MEDICINES CONTROL COUNCIL

DEPARTMENT OF HEALTH
Republic of South Africa

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BIOSTUDIES

This guideline is intended to provide recommendations to applicants wishing to submit applications for the registration of medicines. It represents the Medicines Control Council's current thinking on the safety, quality and efficacy of medicines. It is not intended as an exclusive approach. Council reserves the right to request any additional information to establish the safety, quality and efficacy of a medicine in keeping with the knowledge current at the time of evaluation. Alternative approaches may be used but these should be scientifically and technically justified. The MCC is committed to ensure that all registered medicines will be of the required quality, safety and efficacy. It is important that applicants adhere to the administrative requirements to avoid delays in the processing and evaluation of applications.

Guidelines and application forms are available from the office of the Registrar of Medicines and the website.

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Pharmacokinetic parameters, Abbreviations and Symbols

API	Active Pharmaceutical Ingredient
BA	Bioavailability
BE	Bioequivalence
FDC	Fixed-dose combination
IPI	Inactive Pharmaceutical Ingredient
C_{max}	maximum plasma concentration
C_{min}	minimum plasma concentration
$C_{max\ (ss)}$	maximum plasma concentration at steady-state
$C_{min\ (ss)}$	minimum plasma concentration at steady-state
C_{av}	average plasma concentration
t_{max}	time to C_{max}
AUC_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_∞	area under the plasma/serum/blood concentration-time curve from time zero to time infinity
AUC_τ	AUC during a dosage interval at steady state
MRT	mean residence time
Ae_t	cumulative urinary excretion from pharmaceutical product administration until time t
Ae_∞	Amount of unchanged API excreted in the urine at infinite time (7-10 half-lives).
$t_{1/2}$	Plasma concentration elimination half-life
% PTF	$(C_{max\ (ss)} – C_{min\ (ss)}) / C_{av}.100$
% Swing	$(C_{max\ (ss)} – C_{min\ (ss)}) / C_{min}.100$
LOQ	Limit of quantification
SOP	Standard Operating Procedure

1 INTRODUCTION

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

Other types of applications may also require demonstration of bioequivalence, including amendments, fixed combinations and line extension applications.

The recommendations on design and conduct given for bioequivalence studies in this guideline may also be applied to comparative bioavailability studies evaluating different formulations used during the development of a new medicinal product containing a new chemical entity and to comparative bioavailability studies included in extension applications that are not based exclusively on bioequivalence data.

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), should be well defined.

Comparison of the therapeutic performances of two pharmaceutical products containing the same API, or active moiety is a critical means of assessing the possibility of using either the innovator, or a multisource (generic) pharmaceutical product. Assuming that in the same subject 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 methods can be used to demonstrate bioequivalence. Also refer to CPMP therapeutic area specific guidelines.

When *in vivo* equivalence studies are necessary and types of studies required

Generally *in vivo* documentation of bioequivalence, through either a pharmacokinetic bioequivalence study, a comparative pharmacodynamic study, or a comparative clinical trial, is regarded as especially important. *In vivo* documentation of bioequivalence is needed when there is a risk that possible differences in bioavailability may result in therapeutic inequivalence.

Examples are listed below.

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

- Critical use medicines;
- narrow therapeutic range (efficacy/safety margins); steep dose-response curve;
- documented evidence for bioavailability problems or bio-inequivalence related to the API or APIs of similar chemical structure or formulations (unrelated to dissolution problems);
- excipients and pharmaceutical processes used in manufacturing known to affect the bioequivalence.
- There is scientific evidence to suggest that polymorphs of API, the excipients and/or the pharmaceutical processes used in manufacturing could affect bioequivalence.

(ii) Non-oral and non-parenteral pharmaceutical products designed to act by systemic absorption (such as transdermal patches, suppositories, testosterone gel, skin-inserted contraceptives).

(iii) Modified release pharmaceutical products designed to act by systemic absorption.

(iv) Fixed-dose combination products with systemic action, where at least one of the active pharmaceutical ingredients requires an *in vivo* study.

(v) Non-solution pharmaceutical products, which are for non-systemic use (e.g. for oral, nasal, ocular, dermal, rectal or vaginal application) and are intended to act without systemic absorption. In these cases, the equivalence is established through, e.g. comparative clinical or pharmacodynamic, dermatopharmacokinetic studies and/or in vitro studies. In certain cases, measurement of the concentration of the API may still be required for safety reasons, i.e. in order to assess unintended systemic absorption.

In vitro studies may be used as waivers except under certain circumstances.

2 DEFINITIONS (see also Pharmaceutical & Analytical Guideline)

2.1 Active moiety (Active)

Active moiety is the term used for the therapeutically active entity in the final formulation of a medicine, irrespective of the form of the API. The active is alternative terminology with the same meaning. For example, if the API is propranolol hydrochloride, the active moiety (and the active) is propranolol.

2.2 Active Pharmaceutical Ingredient (API)

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

2.3 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.

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.* intravenous), and the “relative bioavailability” as compared with another form administered by the same or another non-intravenous route (e.g. tablets vs. oral solution).

2.4 Bioequivalence

Two pharmaceutical products are bioequivalent if they are pharmaceutically equivalent or pharmaceutical alternatives and if their bioavailabilities in terms of peak (Cmax and Tmax) and total exposure (AUC) after administration of the same molar dose under the same conditions are similar to such a degree that their effects with respect to both efficacy and safety can be expected to be essentially the same.

Bioequivalence focuses on the equivalence of release of the active pharmaceutical ingredient from the pharmaceutical product and its subsequent absorption into the systemic circulation.

Comparative studies using clinical or pharmacodynamic end points may also be used to demonstrate bioequivalence.

2.5 Fixed-dose combination (FDC)

A combination of two or more active pharmaceutical ingredients in a fixed ratio of doses.

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

2.6 Multisource (Generic) Pharmaceutical Product

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

2.7 Pharmaceutical alternatives

Medicinal products are pharmaceutical alternatives if they contain the same active moiety but differ either in chemical form (e.g. salt, ester**,** ether, isomer, mixtures of isomers, complexes or derivatives) of that moiety or in the dosage form or strength, administered by the same route of administration but are otherwise not pharmaceutically equivalent.
Pharmaceutical alternatives do not necessarily imply bioequivalence.

2.8 Pharmaceutical Dosage Form *(compare 2.10 Pharmaceutical Product)*

A pharmaceutical dosage form is the form of the completed pharmaceutical product e.g. tablet, capsule, injection, elixir, suppository.

2.9 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 intended to be administered by the same route.

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

2.10 Pharmaceutical Product

Any preparation for human (or animal) 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.

2.11 Proportionally Similar Dosage Forms/Products *(refer 5.1.1)*

Pharmaceutical products are considered proportionally similar in the following cases:

2.11.1 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).

2.11.2 When the APIs and IPIs 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 Amendments guideline.

2.11.3 When the pharmaceutical products contain a low concentration of the APIs (e.g. less than 5 %) 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 Amendments guideline.

2.12 Therapeutic Equivalence

Two pharmaceutical products are therapeutically equivalent if they are pharmaceutically equivalent or are pharmaceutical alternatives and, after administration in the same molar dose, their effects with respect to both efficacy and safety are essentially the same, as determined from appropriate bioequivalence, pharmacodynamic, clinical or *in vitro* studies.

3 DESIGN AND CONDUCT OF STUDIES FOR ORALLY ADMINISTERED PHARMACEUTICAL PRODUCTS

A bioequivalence study is basically a comparative bioavailability study designed to establish whether or not there is bioequivalence between test and reference products. In the following sections, requirements for the design and conduct of bioavailability or bioequivalence studies are formulated.

3.1 DESIGN

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

However, under certain circumstances and 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, and replicate designs e.g. for substances with highly variable pharmacokinetic characteristics could be considered.

In general, single dose studies will suffice, but there are situations in which steady-state studies may be required in which case the steady-state study design should be motivated. 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. 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 as due to the recent development in the bio-analytical methodology, it is unusual that parent moiety cannot be measured accurately and precisely.

To avoid carry-over effects, treatments should be separated by adequate wash-out periods. Normally at least 5 elimination half-lives are necessary. 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).

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. 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.

3.1.1 Selection of dose

In bioequivalence studies the molar equivalent dose of multisource and comparator product must be used.

Generally the marketed strength with the greatest sensitivity to bioequivalence assessment should be administered as a single dose. This will usually be the highest marketed strength. A higher dose (i.e. more than one dosage unit) may be employed when analytical difficulties exist. In this case the total single dose should not exceed the maximum daily dose of the dosage regimen. *Refer to Pharmaceutical & Analytical guideline "Reference Products".*

Alternatively the application of area under the curve (AUC) truncated to three times the median T_{max} of the reference formulation would avoid problems of lack of assay sensitivity in many cases. In certain cases a study performed with a lower strength can be considered acceptable if this lower strength is chosen for reasons of safety/tolerability. *Ref 12 Annex 7 Section 6.1*

3.1.1.1 Non-linear pharmacokinetics

For medicines 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 required. If the non-linearity is not caused by limited solubility but is due to e.g. saturation of uptake transporters and provided that biowaiver requirements *(refer section 5 of this guideline)* 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).

3.1.1.2 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.

3.1.2 Fed or fasting conditions

Co-administration of food with oral pharmaceutical products may influence drug/API BA and/or BE. Food-effect BA studies focus on the effects of food on the release of the API from the pharmaceutical product as well as the absorption of the API, and should be done for all new chemical entities.

Bioequivalence studies for immediate release dosage forms should be done under fasting conditions, unless food effects influence/affect bioavailability. If the reference product dosage directions specifically state administration with food, the study should be designed taking in consideration any possible food effects. If the reference product dosage directions state either “with or without food” or make no statement with respect to food, the study needs only be done under fasting conditions.

Bioequivalence studies for modified release dosage forms should demonstrate any influence of food in order to exclude any possibility of dose dumping; hence, both fed and fasted studies are required.

For products with specific formulation characteristics (e.g. micro-emulsions, 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 general, meals that are high in total calories and fat content are more likely to affect the GI physiology and thereby result in a larger effect on the BA of an API or pharmaceutical product. Therefore, the use of high-calorie and high-fat meals during food-effect BA and fed BE studies is recommended. If no specific recommendation is given in the originator SPC, 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 (%)).

3.2 SUBJECTS

3.2.1 Number of Subjects

It is recommended that the number of subjects should be justified on the basis of providing at least 80 % power of meeting the acceptance criteria. The minimum number of subjects should not be less than 12. If 12 subjects do not provide 80 % power, more subjects should be included.

A minimum of 20 subjects is required for modified release oral dosage forms.

3.2.1 Number of Subjects - continued

The number of subjects required to provide an 80 % power of meeting and passing the acceptance criteria for the 0,8 to 1,25 acceptable interval, can be determined from Reference 1.

Alternatively, the sample size can be calculated using appropriate power equations, which should be presented in the protocol.

The provision for add-ons should be made in the protocol *a priori* clearly reflecting the maximum number of subjects to be included.

3.2.2 Drop-outs and withdrawals

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

Sponsors who wish to replace drop-outs during the study or consider an add-on design should indicate this intention in the protocol.

It is more appropriate to recruit into the study more subjects than the sample size calculation requires.

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

If the bioequivalence study was performed with the appropriate size but bioequivalence cannot be demonstrated because of a result of a larger than expected random variation or a relative difference, an add-on subject study can be performed using not less than half the number of subjects in the initial study. Combining is acceptable only in the case when the same protocol was used and preparations from the same batches were used.

Add-on designs must be carried out strictly according to the study protocol and SOPs, and must be given appropriate statistical treatment, including consideration of consumer risk.

3.2.3 Selection of Subjects

The subject 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 there are 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, subjects should exhibit the following characteristics:

a) **Sex:** Subjects may be selected from either sex. However, the risk to women of childbearing potential should be considered on an individual basis.

b) **Age:** Subjects should be between 18 and 55 years of age.

c) **Mass:** Subjects 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), or within 15 % of the ideal body mass, or any other recognised reference.]

d) **Informed Consent**: All subjects participating in the study should be capable of giving informed consent.

3.2.3 Selection of Subjects - continued

e) **Medical Screening:** Subjects 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 API'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:** Subjects 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.

3.2.4 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.

3.2.5 Genetic Phenotyping

Phenotyping and/or genotyping of subjects can be considered for exploratory bioavailability studies. It may also be considered in crossover studies (e.g. bioequivalence, dose proportionality, food interaction studies) for safety or pharmacokinetic reasons.

If an API is known to be subject to major genetic polymorphism, studies could be performed in cohorts of subjects of known phenotype or genotype for the polymorphism in question.

3.3 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.

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

3.3.2 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 (e.g. 200 ml).

3.3.3 Food and Fluid Intake: In fasted studies the period of fasting prior to dosing should be standardised and supervised. All meals and fluids taken after dosing should also be standardised in regard to composition and time of administration and in accordance with any specific requirements for each study.

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

3.3.4 Concomitant Medication: Subjects 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).

3.3.5 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.

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

3.3.7 **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).

3.4 SAMPLE COLLECTION AND SAMPLING TIMES

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

Sampling schedule:

A sufficient number of samples to adequately describe the plasma concentration-time profile should be collected. The sampling schedule should include frequent sampling around predicted t_{max} to provide a reliable estimate of peak exposure.

The sampling schedule should be planned to avoid C_{max} being the first point of a concentration time curve and 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/APIs (> 24 hours) the study should cover a minimum of 72 hours, unless 80 % is recovered before 72 hours. For moieties demonstrating high inter-subject variability in distribution and clearance the use of AUC truncation warrants caution. In these circumstances sampling periods beyond 72 hours may be required.

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 API/moiety.

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

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

3.4.1 When blood is collected:

a) The duration of blood sampling in a study should be sufficient to account for at least 80 % of the known AUC to infinity (AUC_{∞}). This period is approximately three terminal half-lives of the drug/API.

b) For most drugs/APIs 12 to 18 samples including a pre-dose sample should be collected per subject per dose.

c) Sample collection should be spaced such that the maximum concentration of drug/API in blood (C_{max}) and the terminal elimination rate constant (K_{el}) can be estimated.

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

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

3.4.2 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 seven times the terminal elimination half-life, so that the amount excreted to infinity (Ae_∞) can be estimated.

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 API administration, should be recorded.

3.4.3 Endogenous substances

For endogenous substances, the sampling schedule should allow characterisation of the endogenous baseline profile for each subject in each period. Often, a baseline is determined from 2-3 samples taken before the study 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 (refer reference 8 section 4.1.5).

3.5 CHARACTERISTICS TO BE INVESTIGATED

3.5.1 Moieties to be measured

3.5.1.1 Products with uncomplicated actives

In most cases evaluation of bioavailability and bioequivalence will be based upon measured concentrations of the parent compound (i.e. the **active**) 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.

For BA studies determination of moieties should be measured in biological fluids to take into account both concentration and activity.

Concentration refers to the relative quantity of the parent active or one or more metabolites in a given volume of an accessible biological fluid such as blood or plasma.

Activity refers to the relative contribution of the parent active and its metabolite(s) in the biological fluids to the clinical safety and/or efficacy of the active

For BA studies, both the parent active and its major active metabolites should be measured, if analytically feasible.

For BE studies, measurement of only the parent active released from the dosage form, rather than the metabolite, is generally recommended. The rationale for this recommendation is that concentration-time profile of the parent active is more sensitive to changes in formulation performance than a metabolite, which is more reflective of metabolite formation, distribution, and elimination.

It is important to state *a priori* in the study protocol which chemical entities (pro-drug, API, metabolite) will be analyzed in the samples.

3.5.1.1 Products with uncomplicated actives - continued

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.

b) If there is a major difficulty with the analytical method.

c) If the parent compound is unstable in the biological matrix.

d) 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.

- The measurement of concentrations of therapeutically active metabolite is acceptable if the substance studied is a pro-drug.
- If an active metabolite is formed as a result of gut wall or other pre-systemic metabolic process(es) and the metabolite contributes meaningfully to safety and/or efficacy, either the metabolite or the parent concentrations must be measured and assessed in accordance with the protocol.

It is important to note that measurement of one analyte, active pharmaceutical ingredient or metabolite, allows the risk of making a Type-I error (the consumer risk) to remain at the 5 % level.

However, if more than one of several analytes is selected retrospectively as the bioequivalence determinant, then the consumer and producer risks change (13).

When measuring the active metabolites wash-out period and sampling times may need to be adjusted in order to adequately characterize the pharmacokinetic profile of the metabolite.

3.5.1.2 Enantiomers versus Racemates

For BA studies, measurement of individual enantiomers may be important.

For BE studies, this guidance recommends measurement of the racemate using an achiral assay.

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

a) the enantiomers exhibit different pharmacodynamic characteristics,

b) the enantiomers exhibit different pharmacokinetic characteristics,

c) primary efficacy and safety activity resides with the minor enantiomer, and

d) non-linear absorption is present (as expressed by a change in the enantiomer concentration ratio with change in the input rate of the drug/API) for at least one of the enantiomers.

In such cases, we recommend that BE factors be applied to the enantiomers separately.

3.5.1.3 Pharmaceutical Products with Complex Mixtures as APIs

Certain pharmaceutical products may contain complex active substances (i.e. active moieties or APIs) that are mixtures of multiple synthetic and/or natural source components). Some or all of the components of these complex active mixtures cannot be characterized with regard to chemical structure and/or biological activity.

Quantification of all active or potentially active components in pharmacokinetic studies to document BA and BE is neither encouraged nor desirable. BA and BE studies should rather be based on a small number of markers of rate and extent of absorption.

Criteria for marker selection could include amount of the moiety in the dosage form, plasma or blood concentrations of the moiety, and biological activity of the moiety relative to other moieties in the complex mixture. This should be determined case-by-case.

3.5.2 Pharmacokinetic parameters

3.5.2.1 Blood/Plasma/Serum Concentration *versus* Time Profiles

The following bioavailability parameters are to be estimated:

a) AUC_t, AUC_∞, C_{max}, t_{max} for plasma concentration *versus* time profiles.

b) AUC_t, C_{max}, C_{min}, fluctuation (% PTF) and swing (% Swing) for studies conducted at steady state.

c) Any other justifiable characteristics (*cf.* Pharmacokinetic parameters, Abbreviations and Symbols).

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

3.5.2.2 Urinary Excretion Profiles

In the case of API's predominantly excreted renally, the use of urine excretion data may be advantageous in determining the extent of drug/API input. However, justification should also be given when this data is used to estimate the rate of absorption.

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.

The following bioavailability parameters are to be estimated:

a) Ae_t, Ae_∞ as appropriate for urinary excretion studies.

b) Any other justifiable characteristics (cf. Appendix I).

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

3.5.2.3 Pharmacodynamic Studies

If pharmacodynamic parameters/effects are used as bioequivalence criteria, the applicant should submit justification for their use. Bioequivalence determinations based on these measurements should be justified in each case. In addition:

a) A dose-response relationship should be demonstrated.

b) Sufficient measurements should be taken to provide an appropriate pharmacodynamic response profile.

c) The complete dose-effect curve should remain below the maximum physiological response.

d) All pharmacodynamic measurements/methods should be validated with respect to specificity, accuracy and reproducibility.

3.6 BIO ANALYSIS

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.

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 (Reference 2):

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.

i) Robustness and ruggedness.

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.

A number of separately prepared Quality Control samples should be analysed with processed test samples at intervals based on the total number of samples.

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.

Any modification of the bioanalytical method, before and during analysis of study specimens, may require adequate revalidation, and all modifications should be reported and the scope of revalidation justified.

3.7 STUDY PRODUCTS

3.7.1 Reference Products

Refer to the Pharmaceutical and Analytical guideline.

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. The Applicant should document how a representative batch of the reference product with regards to dissolution and assay content has been selected. It is advisable to investigate more than one single batch of the reference product when selecting reference product batch for the bioequivalence study.

3.7.2 Test product

The characterisation and specification of critical quality attributes of the test product, such as dissolution and impurity profiles, should be established from the test and reference batches respectively, i.e. the clinical batches for which bioequivalence has been demonstrated.

3.7.3 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 the MCC.

3.7.4 Sample handling

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

It should be possible to identify unequivocally the identity of the product administered to each subject at each trial period. Packaging, labelling and administration of the products to the subjects should therefore be documented in detail. This documentation should include all precautions taken to avoid and identify potential dosing mistakes. The use of labels with a tear-off portion is recommended.

3.8 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.

3.8.1 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 means (Test/Reference) for the parameters under consideration.

Pharmacokinetic parameters derived from measures of concentration, e.g. AUC_t, AUC_∞ and C_{max} should be analysed using ANOVA. Data for these parameters should be transformed prior to analysis using a logarithmic transformation.

If t_{max} is 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/coefficient of variation, as well as ranges for pharmacokinetic parameters (minimum and maximum), should be provided.

Submit disk with raw data formatted appropriately for evaluation. Refer to Section 3.9.3 a) for the appropriate formatting of the disk.

3.8.2 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.

a) Single-Dose Studies

In single-dose studies designed to determine average bioequivalence, acceptance criteria for the main bioequivalence parameters are as follows:

i) AUC_t - ratio

The 90 % confidence interval for the test/reference ratio should lie within the acceptance interval of 0,80-1,25 (80 – 125 %).

In certain cases an alternative approach may be acceptable.

Justification for the use of alternative methods, e.g. scaled average bioequivalence (ABE) based on sound scientific principles for the evaluation of the bioequivalence of highly variable drugs/APIs, has been described in the literature (References 3 and 4). Use of alternative methods should be stated *a priori* in the protocol and cannot be added retrospectively.

ii) C_{max} - ratio

The 90 % confidence interval for the test/reference ratio should lie within an acceptance interval of 75 – 133 %, calculated using log-transformed data, except for narrow therapeutic range API's when an acceptance interval of 80 – 125 % will apply.

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 (See references 3 and 4).

b) Steady-State Studies

i) Immediate Release Dosage Forms

The acceptance criteria are the same as for single dose studies.

b) Steady-State Studies – continued

ii) Controlled/Modified Release Dosage Forms

The acceptance criteria are as follows:

- **AUC_t - ratio**

  The 90 % confidence interval for the test/reference ratio should lie within the acceptance interval of 0,80-1,25 (80 – 125 %).

- **$C_{max\ (ss)}$ and $C_{min\ (ss)}$**

  The 90 % confidence interval for the test/reference ratio should lie within the acceptance interval of 0,75-1,33 (75 – 133 %), calculated using log-transformed data.

- **% Swing and % PTF**

  The 90 % confidence interval for the test/reference ratio should lie within the acceptance interval of 0,80-1,25 (80 – 125 %), calculated using log transformed data.

3.9 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.

A comprehensive Table of Contents (ToC) of the study report including the sub-reports and major sub-sections / appendices should be included. The items listed in the ToC should include at least all the aspects addressed under each of the reports headings reflected below.

Each report must have clearly labelled tabs to indicate each sub-report and major sub-section / appendices of the Biostudy.

The ToC of the report appendices should reflect the appropriate page number or the location must be indicated with clearly labelled tabs. A range of pages is not acceptable.

All case report forms must be removed. The Informed consent form in English only must be included with the protocol together with a certification of the translation where relevant. Refer General Information guideline 2.4.

3.9.1 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 the all investigator(s), the site of the study and the period of its execution.

e) The names and batch numbers of the products being tested.

f) The name and address of the applicant of both the reference and the test products.

g) Expiry date of the reference product and the date of manufacture of the test product used in the study.

h) CoAs, assay and dissolution profiles for test and reference product biostudy batches. The dissolution profiles should be determined in at least the main medium / final product specification dissolution medium and reported on in accordance with the Dissolution guideline.

i) 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) Subjects who drop out or are withdrawn from the study should be identified and their withdrawal fully documented and accounted for.

3.9.2 Analytical Report

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

a) The analytical method and full analytical validation report. Relevant but not excessive, representative chromatograms to support verification of analytical methods should be included.

b) All individual subject 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 subjects (for a minimum of 4 subjects, whichever is the greater, to a maximum of 8 subjects) including chromatograms for the associated standards and quality control samples.

The chromatograms and chromatogram details must be clearly legible (comply with the legibility requirements General Information guideline 4.2 and the definition of legibility in the regulations), be clearly indexed stating the subject and page numbers. The legend or sample coding system must be included and clearly identified.

The selected chromatograms should pertain to analytical runs, or, if individual subjects, to subjects in consecutive alphabetical or chronological order.

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.

3.9.3 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).

This data should be submitted in hard copy and also formatted electronically in a format compatible for processing by SAS software. Individual subject data should be in rows and arranged in columns, which reflect the subject number, phase number, sequence, formulation, and sample concentration *versus* time data per treatment.

b) The method(s) and programmes used to derive the pharmacokinetic parameters from the raw data.

c) A detailed ANOVA and/or non-parametric analysis, the point estimates and corresponding confidence intervals for each parameter of interest.

d) Tabulated summaries of pharmacokinetic and statistical data.

e) The statistical report should contain sufficient detail to enable the statistical analysis to be repeated, e.g. individual demographic data, randomisation scheme, individual subject concentration vs. time data, values of pharmacokinetic parameters for each subject, descriptive statistics of pharmacokinetic parameters for each formulation and period.

3.9.4 Quality Assurance (QA)

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

b) The applicant should 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) An independent monitor's statement must be included.

3.10 VALIDITY OF BIOSTUDIES

The bioavailability/bioequivalence study(ies) should comply with the requirements of current standards as determined by Council. Sponsor and investigational sites, facilities and laboratories, and all data (including source data) and documentation and reports concerning the data including participant files must be available for verification by the Inspectorate – *cf* SA GCP.

4 BIOAVAILABILITY AND BIOEQUIVALENCE REQUIREMENTS

It is incumbent upon the applicant to demonstrate in the dossier (not in the BE report) that the excipients in the pharmaceutically equivalent product are essentially the same and in comparable concentrations as those in the reference product. In the event that this information about the reference product cannot be provided by the applicant, it is incumbent upon the applicant to perform *in vivo* or *in vitro* studies to demonstrate that the differences in excipients do not affect product performance.

4.1 ORALLY ADMINISTERED PHARMACEUTICAL PRODUCTS INTENDED FOR SYSTEMIC ACTION

4.1.1 Solutions

Pharmaceutically equivalent solutions for oral use (including syrups, elixirs, tinctures or other soluble forms but not suspensions), containing the active pharmaceutical ingredient in the same molar concentration as the comparator product, and containing only excipient(s) known to have no effect on gastrointestinal (GI) transit, GI permeability and hence absorption or stability of the active pharmaceutical ingredient in the GI tract are considered to be equivalent without the need for further documentation of equivalence other than the comparative data indicating compliance with the aforementioned.

Pharmaceutically equivalent powders for reconstitution as solution, meeting the solution criteria above, are considered to be equivalent without the need for further documentation of equivalence other than the comparative data indicating compliance with the above.

4.1.2 Suspensions

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

4.1.3 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 5 below and Dissolution guideline).

4.1.4 Modified Release Products

Modified release products include delayed release products and extended (controlled) release products (as defined in the P&A guideline).

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 (see 5 below and Dissolution guideline).

4.1.5 Fixed-dose combination products (including co-packaged products)

Combination products should in general be assessed with respect to bioavailability and bioequivalence of APIs 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 a pharmacokinetic and / or pharmacodynamic active-active interaction could be detected.

In general approval of FDC will be considered in accordance with the WHO Technical report series 929 “Guidelines for registration of fixed-dose combination medicinal products 2005” or the latest revision.

Fixed-dose combinations for antiretrovirals will be considered in accordance with the FDA "Guidance for Industry: Fixed Dose Combinations, Co-Packaged Drug Products, and Single-Entity Versions of Previously Approved Antiretrovirals for the Treatment of HIV" October 2006 or the latest revision.

4.1.6 Miscellaneous Oral Dosage Forms

Rapidly dissolving pharmaceutical 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 API/moiety.

4.2 MEDICINES INTENDED FOR LOCAL ACTION

Non-solution pharmaceutical products, which are for non-systemic use (oral, nasal, ocular, dermal, rectal, vaginal, etc., application) and are intended to act without systemic absorption. In these cases, the bioequivalence is established through comparative clinical or pharmacodynamic, dermatopharmacokinetic studies and/or *in vitro* studies. In certain cases, active concentration measurement may still be required for safety reasons in order to assess unintended systemic absorption.

4.3 PARENTERAL SOLUTIONS

It is incumbent upon the applicant to demonstrate in the dossier (not in the BE report) that the excipients in the pharmaceutically equivalent product are essentially the same and in comparable concentrations as those in the reference product. In the event that this information about the reference product cannot be provided by the applicant, it is incumbent upon the applicant to perform *in vivo* or *in vitro* studies to demonstrate that the differences in excipients do not affect product performance.

The influence of pH on precipitation should be clearly addressed and the absence of formation of sub-visible particulate matter over the physiological pH range be demonstrated.

4.3.1 Aqueous solutions

Aqueous solutions to be administered by parenteral routes (intravenous, intramuscular, subcutaneous) containing the same active pharmaceutical ingredient(s) in the same molar concentration and the same or similar excipients in comparable concentrations as the comparator product are considered to be equivalent without the need for further documentation.

Certain excipients (e.g. buffer, preservative, antioxidant) may be different provided the change in these excipients is not expected to affect the safety and/or efficacy of the medicine product.

4.3.2 Powders for reconstitution

Pharmaceutically equivalent products that are powders for reconstitution as solution meeting criterion for 4.3.1 above are considered to be equivalent without the need for further documentation.

4.3.3 Micellar and emulsion dosage forms for intravenous use

Emulsions*:* Emulsions normally do not qualify for a biowaiver. However, emulsion formulations may be considered eligible for a biowaiver where:

(a) the 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 as stated above, 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 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 active 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.

4.3.4 Other

For all other parenterals bioequivalence studies are required.

For intramuscular dosage forms, monitoring is required until at least 80 % of the AUC_{∞} has been covered.

4.4 TOPICAL PRODUCTS

Pharmaceutically equivalent topical products prepared as aqueous solutions containing the same active pharmaceutical ingredient(s) in the same molar concentration and essentially the same excipients in comparable concentrations are considered to be equivalent without the need for further documentation.

It is incumbent upon the applicant to demonstrate in the dossier (not in the BE report) that the excipients in the pharmaceutically equivalent product are essentially the same and in comparable concentrations as those in the reference product. In the event that this information about the reference product cannot be provided by the applicant, it is incumbent upon the applicant to perform *in vivo* or *in vitro* studies to demonstrate that the differences in excipients do not affect product performance.

4.4.1 Local Action

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

Simple topical solutions with bacteriostatic, bactericidal, antiseptic and/or antifungal claims may qualify for a waiver based on appropriate validated *in vitro* test methods, e.g. microbial growth inhibition zones.

For other topical formulations clinical data (comparative clinical efficacy) will be required.

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.

4.4.2 Systemic Action

For other locally applied products with systemic action, e.g. transdermal products, a bioequivalence study is always required.

4.5 PRODUCTS INTENDED FOR OTHER ROUTES OF ADMINISTRATION

It is incumbent upon the applicant to demonstrate in the dossier (not in the BE report) that the excipients in the pharmaceutically equivalent product are essentially the same and in comparable concentrations as those in the reference product. In the event that this information about the reference product cannot be provided by the applicant, it is incumbent upon the applicant to perform *in vivo* or *in vitro* studies to demonstrate that the differences in excipients do not affect product performance.

4.5.1 Otic, ophthalmic, nasal and cutaneous products

Pharmaceutically equivalent products prepared as aqueous or oily solutions, e.g. eye drops, ear drops, nasal sprays or cutaneous solutions, and containing the same active pharmaceutical ingredient(s) in the same molar concentration and essentially the same excipients in comparable concentrations are considered to be equivalent without the need for further documentation.

Certain excipients (e.g. preservative, buffer, substance to adjust tonicity or thickening agent) may be different provided use of these excipients is not expected to affect safety and/or efficacy of the product.

4.5.2 Aerosols, nebulisers, nasal sprays

Pharmaceutically equivalent solutions for aerosol or nebuliser inhalation or nasal sprays, tested to be administered with or without essentially the same device, prepared as aqueous solutions, containing the same active pharmaceutical ingredient(s) in the same concentration and essentially the same excipients in comparable concentrations are considered to be equivalent without the need for further documentation.

The pharmaceutical product may include different excipients provided their use is not expected to affect safety and/or efficacy of the product.

Particle size distribution may be used in support of proof of efficacy for inhalations. The Anderson sampler or equivalent apparatus should be used. In addition appropriate information should be submitted to provide evidence of clinical safety and efficacy.

4.5.3 Gases

Pharmaceutically equivalent gases are considered to be equivalent without the need for further documentation.

4.6 VARIATIONS OR AMENDMENTS

For all changes that require proof of efficacy in accordance with the Amendments guideline, the requirements of this guideline will be applicable.

5 WAIVERS OF *IN VIVO* BIOEQUIVALENCE STUDIES FOR ORAL SOLID DOSAGE FORMS

Biowaivers can be considered based on the following:

5.1 *IN VITRO* STUDIES - DISSOLUTION PROFILE COMPARISON *(see Dissolution Guideline)*

For biowaiver purposes the dissolution profiles, in three media and the main/specification dissolution medium if not one of the three dissolution media, as described in the Dissolution Guideline, of the test and the reference product should be tested for similarity.

The f_2 similarity factor should be used to compare dissolution profiles from different products and/or strengths of a product. An f_2 value $\geq$ 50 indicates a sufficiently similar dissolution profile such that further *in vivo* studies are not necessary. For an f_2 value < 50, it may be necessary to conduct an *in vivo* study. However, when both test and reference products dissolve 85 % or more of the label amount of the API in ≤15 minutes similarity is accepted without the need to calculate f2 values.

5.1.1 Proportionally similar formulations *(section 2.11 of this guideline)*

A prerequisite for qualification for a biowaiver based on dose-proportionality of formulations is that

- the multisource product at one strength has been shown to be bioequivalent to the corresponding strength of the reference product.
- the further strengths of the multisource product are proportionally similar in formulation to that of the studied strength.

When both of these criteria are met and all the dissolution profiles of the further dosage strengths are shown to be similar to the one of the studied strength on a percentage released vs. time basis, the biowaiver procedure can be considered for the further strengths.

5.1.1.1 Immediate release tablets

When the pharmaceutical product is the same dosage form but of a different strength and is proportionally similar in its API and IPIs, a biowaiver may be acceptable.

5.1.1.2 Modified Release Products *see 4.1.4 above*

(refer to Pharmaceutical & Analytical Guideline for definition)

Beaded Capsules - Lower Strength

For extended release beaded capsules where the strength differs only in the number of beads containing the API, 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 and media described in the Dissolution guideline.

Tablets – Lower strength

For extended release tablets when the pharmaceutical product is:

a) in the same dosage form but in a different strength, and

b) is proportionally similar in its APIs and IPIs, and

c) has the same drug/API 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.

When the highest strength (generally, as usually the highest strength is used unless a lower strength is chosen for reasons of safety) of the multisource product is bioequivalent to the highest strength or dose[1] of the reference product, and other strengths are proportionally similar in formulations and the dissolution profiles are similar between the dosage strengths, biowaiver can be considered to lower / other strengths.

5.1.2 Reference Products registered in South Africa but procured in another country, the health regulatory authority of which the MCC aligns itself with *(refer also to Pharmaceutical & Analytical and Dissolution Guidelines)*

Bioequivalence studies submitted where a foreign reference product has been used, will require demonstration of equivalence between the foreign product and the innovator product marketed in South Africa. If the reference product is not the current innovator product available on the SA market, then the reference product may be procured from another country provided that it complies with the requirements specified in the Pharmaceutical & Analytical guideline.

Dissolution profiles of the test and reference products should be compared for similarity as described in the Dissolution Guideline for each of the three specified media irrespective of the solubility and/or stability profiles. Further evidence in the main/specification dissolution medium, if not one of the required dissolution media, should be provided.

5.1.3 Amendments

Although this guideline comments primarily on registration requirements for multisource pharmaceutical products, *in vitro* dissolution testing may also be suitable to confirm similarity of product quality and performance characteristics with minor formulation or manufacturing changes after approval.

[1] Dose included in the dosage range of the MCC approved package insert of the innovator product registered in South Africa.

5.2 BIOPHARMACEUTICS CLASSIFICATION SYSTEM (BCS) BIOWAIVER *(refer also to Dissolution Guideline)* [2]

The Biopharmaceutics Classification System (BCS) is based on aqueous solubility and intestinal permeability of the API. It classifies the API into one of four classes:

Class 1 - High Solubility, High Permeability

Class 2 - Low Solubility, High Permeability

Class 3 - High Solubility, Low Permeability

Class 4 - Low Solubility, Low Permeability

Combining the dissolution characteristics of the pharmaceutical product with these two properties of the API, the three major factors that govern the rate and extent of absorption from immediate release solid dosage forms are taken into account (14).

With respect to dissolution properties, immediate release dosage forms can be categorized as having "very rapid", "rapid", or "not rapid" dissolution characteristics.

On the basis of scientific principles of solubility and permeability and dissolution characteristics of the dosage form, the BCS approach provides an opportunity to waive *in vivo* pharmacokinetic bioequivalence testing for certain categories of immediate release pharmaceutical products (15).

Oral pharmaceutical products *not* eligible for a so-called "biowaiver" based on the BCS approach are described under Sections 1 and 4 above.

The BCS (Biopharmaceutics Classification System)-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.

Applying for a BCS-based biowaiver is restricted to highly soluble APIs with known human absorption and considered not to have a narrow therapeutic index (see Introduction i)). 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.

5.2.1 API

Generally, sound peer-reviewed literature may be acceptable for known compounds to describe the API 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). 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 not belong to the group of 'narrow therapeutic index' APIs (see Introduction i)) on narrow therapeutic index drugs).

[2] For classification of APIs appearing in the WHO EML, refer to WHO Technical Report Series, No. 937, 2006 Annex 8

5.2.1 API – continued

The demonstration of complete absorption in humans is preferred for BCS-based biowaiver applications. For this purpose complete absorption is considered to be established where measured extent of absorption is ≥ 85 %. Complete absorption is generally related to high permeability.

In addition highly soluble APIs 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 sect. 5.2.3 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.

5.2.2 Product

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 API has minimum solubility. The use of any surfactant is not acceptable.

5.2.3 Excipients

Although the impact of excipients in immediate release dosage forms on bioavailability of highly soluble and completely absorbable APIs (i.e., BCS-class I) is considered rather unlikely it cannot be completely excluded. Therefore, even in the case of class I APIs 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 APIs 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 APIs, well-established excipients in usual amounts should be employed and possible interactions affecting 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, 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 API (e.g. complexation)
- API 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.

In the event that this information about the reference product is not disclosed, it is incumbent upon the applicant to conduct appropriate studies or reverse engineering to derive the information.

5.2.4 Fixed Combinations (FCs)

BCS-based biowaiver are applicable for immediate release FC products if all active substances in the FC belong to BCS-class I or III and the excipients fulfil the requirements outlined above.

Otherwise *in vivo* bioequivalence testing is required.

5.2.5 Biowaivers based on BCS can be granted under the following conditions:

5.2.5.1 The appropriateness of the biowaiver is addressed, i.e. confirmation with supporting references, that no characteristic which requires an *in vivo* bioequivalence study is applicable. *See sections 1 and 4 above.*

In addressing the appropriateness of the BCS biowaiver the benefit-risk balance / ratio of an inappropriate biowaiver decision, clinical indications, food effect and any other relevant aspect e.g. site-specific absorption, risk for transport protein interactions at the absorption site, excipient composition and therapeutic risks, should be included.

Also refer *to reference 12, WHO Technical Report Series 937 Annex 7 Section 9.2 and Annex 8.*

Generally the risks of an inappropriate biowaiver decision should be more critically reviewed for products containing BCS class III than for BCS class I APIs.

5.2.5.2 Dosage forms containing APIs which are highly soluble, and highly permeable/have complete absorption (i.e. BCS class 1), and are rapidly dissolving are eligible for a biowaiver based on the BCS provided that, in addition to the critical review of 5.2.5.1:

- excipients that might affect bioavailability are qualitatively and quantitatively the same. In general, the use of the same excipients in similar quantities is preferred, and
- the BCS class I dosage form is *rapidly dissolving* (as defined in the Dissolution Guideline, i.e. no less than 85 % of the labelled quantity of the API dissolves in 30 minutes) and
- the dissolution profile of the multisource product is similar to that of the reference product at pH 1,2, pH 4,5 and pH 6,8 buffer using the paddle method at 50 or 75 rpm or the basket method at 100 rpm (as described in the Dissolution Guideline) and meets the criteria of dissolution profile similarity, $f2 \geq 50$ (or equivalent statistical criterion).

  If both the reference and the multisource dosage forms are *very rapidly dissolving*, i.e. 85 % or more dissolution at 15 minutes or less in all 3 media under the above test conditions, the two products are deemed equivalent and a profile comparison is not necessary.

5.2.5.3 Dosage forms containing APIs which are highly soluble, and have low permeability/ limited absorption (i.e. BCS class III), and are *very rapidly* dissolving are eligible for a biowaiver based on the BCS provided that, in addition to the critical review in 5.2.5.1:

- excipients that might affect bioavailability are qualitatively and quantitatively the same and other excipients are qualitatively the same and quantitatively very similar, and
- the BCS class III dosage form is very rapidly dissolving (as defined in the Dissolution guideline, i.e. no less than 85 % of the labelled quantity of the API dissolves in 15 minutes), and
- very rapid (> 85 % within 15 min) in vitro dissolution of the test and reference product has been demonstrated at pH 1,2, pH 4,5 and pH 6,8 buffer using the paddle method at 50 or 75 rpm or the basket method at 100 rpm (as described in the Dissolution Guideline).

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UPDATE HISTORY

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Version 2 Amended sections 1, 2, 3.1, 3.2.2 – 3.2.5, 3.4, 3.5, 3.8.1, 3.9.4, 3.10, 4, 4.1, 4.2, 4.3, 4.4, 4.5, 5, 5.1, 5.2, 6, App 1	June 2006
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Version 6 : Clarification in line with Bioequivalence CPMP/EWP/QWP/1401/98 Rev. 1/Corr ** of 1 August 2010 and updating of requirements in 3.2.2; 3.7; 5.2; updating of reference	March 2014/June 2015
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