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GUIDELINE ON THE INVESTIGATION OF BIOEQUIVALENCE

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This guideline will replace the "Note for guidance on the investigation of bioavailability and bioequivalence" CPMP/QWP/EWP/1401/98 and the related questions in the Q&A document (CHMP/EWP/40326/06). This guideline includes recommendations on BCS-based biowaivers.

* The correction includes changes in section 4.1.4 "Study conduct", sub-section "Fasting or fed conditions", last paragraph (page 10): replacement of the unit "calories" against "kcal"; correction in section 4.1.8 "Evaluation", sub-section "Parameters to be analysed and acceptance limits", last paragraph (page15): deletion of "for AUC" in the cross-reference to section 4.1.9.

** The correction concerns a typographical correction in Appendix II – paragraph on "Non-oral immediate release dosage forms with systemic action".

KEYWORDS	*Bioequivalence, pharmacokinetics, biowaiver, in vitro dissolution, generics*

GUIDELINE ON THE INVESTIGATION OF BIOEQUIVALENCE

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EXECUTIVE SUMMARY

This guideline specifies the requirements for the design, conduct, and evaluation of bioequivalence studies for immediate release dosage forms with systemic action.

1. INTRODUCTION

1.1 Background

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

In bioequivalence studies, the plasma concentration time curve is generally used to assess the rate and extent of absorption. Selected pharmacokinetic parameters and preset acceptance limits allow the final decision on bioequivalence of the tested products. AUC, the area under the concentration time curve, reflects the extent of exposure. C_{max}, the maximum plasma concentration or peak exposure, and the time to maximum plasma concentration, t_{max}, are parameters that are influenced by absorption rate.

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

1.2 Generic medicinal products

In applications for generic medicinal products according to Directive 2001/83/EC, Article 10(1), the concept of bioequivalence is fundamental. The purpose of establishing bioequivalence is to demonstrate equivalence in biopharmaceutics quality between the generic medicinal product and a reference medicinal product in order to allow bridging of preclinical tests and of clinical trials associated with the reference medicinal product. The current definition for generic medicinal products is found in Directive 2001/83/EC, Article 10(2)(b), which states that a generic medicinal product is a product which has the same qualitative and quantitative composition in active substances and the same pharmaceutical form as the reference medicinal product, and whose bioequivalence with the reference medicinal product has been demonstrated by appropriate bioavailability studies. The different salts, esters, ethers, isomers, mixtures of isomers, complexes or derivatives of an active substance are considered to be the same active substance, unless they differ significantly in properties with regard to safety and/or efficacy. Furthermore, the various immediate-release oral pharmaceutical forms shall be considered to be one and the same pharmaceutical form.

1.3 Other types of application

Other types of applications may also require demonstration of bioequivalence, including variations, fixed combinations, extensions and hybrid 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 or hybrid applications that are not based exclusively on bioequivalence data.

2. SCOPE

This guideline focuses on recommendations for bioequivalence studies for immediate release formulations with systemic action. It also sets the relevant criteria under which bioavailability studies need not be required (either waiver for additional strength, see section 4.1.6, a specific type of formulation, see Appendix II or BCS based Biowaiver, see Appendix III).

Specific recommendations regarding bioequivalence studies for modified release products, transdermal products and orally inhaled products are given in other guidelines (see section 3).

The scope is limited to chemical entities. Recommendation for the comparison of biologicals to reference medicinal products can be found in guidelines on similar biological medicinal products.

In case bioequivalence cannot be demonstrated using drug concentrations, in exceptional circumstances pharmacodynamic or clinical endpoints may be needed. This situation is outside the scope of this guideline and the reader is referred to therapeutic area specific guidelines.

Although the concept of bioequivalence possibly could be considered applicable for herbal medicinal products, the general principles outlined in this guideline are not applicable to herbal medicinal products, for which active constituents are less well defined than for chemical entities.

Furthermore, this guideline does not cover aspects related to generic substitution as this is subject to national regulation.

3. LEGAL BASIS

This guideline applies to Marketing Authorisation Applications for human medicinal products submitted in accordance with the Directive 2001/83/EC as amended, under Art. 10 (1) (generic applications). It may also be applicable to Marketing Authorisation Applications for human medicinal products submitted under Art. 8(3) (full applications), Art. 10b (fixed combination), Art. 10(3) (hybrid applications) of the same Directive, and for extension and variation applications in accordance with Commission Regulations (EC) No 1084/2003 and 1085/2003 as well.

This guideline should be read in conjunction with the Annex I of Directive 2001/83/EC as amended, as well as European and ICH guidelines for conducting clinical trials, including those on:

- General Considerations for Clinical Trials (ICH topic E8, CPMP/ICH/291/95)
- Guideline for Good Clinical Practice (ICH E6 (R1), CPMP/ICH/135/95)
- Statistical Principles for Clinical Trials (ICH E9, CPMP/ICH/363/96)
- Structure and Content of Clinical Study Reports (ICH E3, CPMP/ICH/137/95)
- CHMP guidance for users of the centralised procedure for generics/hybrid applications (EMEA/CHMP/225411/2006)
- Pharmacokinetic studies in man (Eudralex, Volume 3, 3CC3a)
- Modified Release Oral and Transdermal Dosage Forms: Sections I and II (CPMP/QWP/604/96, CPMP/EWP/280/96)
- Fixed Combination Medicinal Products (CPMP/EWP/240/95 Rev 1)
- Requirements for clinical documentation for orally inhaled products (OIP) including the requirements for demonstration of therapeutic equivalence between two inhaled products for use in the treatment of Asthma and Chronic Obstructive Pulmonary Disease (COPD) (CPMP/EWP/4151/00 rev 1)
- Clinical Requirements for Locally Applied, Locally Acting Products containing Known Constituents (CPMP/EWP/239/95)

The guideline should also be read in conjunction with relevant guidelines on pharmaceutical quality. The test products used in the bioequivalence study must be prepared in accordance with GMP-regulations including Eudralex volume 4.

Bioequivalence trials conducted in the EU/EEA have to be carried out in accordance with Directive 2001/20/EC. Trials conducted outside of the Union and intended for use in a Marketing Authorisation Application in the EU/EEA have to be conducted to the standards set out in Annex I of the community code, Directive 2001/83/EC as amended.

Companies may also apply for CHMP Scientific Advice, via the EMEA, for specific queries not covered by existing guidelines.

4. MAIN GUIDELINE TEXT

4.1 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 enantioselective analysis and the possibility of waiver for additional strengths (see sections 4.1.4, 4.1.5 and 4.1.6).

Module 2.7.1 should list all relevant studies carried out with the product applied for, i.e. bioequivalence studies comparing the formulation applied for (i.e. same composition and manufacturing process) with a reference medicinal product marketed in EU. Studies should be included in the list regardless of the study outcome. Full study reports should be provided for all studies, except pilot studies for which study report synopses (in accordance with ICH E3) are sufficient. Full study reports for pilot studies should be available upon request. Study report synopses for bioequivalence or comparative bioavailability studies conducted during formulation development should also be included in Module 2.7. Bioequivalence studies comparing the product applied for with non-EU reference products should not be submitted and do not need to be included in the list of studies.

4.1.1 Study design

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

Standard design

If two formulations are compared, a randomised, two-period, two-sequence single dose crossover design is recommended. 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 subjects at the beginning of the second period. Normally at least 5 elimination half-lives are necessary to achieve this.

Alternative designs

Under certain circumstances, provided the study design and the statistical analyses are scientifically sound, alternative well-established designs could be considered such as parallel design for substances with very long half-life and replicate designs e.g. for substances with highly variable pharmacokinetic characteristics (see section 4.1.10).

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 C_{max}, this will only be acceptable if the applicant can adequately justify that the sensitivity of the analytical method cannot be improved and that it is not possible to reliably measure the parent compound after single dose administration taking into account also the option of using a supra-therapeutic dose in the bioequivalence study (see also section 4.1.6). Due to the recent development in the bioanalytical methodology, it is unusual that parent drug cannot be measured accurately and precisely. Hence, use of a multiple dose study instead of a single dose study, due to limited sensitivity of the analytical method, will only be accepted in exceptional cases.

In 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).

4.1.2 Reference and test product

<u>Reference Product</u>

For Article 10(1) and 10(3) marketing authorisation applications reference must be made to the dossier of a reference medicinal product for which a marketing authorisation is or has been granted in the Union on the basis of a complete dossier in accordance with Articles 8(3), 10a, 10b or 10c of Directive 2001/83/EC, as amended. The product used as reference product in the bioequivalence study should be part of the global marketing authorisation of the reference medicinal product (as defined in Article 6(1) second subparagraph of Directive 2001/83/EC). The choice of the reference medicinal product identified by the applicant in Module 1.2 Application form for which bioequivalence has been

demonstrated by appropriate bioavailability studies, should be justified in section 1.5.2 “Information for generic, hybrid or bio-similar applications”.

Test products in an application for a generic or hybrid product or an extension of a generic/hybrid product are normally compared with the corresponding dosage form of a reference medicinal product, if available on the market.

In an application for extension of a medicinal product which has been initially approved under Art. 8(3) of Directive 2001/83/EC and when there are several dosage forms of this medicinal product on the market, it is recommended that the dosage form used for the initial approval of the concerned medicinal product (and which was used in clinical efficacy and safety studies) is used as reference product, if available on the market.

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.

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.

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.

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.

   In case of a production batch smaller than 100,000 units, a full production batch will be required.

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 I).

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

   If full scale production batches are not available at the time of submission, the applicant should not market a batch until comparative dissolution profile testing has been completed.

   The results should be provided at a Competent Authority’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.

Packaging of study products

The reference and test products should be packed in an individual way for each subject and period, either before their shipment to the trial site, or at the trial site itself. Packaging (including labelling) should be performed in accordance with good manufacturing practice, including Annex 13 to the EU guide to GMP. Where necessary and in accordance with local regulations, sites should be authorised, as provided for in Article 13(1) of Directive 2001/20/EC, except where the provisions of Article 9(2)

of Directive 2005/28/EC apply. Third country sites should be able to demonstrate standards equivalent to these GMP requirements compliant with local requirements.

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.

4.1.3 Subjects

Number of subjects

The number of subjects to be included in the study should be based on an appropriate sample size calculation. The number of evaluable subjects in a bioequivalence study should not be less than 12.

Selection of subjects

The subject population for bioequivalence studies should be selected with the aim of permitting detection of differences between pharmaceutical products. In order to reduce variability not related to differences between products, the studies should normally be performed in 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. Subjects should be 18 years of age or older and preferably have a Body Mass Index between 18.5 and 30 kg/m^2.

The subjects should be screened for suitability by means of clinical laboratory tests, a medical history, and a physical examination. Depending on the drug's therapeutic class and safety profile, special medical investigations and precautions may have to be carried out before, during and after the completion of the study. Subjects could belong to either sex; however, the risk to women of childbearing potential should be considered. Subjects should preferably be non-smokers and without a history of alcohol or drug abuse. Phenotyping and/or genotyping of subjects may be considered for safety or pharmacokinetic reasons.

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 investigated active substance is known to have adverse effects, and the pharmacological effects or risks are considered unacceptable for healthy volunteers, it may be necessary to include patients instead, under suitable precautions and supervision.

4.1.4 Study conduct

Standardisation

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

The time of day for ingestion should be specified. Subjects should fast for at least 8 hours prior to administration of the products, unless otherwise justified. As fluid intake may influence gastric passage for oral administration forms, the test and reference products should be administered with a standardised volume of fluid (at least 150 ml). It is recommended that water is allowed as desired except for one hour before and one hour after drug administration and no food is allowed for at least 4 hours post-dose. Meals taken after dosing should be 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

subjects should start the meal 30 minutes prior to administration of the drug product and eat this meal within 30 minutes.

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

The subjects should abstain from food and drinks, which may interact with circulatory, gastrointestinal, hepatic or renal function (e.g. alcoholic drinks or certain fruit juices such as grapefruit juice) during a suitable period before and during the study. Subjects should not take any other concomitant medication (including herbal remedies) for an appropriate interval before as well as during the study. Contraceptives are, however, allowed. In case concomitant medication is unavoidable and a subject 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 subjects for safety or tolerability reasons (e.g. opioid antagonists, anti-emetics). In that scenario, the risk for a potential interaction or bioanalytical interference affecting the results must be addressed.

Medicinal products that according to the 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 recommended to be administered concomitantly.

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).

Sampling times

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. In particular, the sampling schedule should be planned to avoid C_{max} being the first point of a concentration time curve. 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 $AUC_{(0-\infty)}$. At least three to four samples are needed during the terminal log-linear phase in order to reliably estimate the terminal rate constant (which is needed for a reliable estimate of $AUC_{(0-\infty)}$). 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.

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 $AUC_{(0-\tau)}$.

If urine is used as the biological sampling fluid, urine should normally be collected over no less than three times the terminal elimination half-life. However, in line with the recommendations on plasma sampling, urine does not need to be collected for more than 72 h. If rate of excretion is to be determined, the collection intervals need to be as short as feasible during the absorption phase (see also section 4.1.5).

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 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 4.1.5).

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 cross-over studies or a four-way cross-over 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 (%)).

4.1.5 Characteristics to be investigated

Pharmacokinetic parameters

Actual time of sampling should be used in the estimation of the pharmacokinetic parameters. In studies to determine bioequivalence after a single dose, $AUC_{(0-t)}$, $AUC_{(0-\infty)}$, residual area, C_{max} and t_{max} should be determined. In studies with a sampling period of 72 h, and where the concentration at 72 h is quantifiable, $AUC_{(0-\infty)}$ 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, λ_z, and $t_{1/2}$.

In studies to determine bioequivalence for immediate release formulations at steady state, $AUC_{(0-\tau)}$, $C_{max,ss,}$ and $t_{max,ss}$ should be determined.

When using urinary data, $Ae_{(0-t)}$ and, if applicable, R_{max} should be determined.

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.

Parent compound or metabolites

General recommendations

In principle, evaluation of bioequivalence should be based upon measured concentrations of the parent compound. The reason for this is that C_{max} of a parent compound is usually more sensitive to detect differences between formulations in absorption rate than C_{max} of a metabolite.

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

The use of a metabolite as a surrogate for an active parent compound is not encouraged. 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 4.1.6). 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 expected to be accepted only in exceptional cases. When using metabolite data as a substitute for active parent drug concentrations, the applicant should present any available data supporting the view that the metabolite exposure will reflect parent drug and that the metabolite formation is not saturated at therapeutic doses.

Enantiomers

The use of achiral bioanalytical methods is generally acceptable. However, the individual enantiomers should be measured when all the following conditions are met:

(1) the enantiomers exhibit different pharmacokinetics
(2) the enantiomers exhibit pronounced difference in pharmacodynamics
(3) the exposure (AUC) ratio of enantiomers is modified by a difference in the rate of absorption.

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.

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

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.

4.1.6 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.

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 4.2).

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.

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.

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.

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.

4.1.7 Bioanalytical methodology

The bioanalytical part of bioequivalence trials should be performed in accordance with the principles of Good Laboratory Practice (GLP). However, as human bioanalytical studies fall outside the scope of GLP, the sites conducting the studies are not required to be monitored as part of a national GLP compliance programme.

The bioanalytical methods used must be well characterised, 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 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 (calibration curve performance), accuracy, precision and stability.

The lower limit of quantitation should be 1/20 of C_{max} or lower, as pre-dose concentrations should be detectable at 5% of C_{max} or lower (see section 4.1.8. *Carry-over effects*).

Reanalysis of study samples should be predefined in the study protocol (and/or SOP) before the actual start of the analysis of the samples. Normally reanalysis of subject samples because of a pharmacokinetic reason is not acceptable. This is especially important for bioequivalence studies, as this may bias the outcome of such a study.

Analysis of samples should be conducted without information on treatment.

4.1.8 Evaluation

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 4.1.2) 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.

Subject accountability

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

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

Reasons for exclusion

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

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 subjects is required.

Examples of reasons to exclude the results from a subject 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 subject.

The permitted reasons for exclusion must be pre-specified in the protocol. If one of these events occurs it should be noted in the CRF as the study is being conducted. Exclusion of subjects 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 subject with lack of any measurable concentrations or only very low plasma concentrations for reference medicinal product. A subject 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 subject). The exclusion of data due to this reason will only be accepted in exceptional cases and may question the validity of the trial.

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

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

As stated in section 4.1.4, $AUC_{(0-t)}$ should cover at least 80% of $AUC_{(0-\infty)}$. Subjects should not be excluded from the statistical analysis if $AUC_{(0-t)}$ covers less than 80% of $AUC_{(0-\infty)}$, but if the percentage is less than 80% in more than 20% of the observations then the validity of the study may need to be discussed. This does not apply if the sampling period is 72 h or more and $AUC_{(0-72h)}$ is used instead of $AUC_{(0-t)}$.

Parameters to be analysed and acceptance limits

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 C_{max}. For these parameters the 90% confidence interval for the ratio of the test and reference products should be contained within the acceptance interval of 80.00-125.00%. 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.

For studies to determine bioequivalence of immediate release formulations at steady state, $AUC_{(0-\tau)}$ and $C_{max,ss}$ should be analysed using the same acceptance interval as stated above.

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 $AUC_{(0-t)}$. R_{max} should be analysed using the same acceptance interval as for C_{max}.

A statistical evaluation of t_{max} is not required. However, if rapid release is claimed to be clinically relevant and of importance for onset of action or is related to adverse events, there should be no apparent difference in median t_{max} and its variability between test and reference product.

In specific cases of products with a narrow therapeutic range, the acceptance interval may need to be tightened (see section 4.1.9). Moreover, for highly variable drug products the acceptance interval for C_{max} may in certain cases be widened (see section 4.1.10).

Statistical analysis

The assessment of bioequivalence is based upon 90% confidence intervals 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.

The pharmacokinetic parameters under consideration should be analysed using ANOVA. The data 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, subject within sequence, period and formulation. Fixed effects, rather than random effects, should be used for all terms.

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

Two-stage design

It is acceptable to use a two-stage approach when attempting to demonstrate bioequivalence. An initial group of subjects can be treated and their data analysed. If bioequivalence has not been demonstrated an additional group can be recruited and the results from both groups combined in a final analysis. If this approach is adopted appropriate steps must be taken to preserve the overall type I error of the experiment and the stopping criteria should be clearly defined prior to the study. The analysis of the first stage data should be treated as an interim analysis and both analyses conducted at adjusted significance levels (with the confidence intervals accordingly using an adjusted coverage probability

which will be higher than 90%). For example, using 94.12% confidence intervals for both the analysis of stage 1 and the combined data from stage 1 and stage 2 would be acceptable, but there are many acceptable alternatives and the choice of how much alpha to spend at the interim analysis is at the company's discretion. The plan to use a two-stage approach must be pre-specified in the protocol along with the adjusted significance levels to be used for each of the analyses.

When analysing the combined data from the two stages, a term for stage should be included in the ANOVA model.

Presentation of data

All individual concentration data and pharmacokinetic parameters should be listed by formulation together with summary statistics such as geometric mean, median, arithmetic mean, standard deviation, coefficient of variation, minimum and maximum. Individual plasma concentration/time curves should be presented in linear/linear and log/linear scale. The method used to derive the pharmacokinetic parameters from the raw data should be specified. The number of points of the terminal log-linear phase used to estimate the terminal rate constant (which is needed for a reliable estimate of AUC_{∞}) should be specified.

For the pharmacokinetic parameters that were subject to statistical analysis, the point estimate and 90% confidence interval for the ratio of the test and reference products should be presented.

The ANOVA tables, including the appropriate statistical tests of all effects in the model, should be submitted.

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

Drop-out and withdrawal of subjects should be fully documented. If available, concentration data and pharmacokinetic parameters from such subjects should be presented in the individual listings, but should not be included in the summary statistics.

The bioanalytical method should be documented in a pre-study validation report. A bioanalytical report should be provided as well. The bioanalytical report should include a brief description of the bioanalytical method used and the results for all calibration standards and quality control samples. A representative number of chromatograms or other raw data should be provided covering the whole concentration range for all standard and quality control samples as well as the specimens analysed. This should include all chromatograms from at least 20% of the subjects with QC samples and calibration standards of the runs including these subjects.

If for a particular formulation at a particular strength multiple studies have been performed some of which demonstrate bioequivalence and some of which do not, the body of evidence must be considered as a whole. Only relevant studies, as defined in section 4.1, need be considered. The existence of a study which demonstrates bioequivalence does not mean that those which do not can be ignored. The applicant should thoroughly discuss the results and justify the claim that bioequivalence has been demonstrated. Alternatively, when relevant, a combined analysis of all studies can be provided in addition to the individual study analyses. It is not acceptable to pool together studies which fail to demonstrate bioequivalence in the absence of a study that does.

4.1.9 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.

4.1.10 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 cross-over design study can be carried out.

Those HVDP for which a wider difference in C_{max} 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 C_{max} 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 C_{max} 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 C_{max} 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

$$* \, CV(\%) = 100\sqrt{e^{s_{WR}^2} - 1}$$

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.

4.2 *In vitro* dissolution tests

General aspects of *in vitro* dissolution experiments are briefly outlined in Appendix I including basic requirements how to use the similarity factor (f_2-test).

4.2.1 *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 (see Appendix I).

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

4.2.2 *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 App. 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).

4.3 Study report

4.3.1 Bioequivalence study report

The report of the bioequivalence study should give the complete documentation of its protocol, conduct and evaluation. It should be written in accordance with the ICH E3 guideline and be signed by the investigator in accordance with Annex I of the Directive 2001/83/EC as amended.

Names and affiliations of the responsible investigator(s), the site of the study and the period of its execution should be stated. Audits certificate(s), if available, should be included in the report.

The study report should include evidence that the choice of the reference medicinal product is in accordance with Article 10(1) and Article 10(2) of Directive 2001/83/EC as amended. This should include the reference product name, strength, pharmaceutical form, batch number, manufacturer, expiry date and country of purchase.

The name and composition of the test product(s) used in the study should be provided. The batch size, batch number, manufacturing date and, if possible, the expiry date of the test product should be stated.

Certificates of analysis of reference and test batches used in the study should be included in an appendix to the study report.

Concentrations and pharmacokinetic data and statistical analyses should be presented in the level of detail described above (section 4.1.8 *Presentation of data*).

4.3.2 Other data to be included in an application

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. Comparative dissolution profiles (see section 4.2) should be provided.

The validation report of the bioanalytical method should be included in Module 5 of the application.

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 subject 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.

4.4 Variation applications

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 APPENDIX III, or on whether an acceptable level A *in vitro / in vivo* correlation has been established (see CPMP/QWP/ 604/96).

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).

For variations of products approved under Art. 8 (3), 10a, 10b or 10c of Directive 2001/83/EC as amended, the comparative medicinal product for use in bioequivalence and dissolution studies is usually that authorised under the currently registered formulation, manufacturing process, packaging etc.

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.

DEFINITIONS

Pharmaceutical equivalence

Medicinal products are pharmaceutically equivalent if they contain the same amount of the same active substance(s) in the same dosage forms that meet the same or comparable standards.

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

Pharmaceutical alternatives

Pharmaceutical alternatives are medicinal products with different salts, esters, ethers, isomers, mixtures of isomers, complexes or derivatives of an active moiety, or which differ in dosage form or strength.

Pharmacokinetic parameters

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

APPENDIX I

Dissolution testing and Similarity of Dissolution Profiles

1. General aspects of dissolution testing as related to bioavailability

During the development of a medicinal product a dissolution test is used as a tool to identify formulation factors that are influencing and may have a crucial effect on the bioavailability of the drug. As soon as the composition and the manufacturing process are defined a dissolution test is used in the quality control of scale-up and of production batches to ensure both batch-to-batch consistency and that the dissolution profiles remain similar to those of pivotal clinical trial batches. Furthermore, in certain instances a dissolution test can be used to waive a bioequivalence study. Therefore, dissolution studies can serve several purposes:

i – Testing on product quality

- To get information on the test batches used in bioavailability/bioequivalence studies and pivotal clinical studies to support specifications for quality control
- To be used as a tool in quality control to demonstrate consistency in manufacture
- To get information on the reference product used in bioavailability/bioequivalence studies and pivotal clinical studies.

ii - Bioequivalence surrogate inference

- To demonstrate in certain cases similarity 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 4.2 and App. III)
- To investigate batch to batch consistency of the products (test and reference) to be used as basis for the selection of appropriate batches for the *in vivo* study.

Test methods should be developed product related based on general and/or specific pharmacopoeial requirements. In case those requirements are shown to be unsatisfactory and/or do not reflect the *in vivo* dissolution (i.e. biorelevance) alternative methods can be considered when justified that these are discriminatory and able to differentiate between batches with acceptable and non-acceptable performance of the product *in vivo*. Current state-of-the-art information including the interplay of characteristics derived from the BCS classification and the dosage form must always be considered.

Sampling time points should be sufficient to obtain meaningful dissolution profiles, and at least every 15 minutes. More frequent sampling during the period of greatest change in the dissolution profile is recommended. 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.

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.

2. Similarity 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 in section 1 above, 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%.

For modified release products, the advice given in the relevant guidance should be followed.

Dissolution similarity may be determined using the f_2 statistic as follows:

$$f_2 = 50 \cdot \log \left[\frac{100}{\sqrt{1 + \frac{\sum_{t=1}^{t=n} \left[\bar{R}(t) - \bar{T}(t) \right]^2}{n}}} \right]$$

In this equation f_2 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 time points should be the same for the two formulations
- Twelve individual values for every time point for each formulation
- Not more than one mean value of > 85% dissolved for any of the formulations.
- The relative standard deviation or coefficient of variation of any product should be less than 20% for the first point and less than 10% from second to last time point.

An f_2 value between 50 and 100 suggests that the two dissolution profiles are similar.

When the f_2 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 f_2 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.

APPENDIX II

Bioequivalence study requirements for different dosage forms

Although this guideline concerns immediate release formulations, Appendix II 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 Appendix III, section III), *in vivo* bioequivalence studies may in some situations not be required as described below and in Appendix III.

Oral immediate release dosage forms with systemic action

For dosage forms such as tablets, capsules and oral suspensions, bioequivalence studies are required unless a biowaiver is applicable (see APPENDIX III). For orodispersable tablets and oral solutions specific recommendations apply, as detailed below.

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 Appendix III). 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.

Oral solutions

If the test product is an aqueous oral solution at time of administration and contains an active substance in the same concentration as an approved oral solution, bioequivalence studies may be waived. However if the excipients may affect gastrointestinal transit (e.g. sorbitol, mannitol, etc.), absorption (e.g. surfactants or excipients that may affect transport proteins), *in vivo* solubility (e.g. co-solvents) or *in vivo* stability of the active substance, a bioequivalence study should be conducted, unless the differences in the amounts of these excipients can be adequately justified by reference to other data. The same requirements for similarity in excipients apply for oral solutions as for Biowaivers (see Appendix III, Section IV.2 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.

Fixed combination dosage forms

Bioequivalence requirements are covered in the "Guideline on Clinical Development of Fixed Combination Medicinal Products". The possibility for a biowaiver of Fixed Combination Medicinal Products is addressed in Appendix III section V.

Non-oral immediate release dosage forms with systemic action

This section applies to e.g. rectal formulations. In general, bioequivalence studies are 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).

Parenteral solutions

Bioequivalence studies are generally not required if the test product is to be administered as an aqueous intravenous solution containing the same active substance as the currently approved product. However, if any excipients interact with the drug substance (e.g. complex formation), or otherwise affect the disposition of the drug substance, a bioequivalence study is required unless both products contain the same excipients in very similar quantity and it can be adequately justified that any difference in quantity does not affect the pharmacokinetics of the active substance.

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

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.

Modified release dosage forms with systemic action

Modified release oral and transdermal dosage forms

Bioequivalence studies are required in accordance with the guideline on Modified Release Oral and Transdermal Dosage Forms: Section II (Pharmacokinetic and Clinical Evaluation) (CPMP/EWP/280/96).

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.

Locally acting locally applied products

For products for local use (after oral, nasal, pulmonary, ocular, dermal, rectal, vaginal etc. administration) intended to act at the site of application, recommendations can be found in other guidelines (CPMP/EWP/4151/00 rev 1, CPMP/EWP/239/95).

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, 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.

Gases

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

APPENDIX III

BCS-based Biowaiver

I. Introduction

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 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.

II. Summary Requirements

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 III) <u>and</u>
- 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 IV.1) <u>and</u>
- 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 IV.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 III) <u>and</u>
- very rapid (> 85 % within 15 min) *in vitro* dissolution of the test and reference product has been demonstrated considering specific requirements (see section IV.1) <u>and</u>
- excipients that might affect bioavailability are qualitatively and quantitatively the same and other excipients are qualitatively the same and quantitatively very similar (see section IV.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.

III. 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 *III.1* and *III.2*). 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 drug substance should not belong to the group of 'narrow therapeutic index' drugs (see section 4.1.9 on narrow therapeutic index drugs).

III.1 Solubility

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

III.2 Absorption

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

Complete drug absorption should be justified based on reliable investigations in human. Data from

- absolute bioavailability or
- mass-balance

studies could be used to support this claim.

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 sect. *IV.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.

IV. Drug Product

IV.1 In vitro Dissolution

IV.1.1 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 4.1.2 of the main guideline text. 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 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.

IV.1.2 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. 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.

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. F_2-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.

IV.2 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 can not 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.

V. 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 in section IV.2. Otherwise *in vivo* bioequivalence testing is required.