed data.

In certain cases, e.g. in the case of highly variable API's, a wider interval or other appropriate measure may be acceptable, but should be stated *a priori* and justified in the protocol. Refer to section 14.4.5 for information on an approach for proving bioequivalence when the Cmax parameter intra-subject variability is high.

To be inside the acceptance interval the lower bound should be ≥80.00% when rounded to two

decimal places and the upper bound should be ≤125.00% when rounded to two decimal places.

Tmax-difference

Statistical evaluation of tmax makes sense only if there is a clinically relevant claim for rapid onset of action or concerns about adverse effects. In such a case comparison of the median and range data for each product should be undertaken.

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

14.4.2. Steady-State Studies

Immediate Release Dosage Forms

The acceptance criteria for the pharmacokinetic parametersAUC0-τ and Cmax,ss are the same as for single dose studies.

Controlled/Modified Release Dosage Forms

The acceptance criteria are as follows:

- **AUC$_\infty$ - ratio**

  The 90 % confidence interval for the test/reference ratio should lie within the acceptance interval of 80.00 – 125.00 %.

- **Cmax (ss)**

  The 90 % confidence interval for the test/reference ratio should lie within the acceptance interval of, 80.00 – 125.00 %, calculated using log-transformed data.

14.4.3.   Urinary analysis

In the rare case where urinary data has been used, Ae (0-t) should be analysed using the same acceptance interval as stated above for AUC (0-t). Rmax should be analysed using the same acceptance interval as for Cmax.

14.4.4.   Narrow therapeutic index drugs

In specific cases of products with a narrow therapeutic index, the acceptance interval for AUC should be tightened to 90.00-111.11%. Where Cmax is of particular importance for safety, efficacy or drug level monitoring the 90.00-111.11% acceptance interval should also be applied for this parameter. It is not possible to define a set of criteria to categorise drugs as narrow therapeutic index drugs (NTIDs) and it must be decided case-by-case if an active substance is an NTID based on clinical considerations.

14.4.5.   Highly variable drugs or drug products

Highly variable drug products (HVDP) are those whose intra-subject variability for a parameter is larger than 30%. If an applicant suspects that a drug product can be considered as highly variable in its rate and/or extent of absorption, a replicate crossover design study can be carried out.

Those HVDP for which a wider difference in Cmax is considered clinically irrelevant based on a sound clinical justification can be assessed with a widened acceptance range. If this is the case the acceptance criteria for Cmax can be widened to a maximum of 69.84 – 143.19%. For the acceptance interval to be widened the bioequivalence study must be of a replicate design where it has been demonstrated that the within-subject variability for Cmax of the reference compound in the study is >30%. The applicant should justify that the calculated intra-subject variability is a reliable estimate and that it is not the result of outliers. The request for widened interval must be prospectively specified in the protocol.

The extent of the widening is defined based upon the within-subject variability seen in the bioequivalence study using scaled-average-bioequivalence according to $[U, L] = \exp[\pm k \cdot S_{WR}]$, 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 within-subject standard deviation of the log-transformed values of Cmax of the reference product. The table below gives examples of how

different levels of variability lead to different acceptance limits using this methodology.

Within-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 (%) $=100 \sqrt{e^{sWR2}} -1$

The geometric mean ratio (GMR) should lie within the conventional acceptance range 80.00-125.00%.

The possibility to widen the acceptance criteria based on high intra-subject variability does not apply to AUC where the acceptance range should remain at 80.00 – 125.00% regardless of variability.

It is acceptable to apply either a 3-period or a 4-period crossover scheme in the replicate design study.

15. STUDY REPORT

The report of a bioavailability or a bioequivalence study should give the complete documentation of its protocol, conduct and evaluation, complying with GCP, GLP and cGMP. It should be written in accordance with the ICH E3 guideline and be signed by the principal investigator(s).

15.1. Clinical Report

In addition to the protocol the clinical section of the bioequivalence study report should include the following:
a) A statement indicating the independence of the ethics committee.
b) Documented proof of ethical approval of the study.
c) A complete list of the members of the ethics committee, their qualifications and affiliations.
d) Names and affiliations of all investigator(s), the site of the study and the period of its execution. Audits certificate(s), if available, should be included in the report.
e) The name, composition, batch size, batch number, manufacturing date and, if possible, the expiry date of the test product(s) being tested.
f) The name, strength, pharmaceutical form, batch number, manufacturer, expiry date and country of purchase–for reference. The study report should include evidence that the choice of the reference medicinal product acceptable in SADC or by respective regulatory authorities
g) Certificates of analysis of reference and test batches used in the study should be included in an appendix to the study report.
h) Comparative dissolution profiles for test and reference products in accordance with the dissolution requirements (see section 19).
i) Certificate of analysis (CoA) of the API used in the test product bio-batch.
j) A summary of adverse events, which should be accompanied by a discussion on the influence of these events on the outcome of the study.
k) A summary of protocol deviations (sampling and non-sampling), which should be accompanied by a discussion on the influence of these adverse events on the outcome of the study.
l) Participants who drop out or are withdrawn from the study should be identified and their withdrawal fully documented and accounted for.

The applicant should submit a signed statement confirming that the test product has the same quantitative composition and is manufactured by the same process as the one submitted for authorisation. A confirmation whether the test product is already scaled-up for production should be submitted.

15.2. Analytical Report

The analytical section of the bioequivalence report should include the following clearly presented:

a) The full analytical validation report.
b) All individual participant concentration data.
c) Calibration data, i.e. raw data and back-calculated concentrations for standards, as well as calibration curve parameters, for the entire study.
d) Quality control samples for the entire study.
e) Chromatograms from analytical runs for 20 % of all participants (or a minimum of 4 participants) including chromatograms for the associated standards and quality control samples.
f) A summary of protocol deviations, which should be accompanied by a discussion on the influence of these deviations on the outcome of the study. Protocol deviations should be justified.

15.3.   Pharmacokinetic and Statistical Report

The pharmacokinetic and statistical section of the bioequivalence report should include the following, which should be clearly presented:
a) All individual plasma concentration *versus* time profiles presented on a linear/linear as well as log/linear scale (or, if appropriate, cumulative urinary excretion data presented on a linear/linear scale).
b) Data sufficiently detailed to enable the pharmacokinetics and the statistical analysis to be repeated, e.g. data on actual times of blood sampling, drug concentrations, the values of the pharmacokinetic parameters for each participant in each period and the randomisation scheme, should be available in a suitable electronic format (e.g. as comma separated and space delimited text files or Excel format) to be provided upon request.
c) The method(s) and programmes used to derive the pharmacokinetic parameters from the raw data.
d) A detailed ANOVA and/or non-parametric analysis, the point estimates and corresponding confidence intervals for each parameter of interest.
e) Tabulated summaries of pharmacokinetic and statistical data.
f) The statistical report should contain sufficient detail to enable the statistical analysis to be repeated, e.g. individual demographic data, randomisation scheme, individual participant concentration *vs*. time data, values of pharmacokinetic parameters for each participant, descriptive statistics of pharmacokinetic parameters for each formulation and period.

15.4.   Quality Assurance (QA)

a) A signed QA statement, confirming release of the document should accompany the study report.

b) A declaration should be made by the applicant to indicate whether the site(s) (clinical and analytical) where the study was performed was subjected to a pre-study audit to ascertain its/their status of GCP and GLP and/or cGMP conditions. All audit certificates should clearly indicate the date of audit and the name(s), address (es) and qualifications of the auditor(s).

c) The applicant should submit an independent monitor's certificate on the clinical portion of the study. This certificate should clearly indicate the date of monitoring and the name, address and qualifications of the monitor, and should be included in the study report.

d) Proof of inspection of the clinical and bioanalytical by regulatory agencies such as those from ICH, PIC/s, SADC or WHO should be provided.

16. BIOAVAILABILITY AND BIOEQUIVALENCE REQUIREMENTS

Although this guideline concerns immediate release formulations, this section provides some general guidance on the bioequivalence data requirements for other types of formulations and for specific types of immediate release formulations.

When the test product contains a different salt, ester, ether, isomer, mixture of isomers, complex or derivative of an active substance than the reference medicinal product, bioequivalence should be demonstrated in in vivo bioequivalence studies. However, when the active substance in both test and reference products is identical (or contain salts with similar properties as defined in section 19.1.1), in vivo bioequivalence studies may in some situations not be required as described below and in section 19.

16.1. Orally Administered Medicinal Products With Systemic Action

For dosage forms such as tablets, capsules and oral suspensions, bioequivalence studies are required unless a biowaiver is applicable (see section 19).

16.1.1. Orodispersible tablets

An orodispersable tablet (ODT) is formulated to quickly disperse in the mouth. Placement in the mouth and time of contact may be critical in cases where the active substance also is dissolved in the mouth and can be absorbed directly via the buccal mucosa. Depending on the formulation, swallowing of the e.g. coated substance and subsequent absorption from the gastrointestinal tract also will occur. If it can be demonstrated that the active substance is not absorbed in the oral cavity, but rather must be swallowed and absorbed through the gastrointestinal tract, then the product might be considered for a BCS based biowaiver (see section 19). If this cannot be demonstrated, bioequivalence must be evaluated in human studies.

If the ODT test product is an extension to another oral formulation, a 3-period study is recommended in order to evaluate administration of the orodispersible tablet both with and without concomitant fluid intake. However, if bioequivalence between ODT taken without water and reference formulation with water is demonstrated in a 2-period study, bioequivalence of ODT taken with water can be assumed.

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

- if the reference 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 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 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 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.

16.1.2. Solutions

A bioequivalence waiver may be granted for oral solutions, elixirs, syrups or other solubilised forms containing the same API(s) in the same concentration(s) as the reference product, and containing no ingredient known to significantly affect absorption of the medicinal ingredient(s). 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. The same requirements for similarity in excipients apply for oral solutions as for Biowaivers (see section 19, section 19.1.2 on excipients).

In those cases where the test product is an oral solution, which is intended to be bioequivalent to another immediate release oral dosage form, bioequivalence studies are required.

16.1.3. Powders for reconstitution

For pharmaceutically-equivalent products which are in the form of powders for reconstitution as a solution and the resultant solution meets criterion for solutions as stated in 16.1.2 or parenteral solutions as in 16.6, exemption from in vivo studies may be granted.

16.1.4. Suspensions

Bioequivalence for a suspension should be treated in the same way as for immediate release solid oral dosage forms.

16.1.5. Immediate Release Products – Tablets and Capsules

In general bioequivalence studies are required. *In vivo* BE studies should be accompanied by *in vitro* dissolution profiles on all strengths of each product. Waivers for *in vivo* bioavailability and bioequivalence studies for immediate release solid oral dosage forms, based on comparative dissolution studies, may be acceptable (see section 19 and Appendix I - Dissolution guideline).

16.1.6. Modified Release Products

Modified release products include delayed release products and extended (controlled) release products. In general, bioequivalence studies are required. In addition to the studies required for immediate release products, a food-effect study is necessary. Multiple dose studies are generally not recommended.

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.

Due to the more complex nature of modified-release products relative to immediate-release products, additional data is required to ensure the bioequivalence of two modified-release products. Factors such as food, which influences medicine bioavailability and in certain cases, pharmacokinetic bioequivalence, must be taken into consideration. The presence of food can affect product performance both by influencing the release of the API from the formulation and by causing physiological changes in the GI tract, In this regard a significant concern with regard to modified- release products is the possibility that food may trigger a sudden and abrupt release of the API leading to "dose dumping". This would most likely be manifested as a premature and abrupt rise in the plasma concentration time profile. Therefore, pharmacokinetic bioequivalence studies conducted under both fasted and fed conditions are required for orally administered, modified-release pharmaceutical products.

Unless single-dose studies are not possible for reasons such as those discussed in section 7.4 single-dose, cross-over bioequivalence studies conducted under both fasted and fed conditions comparing the highest strength of the generic product and the reference product must be performed to demonstrate bioequivalence. Single-dose studies are preferred to multiple-dose studies as single-dose studies are considered to provide more sensitive measurement of the release of API from the pharmaceutical product into the systemic circulation. In addition to single-dose studies, multiple-dose studies may be considered necessary for extended-release dosage forms with a tendency to accumulate.

The reference product in this study should be a pharmaceutically equivalent, modified-release product. The pharmacokinetic bioequivalence criteria for modified-release products are essentially the same as for conventional-release dosage forms except acceptance criteria should also be applied to Cmin (Ctau). As delivery mechanisms of pharmaceutical products become more complex, e.g. products with an immediate-release and modified-release component, additional parameters such as partial AUC measures may be necessary to ensure the bioequivalence of two products.

The fed-state bioequivalence study should be conducted after the administration of an appropriate standardized meal at a specified time (usually not more than 30 minutes) before

taking the medicine. A meal that will promote the greatest change in GI tract conditions relative to the fasted state should be employed. Refer to section 11.2 for more recommendations for the content of the meal. The composition of the meal should take local diet and customs into consideration. The composition and caloric breakdown of the test meal should be provided in the study protocol and report.

16.2.   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 IM or SC administration, demonstration of bioequivalence follows the rules for extra vascular modified release formulations, e.g. transdermal dosage forms as per corresponding guideline.

16.3.   Miscellaneous Oral Dosage Forms

Rapidly dissolving drug products, such as buccal and sublingual dosage forms, should be tested for *in vitro* dissolution and *in vivo* BA and/or BE. Chewable tablets should also be evaluated for *in vivo* BA and/or BE. Chewable tablets (as a whole) should be subject to *in vitro* dissolution because a patient, without proper chewing, might swallow them. In general, *in vitro* dissolution test conditions for chewable tablets should be the same as for non-chewable tablets of the same active ingredient/moiety.

16.4.   Fixed Dose Combinations (FDC)

Combination products should in general be assessed with respect to bioavailability and bioequivalence of individual active substances either separately (in the case of a new combination) or as an existing combination. The study in case of a new combination should be designed in such a way that the possibility of medicine-medicine interaction could be detected. Bioequivalence study is required for fixed-combination products with systemic action, where at least one of the APIs requires an in vivo study. The possibility for a biowaiver of Fixed Combination Medicinal Products is addressed in section 19.1.3.

16.5.   Orally Administered Drugs With Local Action

Generally BE studies with clinical efficacy and safety endpoints and/or suitably designed and validated *in vitro* studies are required. Where these are not available justification should be provided.

16.6.   Parenteral Solutions

The applicant is not required to submit a bioequivalence study if the product is to be administered as an aqueous intravenous solution containing the same API in the same concentration 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 parenteral routes other than intravenous, e.g. intramuscular or subcutaneous - if the test product is of the same type of solution (aqueous) as the reference product, contains the same concentration of the same API, and the same or comparable excipients as the reference, then bioequivalence testing is not required; provided that the formulation does not contain an excipient(s) known to significantly affect absorption of the active ingredient(s). 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. The same principles are applicable for parenteral oily solutions, but in this case the use of the same oily vehicle is essential. Similarly, for micellar solutions, the same qualitative and quantitative composition of the surfactant is necessary to waive in vivo studies and the change of other excipients should be critically reviewed.

For all other parenterals bioequivalence studies are required.

For intramuscular dosage forms, monitoring is required until at least 80 % of the $AUC\infty$ has been covered.

16.7.   Liposomal, micellar and emulsion dosage forms for intravenous use

Liposomal formulations: Pharmacokinetic issues related to liposomal formulations for iv 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 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.

16.8.   Topical Products

16.8.1. Local Action

For topical preparations containing corticosteroids intended for application to the skin and scalp, the human vasoconstrictor test (blanching test) is recommended to prove bioequivalence. Validated visual and/or chromometer data will be necessary.

For topical formulations, other than simple solutions with bacteriostatic, bactericidal, antiseptic and/or antifungal claims, clinical data (comparative clinical efficacy) will be required. Microbial growth inhibition zones will not be acceptable as proof of efficacy. Simple solutions, however, may qualify for a waiver based on appropriate *in vitro* test methods.

Proof of release by membrane diffusion will not be accepted as proof of efficacy, unless data are presented that show a correlation between release through a membrane and clinical efficacy.

Whenever systemic exposure resulting from locally applied/locally acting medicinal products entails a risk of systemic adverse reactions, systemic exposure should be measured.

For 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, local availability studies and/or in vitro studies. In certain cases, measurement

of the concentration of the API may still be required for safety reasons, i.e. in order to assess unintended systemic absorption.

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 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 and 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 product, i.e. the upper limit of the 90% confidence interval should not exceed the upper bioequivalence acceptance limit 125.00.

16.8.2. Gases

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

16.8.3. Systemic Action

For locally applied products with systemic action, e.g. transdermal products, rectal formulations, a bioequivalence study is always required. A biowaiver can be considered in the case of a solution, which contains an active substance in the same concentration as an approved solution and with the same qualitative and similar quantitative composition in excipients (conditions under oral solutions may apply in this case).

16.9.   Products Intended For Other Routes Of Administration

Products for local use (e.g. oral, nasal, inhalation, ocular, dermal, rectal, vaginal) intended to act without systemic absorption, the approach to determine bioequivalence based on systemic measurements is not applicable and pharmacodynamic or comparative clinical studies are required. However, pharmacokinetic studies may be required as measures of safety.

16.10.   Variations or Post-Registration Amendments

For all post-registration changes that require proof of efficacy in accordance with the Post-registration amendment guideline, the requirements of this guideline will be applicable.

If a product has been reformulated from the formulation initially approved or the manufacturing method has been modified in ways that may impact on the bioavailability, an in vivo bioequivalence study is required, unless otherwise justified. Any justification presented should be based upon general considerations, e.g. as per section 19, or on whether an acceptable level A in vitro / in vivo correlation has been established.

In cases where the bioavailability of the product undergoing change has been investigated and an acceptable level A correlation between in vivo performance and in vitro dissolution has been established, the requirements for in vivo demonstration of bioequivalence can be waived if the dissolution profile in vitro of the new product is similar to that of the already approved medicinal product under the same test conditions as used to establish the correlation (see Appendix I).

When variations to a generic or hybrid product are made, the comparative medicinal product for the bioequivalence study should normally be a current batch of the reference medicinal product. If a valid reference medicinal product is not available on the market, comparison to the previous formulation (of the generic or hybrid product) could be accepted, if justified. For variations that do not require a bioequivalence study, the advice and requirements stated in other published regulatory guidance should be followed.

17. COMPARATIVE PHARMACODYNAMIC STUDIES

Studies in healthy volunteers or patients using pharmacodynamic measurements may be used for establishing equivalence between two pharmaceutical products when the pharmacokinetic approach is not feasible. Pharmacodynamic studies are not recommended for orally administered, pharmaceutical products for systemic action when the API is absorbed into the systemic circulation and a pharmacokinetic approach can be used to assess systemic exposure and establish bioequivalence. This is because the sensitivity to detect differences between products in their biopharmaceutical quality, medicine release and absorption is lower with pharmacodynamic or clinical end-points. As the dose–response curve for pharmacodynamics or clinical end-points is usually flatter than the relationship between dose and PK parameters, it is essential to ensure the internal validity of the study by showing assay sensitivity, i.e. the ability to distinguish the response obtained by adjacent doses (two-fold or even four-fold difference in dose), which implies the administration of two different doses of at least one of the product, test or reference, and a statistical comparison based on the dose-scale analysis (i.e. relative potency). On the contrary, if a conventional response-scale analysis is conducted, it is essential to perform the comparison at the dose level where the dose response is steepest, which may require a previous pilot study for its identification. Furthermore, variability in pharmacodynamic measures is usually greater than that in pharmacokinetic measures. In addition, pharmacodynamic measures are often subject to significant placebo effects, which add to the variability and complicate experimental design. The result is often that huge numbers of patients would have to be enrolled in pharmacodynamic studies to achieve adequate statistical power. Pharmacodynamic bioequivalence studies may become necessary if quantitative analysis of the API and/or metabolite(s) in plasma or urine cannot be made with sufficient accuracy and sensitivity; however, this is extremely unlikely given current technology. Furthermore, pharmacodynamic equivalence studies in humans are required if measurements of API concentrations cannot be used as surrogate end-points for the demonstration of efficacy and safety of the particular pharmaceutical product such as pharmaceutical products designed to act locally. However, local availability studies based on pharmacokinetic studies alone or in combination with in vitro dissolution studies, are being considered as surrogate end-points for the demonstration of equivalent biopharmaceutical quality and release at the site of action for some products acting locally. In addition, however, pharmacokinetic bioequivalence studies are also required in order to demonstrate equivalent systemic exposure for systemic safety purposes.

If pharmacodynamic studies are to be used they must be performed as rigorously as bioequivalence studies and the principles of GCP must be followed (4).

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

- participants 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 products should be the same as described in section 9. In studies in which continuous variables can be recorded the time-course of the intensity of the medicine 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. However, 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.

18. CLINICAL TRIALS

In some instances (see section 16.8.1) plasma concentration time–profile data may be not suitable for assessing equivalence between two formulations. Although in some cases pharmacodynamic equivalence studies can be an appropriate tool for establishing equivalence, in others this type of study cannot be performed because of a lack of meaningful pharmacodynamic parameters that can be measured; a comparative clinical trial then has to be performed to demonstrate equivalence between two formulations. In cases when equivalence can be assessed by a pharmacokinetic bioequivalence study, this is preferred because the analogous clinical trial would be less sensitive. Huge numbers of participants are required to achieve adequate statistical power. For example, it has been calculated that 8600 patients would be required to give adequate statistical power to detect a 20% improvement in response to the study medicine compared with placebo (11). Similarly it was calculated that myocardial infarct patients would be required to show a 16% reduction in risk. A comparison of two formulations of the same API based on such end-points would require even greater numbers of participants (12).

If a clinical equivalence study is considered as being undertaken to prove equivalence, the same statistical principles apply as for the pharmacokinetic bioequivalence studies, although a 95% confidence interval might be necessary for pharmacodynamic and clinical end-points in contrast to the 90% confidence level employed conventionally for pharmacokinetic studies. The number of patients to be included in the study will depend on the variability of the target parameters and the acceptance range and is usually much higher than the number of participants needed in pharmacokinetic bioequivalence studies. The methodology for establishing equivalence between pharmaceutical products by means of a clinical trial in patients with a therapeutic end-point has not yet evolved as extensively as for pharmacokinetic bioequivalence trials. However, some important items that need to be defined in the protocol can be identified as follows:

- 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 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. In contrast to pharmacokinetic bioequivalence studies (where a conventional acceptance range is applied) the size of the acceptance range in clinical trials should be set individually according to the therapeutic class and indication(s);

- the presently used statistical method is the confidence interval approach.

- the confidence intervals can be derived from either parametric or nonparametric 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 as described in section 9.

19. COMPARATIVE IN VITRO TESTING

19.1.   BCS Biowaivers

In vitro testing and BCS– based biowaivers for immediate release pharmaceutical products can also assure equivalence between the generic product and the reference product. The BCS is a scientific framework for classifying medicinal substances based on their aqueous solubility and intestinal permeability. When combined with the dissolution of the drug product and critical examination of excipients of the drug product, the BCS takes into account the major factors that govern the rate and extend of medicine absorption (exposure) from immediate release solid oral dosage forms; excipients composition, dissolution, solubility and intestinal permeability. The BCS based biowaiver approach is meant to reduce in vivo bioequivalence studies, i.e., it may represent a surrogate for in vivo bioequivalence. In vivo bioequivalence studies may be exempted if an assumption of equivalence in in vivo performance can be justified by satisfactory in vitro data. According to the BCS, medicinal substances are classified as follows:

Class 1: High solubility – High permeability

Class 2: Low solubility – High permeability

Class 3: High solubility – Low permeability

Class 4: Low solubility – Low permeability

In addition, IR solid oral dosage forms are characterised as having rapid or slow dissolution.

Applying for a BCS-based biowaiver is restricted to highly soluble drug substances with known human absorption and considered not to have a narrow therapeutic index (see section 4.1.9). The concept is applicable to immediate release, solid pharmaceutical products for oral administration and systemic action having the same pharmaceutical form. However, it is not applicable for sublingual, buccal, and modified release formulations. For orodispersible formulations the BCS-based biowaiver approach may only be applicable when absorption in the oral cavity can be excluded.

BCS-based biowaivers are intended to address the question of bioequivalence between specific test and reference products. The principles may be used to establish bioequivalence in applications for generic medicinal products, extensions of innovator products, variations that require bioequivalence testing, and between early clinical trial products and to-be-marketed products.

BCS-based biowaiver are applicable for an immediate release drug product if

- the drug substance has been proven to exhibit high solubility and complete absorption (BCS-class I; for details see section 19.1.1) and

- either very rapid (> 85 % within 15 min) or similarly rapid (85 % within 30 min) in vitro dissolution characteristics of the test and reference product has been demonstrated considering specific requirements (see section 19.1.2) and

- excipients that might affect bioavailability are qualitatively and quantitatively the same. In general, the use of the same excipients in similar amounts is preferred (see section 19.1.2).

BCS-based biowaiver are also applicable for an immediate release drug product if

- the drug substance has been proven to exhibit high solubility and limited absorption (BCS-class III; for details see section 19.1.1) and

- very rapid (> 85 % within 15 min) in vitro dissolution of the test and reference product has been demonstrated considering specific requirements (see section 19.1.2) and

- excipients that might affect bioavailability are qualitatively and quantitatively the same and other excipients are qualitatively the same and quantitatively very similar as defined by the quality limits on allowable quantitative changes in excipients for a variation (see section 19.1.2).

Generally the risks of an inappropriate biowaiver decision should be more critically reviewed (e.g. site-specific absorption, risk for transport protein interactions at the absorption site, excipient composition and therapeutic risks) for products containing BCS class III than for BCS class I drug substances.

19.1.1. Drug Substance

Generally, sound peer-reviewed literature may be acceptable for known compounds to describe the drug substance characteristics of importance for the biowaiver concept.

Biowaiver may be applicable when the active substance(s) in test and reference products are identical. Biowaiver may also be applicable if test and reference contain different salts provided that both belong to BCS-class I (high solubility and complete absorption; see sections 19.1.1). 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 product, since these differences may lead to different bioavailabilities not deducible by means of experiments used in the BCS-based biowaiver concept.

The API should be uncomplicated, i.e. it does not exhibit any of the following:

- A narrow therapeutic range or safety margin, e.g. it does not require careful dosage titration or patient monitoring.
- A risk of serious undesired effects.
- Complicated or variable pharmacokinetics, e.g., non linear pharmacokinetics, variable or incomplete absorption, an absorption window, i.e. site-specific absorption, substantial first-pass metabolism (>40 %),
- There is no documented evidence of bioavailability problems related to the API(s) or the pharmaceutical product, or products of similar chemical structure or formulations.

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. A minimum of three replicates determinations at each pH condition is recommended 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 established where measured extent of absorption is $\geq$ 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.

When data from mass balance studies are used to support complete absorption, it must be ensured that the metabolites taken into account in determination of fraction absorbed are formed after absorption. Hence, when referring to total radioactivity excreted in urine, it should be ensured that there is no degradation or metabolism of the unchanged drug substance in the gastric or intestinal fluid. Phase 1 oxidative and Phase 2 conjugative metabolism can only occur after absorption (i.e. cannot occur in the gastric or intestinal fluid). Hence, data from mass balance studies support complete absorption if the sum of urinary recovery of parent compound and urinary and faecal recovery of Phase 1 oxidative and Phase 2 conjugative drug metabolites account for ≥85 % of the dose.

In addition highly soluble drug substances with incomplete absorption, i.e. BCS-class III compounds, could be eligible for a biowaiver provided certain prerequisites are fulfilled regarding product composition and in vitro dissolution (see also 19.1.2 Excipients). The more restrictive requirements will also apply for compounds proposed to be BCS class I but where complete absorption could not convincingly be demonstrated.

Reported bioequivalence between aqueous and solid formulations of a particular compound administered via the oral route may be supportive as it indicates that absorption limitations due to (immediate release) formulation characteristics may be considered negligible. Well-performed in vitro permeability investigations including reference standards may also be considered supportive to in vivo data.

19.1.2. Drug Product

In vitro Dissolution

General aspects

Investigations related to the medicinal product should ensure immediate release properties and prove similarity between the investigative products, i.e. test and reference show similar in vitro dissolution under physiologically relevant experimental pH conditions. However, this does not establish an in vitro/in vivo correlation. In vitro dissolution should be investigated within the range of pH 1 – 6.8 (at least pH 1.2, 4.5, and 6.8). Additional investigations may be required at pH values in which the drug substance has minimum solubility. The use of any surfactant is not acceptable.

Test and reference products should meet requirements as outlined in section 9. In line with these requirements it is advisable to investigate more than one single batch of the test and reference products.

Comparative in vitro dissolution experiments should follow current compendial standards. Hence, thorough description of experimental settings and analytical methods including validation data should be provided. It is recommended to use 12 units of the product for each experiment to enable statistical evaluation. Usual experimental conditions are e.g.:

- Apparatus: paddle or basket

- Volume of dissolution medium: 900 ml or less

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

- Agitation: paddle apparatus - usually 50 or 75 rpm

    basket apparatus - usually 100 rpm

- Sampling schedule: e.g. 10, 15, 20, 30 and 45 min

- Buffer: pH 1.0 – 1.2 (usually 0.1 N HCl or SGF without enzymes), pH 4.5, and pH 6.8 (or SIF without enzymes); (pH should be ensured throughout the experiment; Ph.Eur. buffers recommended)

- Other conditions: no surfactant; in case of gelatin capsules or tablets with gelatin coatings the use of enzymes may be acceptable.

Complete documentation of in vitro dissolution experiments is required including a study protocol,

batch information on test and reference batches, detailed experimental conditions, validation of experimental methods, individual and mean results and respective summary statistics.

In the case of generic products, the reference product should be a conventional, immediate-release oral dosage form and the test and reference products should exhibit similar dissolution profiles.

Dosage forms should not be intended for absorption in the oral cavity, e.g. sublingual or buccal tablets.

BCS based biowaivers are intended only for BE studies. They do not apply to food effect BA studies or similar pharmacokinetic studies.

The reference product should be a conventional, immediate-release oral dosage form.

Evaluation of in vitro dissolution results

Drug products are considered 'very rapidly' dissolving when more than 85 % of the labelled amount is dissolved within 15 min using a paddle apparatus at 75 rpm or basket apparatus at 100 rpm in a volume of 900 mL or less in each of the three media (at least pH 1.2 HCl solution, 4.5 acetate buffer, and 6.8 phosphate buffer). In cases where this is ensured for the test and reference product the similarity of dissolution profiles may be accepted as demonstrated without any mathematical calculation. Surfactant should not be employed.

Absence of relevant differences (similarity) should be demonstrated in cases where it takes more than 15 min but not more than 30 min to achieve almost complete (at least 85 % of labelled amount) dissolution. F2-testing (see App. I) or other suitable tests should be used to demonstrate profile similarity of test and reference. However, discussion of dissolution profile differences in terms of their clinical/therapeutical relevance is considered inappropriate since the investigations do not reflect any in vitro/in vivo correlation.

Excipients

Although the impact of excipients in immediate release dosage forms on bioavailability of highly soluble and completely absorbable drug substances (i.e., BCS-class I) is considered rather unlikely it cannot be completely excluded. Therefore, even in the case of class I drugs it is advisable to use similar amounts of the same excipients in the composition of test like in the reference product.

If a biowaiver is applied for a BCS-class III drug substance, excipients have to be qualitatively the same and quantitatively very similar in order to exclude different effects on membrane transporters.

As a general rule, for both BCS-class I and III drug substances well-established excipients in usual amounts should be employed and possible interactions affecting drug bioavailability and/or solubility characteristics should be considered and discussed. A description of the function of the excipients is required with a justification whether the amount of each excipient is within the normal range. Excipients that might affect bioavailability, like e.g. sorbitol, mannitol, sodium lauryl sulfate 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
- interaction with membrane transporters

Excipients that might affect bioavailability should be qualitatively and quantitatively the same in the test product and the reference product.

19.1.3. Fixed Dose Combinations (FDCs)

BCS-based biowaiver are applicable for immediate release FDC products if all active substances in the FDC belong to BCS-class I or III and the excipients fulfil the requirements outlined in section IV.2. Otherwise in vivo bioequivalence testing is required.

19.2.   In vitro dissolution tests in support of biowaiver of strengths

Appropriate in vitro dissolution should confirm the adequacy of waiving additional in vivo bioequivalence testing. Accordingly, dissolution should be investigated at different pH values as outlined in the previous section (normally pH 1.2, 4.5 and 6.8) unless otherwise justified. Similarity of in vitro dissolution (see Appendix I) should be demonstrated at all conditions within the applied product series, i.e. between additional strengths and the strength(s) (i.e. batch (es)) used for bioequivalence testing.

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 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).

20. Reference

1. US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER). Guidance for industry: Bioavailability and bioequivalence studies for orally administered drug products-general considerations. 2003.
2. European Medicines Agency, Committee for Medicinal Products for Human Use (CHMP). Guideline on the investigation of bioequivalence. 2010.
3. World Health Organization, Multisource (generic) pharmaceutical products: Guidelines on registration requirements to establish interchangeability, Revision, April 2014. (draft for comment)
4. Guidelines for good clinical practice for trials on pharmaceutical products. In: The use of essential drugs. Sixth report of the WHO Expert Committee. Geneva, World Health Organization, 1995 (WHO Technical Report Series, No. 850): 97–137.
5. Guidelines for organizations performing in vivo bioequivalence studies. In: WHO Expert Committee on Specifications for Pharmaceutical Preparations. Fortieth report. Geneva, World Health Organization (WHO Technical Report Series, No. 937), 2006, Annex 9.
6. Julious SA. Sample sizes for clinical trials with normal data. Statistics in Medicine, 2004, 23(12): 1921-1986.
7. Revision/update of the guidance on the selection of comparator pharmaceutical products for equivalence assessment of interchangeable multisource (generic) products. (In preparation for WHO Technical Report Series.)
8. Schuirmann DJ. A comparison of the two one-sided tests procedure and the power approach for assessing the equivalence of average bioavailability. Journal of Pharmacokinetics and Biopharmaceutics, 1987, 15:657–680.
9. Westlake WJ. Bioavailability and bioequivalence of pharmaceutical formulations. In: Peace KE, ed. Biopharmaceutical statistics for drug development. New York, Marcel Dekker, Inc., 1988: 329–352.
10. Pocock SJ. Group sequential methods in the design and analysis of clinical trials. Biometrika, 1977, 64(2): 191-199.
11. Yusif et al. Journal of the American Medical Association, 1988, 260: 2259–2263.
12. The Studies of Left V entricular Dysfunction (SOL VD) Investigators. Effect of enalapril on survival in patients with reduced left ventricular ejection fractions and congestive heart failure. New England Journal of Medicine, 1991, 325:293–302.

21. APPENDIX 1 – DISSOLUTION REQUIREMENTS

INTRODUCTION

This guideline describes the setting of dissolution specifications as a quality control requirement and also describes how to conduct dissolution testing in support of a request for a waiver for bioequivalence testing.

Although intrinsic dissolution of the active pharmaceutical ingredient (API) is an important consideration when formulating solid oral dosage forms, the dissolution behaviour of solid oral dosage forms provides important information to ensure drug product quality. Hence, dissolution testing has been established as an extremely valuable tool to monitor batch-to-batch consistency. The primary utility of a dissolution test is, therefore, to establish dissolution specifications for relevant drug products for the purposes of quality assurance.

Dissolution testing can also be useful in providing information on drug product quality following certain post-approval changes made to the product, such as changes in formulation, manufacturing process, site of manufacture and the scale-up of the manufacturing process.

In addition, where solid oral dosage forms have been proportionally formulated in different strengths, and the drug follows linear kinetics, dissolution data can be used in support of a biowaiver for lower strengths of such dosage forms, provided an acceptable bioequivalence study has been carried out on one strength, usually the highest strength.

Drug absorption from oral dosage forms depends on adequate release of the active pharmaceutical ingredient (API) from the product. Physico-chemical factors, such as dissolution or solubility of the drug under physiologic conditions, and its permeability through the membranes of the gastrointestinal tract, play pivotal roles in this respect. Due to the critical nature of these factors, dissolution of a drug product *in vitro* can, in certain instances, be relevant to anticipate the *in vivo* characteristics/results.

In summary, dissolution testing can serve several purposes:
a) Quality assurance
  * To get information on the test batches used in BA/BE studies and pivotal clinical studies to support specifications for quality control
  * To be used as a tool in quality to demonstrate consistency in manufacture
  * To get information on the reference product used in BA/BE studies and pivotal clinical studies

b) Bioequivalence surrogate inference
  * To demonstrate similarities between different formulations of an active substance and the reference medicinal product (biowaivers e.g., variations, formulation changes during development and generic medicinal products; see section 19 and Appendix I)
  * To Demonstrate equivalence between two strengths on which one of the strengths has been demonstrated to be equivalent to an acceptable reference product
  * To support variation/ post approval changes
  * To collect information on batch to batch consistency of the products to be used as basis for the selection of appropriate batches for the in vivo study

SETTING DISSOLUTION SPECIFICATIONS

a) For new drug products, dissolution specifications should be based on data obtained from acceptable clinical, pivotal bioavailability and/or bioequivalence batches.

b) In the case of generic products, the dissolution specifications are generally the same as the reference product.

  These specifications should be confirmed by comparison of the dissolution performance of the generic product and reference product from an acceptable bioequivalence study.

If the dissolution performance of the generic pharmaceutical product is substantially different from that of the reference product and the *in vivo* data remain acceptable, a different dissolution specification for the product may be set.

c) A single point specification for immediate release dosage forms and a multipoint specification for modified release dosage forms are generally applicable for quality control, batch release and stability testing purposes.

Once dissolution specifications are set, the drug product should comply with those specifications throughout its shelf life.

Testing should continue through the three stages of testing unless the product conforms at stage 1 or 2.

Setting dissolution specifications for generic products may be classified in three categories as described below.

PHARMACOPOEIAL PRODUCT DISSOLUTION TEST AVAILABLE

In this instance the quality control dissolution test should be the test described in the BP, USP or EP. Use of any other pharmacopoeia should be justified.

It is recommended that a dissolution profile be generated by taking samples at 15-minute intervals, or less, using the specified pharmacopoeial method for test and reference products (12 units each). More frequent sampling during the period of greatest change in the dissolution profile is recommended. For rapidly dissolving products, where complete dissolution is within 30 minutes, generation of an adequate profile by sampling at 5- or 10-minute intervals may be necessary.

Additional dissolution data may also be required when scientifically justified, e.g. when the pharmacopoeia does not specify a dissolution test for all API's in a combination product.

If appropriate the pharmacopoeial specification may be adopted.

PHARMACOPOEIAL PRODUCT DISSOLUTION TEST NOT AVAILABLE

Comparative dissolution testing, using test and reference products under a variety of test conditions, is recommended.

The test conditions may include different dissolution media (pH 1 to 6,8), addition of surfactant, or use of an official basket or paddle apparatus with varying agitation.

In all cases, profiles should be generated as previously recommended.

The medium, which exhibits optimum discrimination, should be selected.

The dissolution specifications should be set based on available bioequivalence and other data. In addition, the method used should be justified and validated.

Dissolution testing methods and conditions from the US FDA Office of Generic Drugs' website are acceptable without verification. Be that as it may, the manufacturer is required to demonstrate discriminatory nature of the dissolution method.

If an active substance is considered highly soluble, it is reasonable to expect that it will not cause any bioavailability problems if, in addition, the dosage system is rapidly dissolved in the physiological pH-range and the excipients are known not to affect bioavailability. In contrast, if an active substance is considered to have a limited or low solubility, the rate-limiting step for absorption may be dosage form dissolution. This is also the case when excipients are controlling the release and subsequent dissolution of the active substance. In those cases a variety of test conditions is recommended and adequate sampling should be performed. For very highly soluble API limits of NLT 75% (Q) in 30 minutes is adequate enough.

SPECIAL CASES

For poorly water-soluble drug products (e.g. glyburide), dissolution testing at more than one time point, and preferably a dissolution profile, is recommended for quality control purposes. Alternatively, the use of

the USP apparatus 4 (Flow-Through Method) should be considered for the development of dissolution specifications for such products.

If a monograph for a multipoint product is not included in the BP, USP, Int.Ph, Eur Ph. the monographs for the individual components should be used to set the dissolution requirements for each.

IN VITRO DISSOLUTION TESTS COMPLEMENTARY TO BIOEQUIVALENCE STUDIES

The results of in vitro dissolution tests at three different buffers (normally pH 1.2, 4.5 and 6.8) and the media intended for drug product release (QC media), obtained with the batches of test and reference products that were used in the bioequivalence study should be reported. Particular dosage forms like ODT (oral dispersible tablets) may require investigations using different experimental conditions. The results should be reported as profiles of percent of labelled amount dissolved versus time displaying mean values and summary statistics.

Unless otherwise justified, the specifications for the in vitro dissolution to be used for quality control of the product should be derived from the dissolution profile of the test product batch that was found to be bioequivalent to the reference product.

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.

IN VITRO DISSOLUTION TESTING IN SUPPORT OF A BIOWAIVER

Requirements for dissolution for BCS based biowaivers and biowaivers for additional strengths are in section 19.

POST-REGISTRATION / APPROVAL AMENDMENTS

When amendments are made to pharmaceutical products, manufacturing procedures, and other associated processes including change of site, their impact on quality should be demonstrated. The following describes the use of dissolution testing as an indicator of quality, which may be applicable as described below.

The following dissolution tests are recommended:

Types of dissolution testing

a)   **Case A**

Dissolution testing should be conducted as a release test according to the original submission, or in accordance with compendial requirements, for that product.

b)   **Case B**

Dissolution testing should be conducted as a multipoint test in the application/compendial medium at intervals such as 15, 30, 45, 60 and 120 minutes, or until an asymptote is reached for the proposed and currently registered formulation.

c)   **Case C**

Dissolution testing should be conducted as a multipoint test in water, 0,1 N HCl and buffer at pH 4,5 and 6,8 for the proposed, and currently registered formulations, at intervals such as 15, 30, 45, 60 and 120 minutes, or until either 90 % of drug from the drug product is dissolved, or an asymptote is reached.

In the case of poorly soluble drugs, comparisons may be made using alternative compendial methods and media that have been appropriately justified.

Types of amendments

a)   **Type A**

In the event that the Type A change made is such that there is unlikely to be an effect on the quality and performance of a dosage form, Case A dissolution testing is appropriate.

b)   Type B

In the event that the changes, which were made, have a significant impact on the quality and performance of a dosage form, Case B dissolution testing is appropriate. However, if the change is made to a product containing a BCS class 1 compound 85 % should be dissolved in 15 minutes in the media used in (accordance with) the application or compendial requirements.

For low permeability, high solubility drugs, dissolution profiles should be generated in the application/compendial medium as previously described for Case B dissolution testing. For high permeability, low solubility compounds, multipoint dissolution profiles should be carried out according to Case C dissolution testing.

Profiles of the currently used product and the proposed product should be proven to be similar, according to the f_2 requirements as describe in this Guideline.

c)   Type C

In the case of changes that are likely to have a significant impact on formulation quality and performance, *in vivo* bioequivalence testing should be conducted unless otherwise justified. Case B or Case C dissolution testing may also be required. Biowaivers may also be considered if a proven *in vitro/in vivo* correlation (IVIVC) has been established.

COMPARISON OF DISSOLUTION PROFILES

Dissolution profile similarity testing and any conclusions drawn from the results (e.g. justification for a biowaiver) can be considered valid only if the dissolution profile has been satisfactorily characterised using a sufficient number of time points.

For immediate release formulations, further to the guidance given previously, comparison at 15 min is essential to know if complete dissolution is reached before gastric emptying.

Where more than 85% of the drug is dissolved within 15 minutes, 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, 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%.

The similarity factor (f_2) is a logarithmic reciprocal square root transformation of the sum of squared errors, and is a measurement of the similarity in the percentage (%) dissolution between the two curves. Dissolution similarity may be determined using the $f2$ statistic as follows

$$f_2 = 50 \cdot \text{Log} \{[1 + (1/n) \sum_{t=1}^{n} (R_t - T_t)^2]^{-0.5} \cdot 100\}$$

In this equation $f2$ is the similarity factor, n is the number of time points, R (t) is the mean percent reference drug dissolved at time t after initiation of the study; T (t) is the mean percent test drug dissolved at time t after initiation of the study. For both the reference and test formulations, percent dissolution should be determined.

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

- A minimum of three time points (zero excluded)
- The dissolution measurements of the test and reference batches should be made under exactly the same conditions. The time points should be the same for the two formulations
- The sampling intervals should be short for a scientifically sound comparison of the profiles (e.g.5, 10, 15,20, 30, 45, (60,90, 120) minutes. The 15-minute time point is critical to determine whether a product is very rapidly dissolving and to determine whether f2 must be calculated.
- For extended release FPPs the time point should be set to cover the entire duration of expected release e.g. 1,2,3,4,5 and 8 hour for 12 hour release and additional interval for long duration of release
- Twelve individual values for every time point for each formulation

- Using the mean dissolution values from both curves at each time interval, calculate the similarity factor (f_2) using the above equation. Not more than one mean value of > 85% dissolved for any of the formulations.
- To allow use of mean data, the relative standard deviation or percent coefficient of variation (CV) of any product should be less than 20% for the first point and less than 10% from second to last time point.
- Only one measurement should be considered after 85 % dissolution of either test or reference product has occurred. In the case where 85% dissolution cannot be reached due to poor solubility of the API, the dissolution should be conducted until an asymptote (plateau) has been reached
- When delayed release products e.g. enteric coated are being compared, the recommended conditions are acid media pH 1.2 for 2 hours and buffer pH 6.8
- When comparing extended release beaded capsules, where different strengths has been achieved soley by means of adjusting the number of beads containg the API, one condition (normally the release condition) will suffice.
- Surfactants should be avoided in comparative dissolution testing. A statement that thaAPI is not soluble in any of the media is not acceptable and profiles in the absence of surfactant should be provided. The rationale for the choice and concentration of surfactant should be provided. The concentration of the surfactant should be such that the discriminatory power of the test will not be compromised.

An f2 value between 50 and 100 suggests that the two dissolution profiles are similar.

When the $f2$ statistic is not suitable, then the similarity may be compared using model-dependent or model-independent methods e.g. by statistical multivariate comparison of the parameters of the Weibull function or the percentage dissolved at different time points.

Alternative methods to the $f2$ statistic to demonstrate dissolution similarity are considered acceptable, if statistically valid and satisfactorily justified.

The similarity acceptance limits should be pre-defined and justified and not be greater than a 10% difference. In addition, the dissolution variability of the test and reference product data should also be similar, however, a lower variability of the test product may be acceptable.

Evidence that the statistical software has been validated should also be provided.

A clear description and explanation of the steps taken in the application of the procedure should be provided, with appropriate summary tables.