Annex 6

Multisource (generic) pharmaceutical products: guidelines on registration requirements to establish interchangeability

Republication of *Multisource (generic) pharmaceutical products: guidelines on registration requirements to establish interchangeability*, WHO Technical Report Series, No. 992, Annex 7 with a new Appendix 2

Background

Following the publication of the *Multisource (generic) pharmaceutical products: guidelines on registration requirements to establish interchangeability* in 2015, it was noted that a text on equilibrium solubility experiments for the purpose of classification of active pharmaceutical ingredients (APIs) according to the Biopharmaceutics Classification System (BCS) would be a useful addition. The method for determination of equilibrium solubility was suggested to be added as an appendix to the above-mentioned guidelines.

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1. Introduction

These guidelines provide recommendations to regulatory authorities when defining requirements for approval of multisource (generic) pharmaceutical products in their respective countries. The guidance provides appropriate in vivo and in vitro requirements to assure interchangeability of the multisource product without compromising the safety, quality and efficacy of the pharmaceutical product.

National regulatory authorities (NRAs) should ensure that all pharmaceutical products subject to their control conform to acceptable standards of safety, efficacy and quality, and that all premises and practices employed in the manufacture, storage and distribution of these products comply with good manufacturing practice (GMP) standards so as to ensure the continued conformity of the products with these requirements until they are delivered to the end user.

All pharmaceutical products, including multisource products, should be used in a country only after approval by the national or regional authority.

Regulatory authorities should require the documentation of a multisource pharmaceutical product to meet the following:

- GMP;
- quality control (QC) specifications;
- pharmaceutical product interchangeability.

Multisource pharmaceutical products need to conform to the same appropriate standards of quality, efficacy and safety as those required of the innovator's (comparator) product. In addition, reasonable assurance must be provided that the multisource product is therapeutically equivalent and interchangeable with the comparator product. For some classes of products, including – most evidently – aqueous parenteral solutions, interchangeability is adequately assured by assessment of the composition, implementation of GMP and evidence of conformity with appropriate specifications including relevant pharmacopoeial specifications. For a wide range of pharmaceutical products the concepts and approaches covered by these guidelines will enable NRAs to decide whether a given multisource product can be approved. This guidance is generally applicable to orally administered multisource products as well as to non-orally administered pharmaceutical products for which systemic exposure measures are suitable for documenting bioequivalence (e.g. transdermal delivery systems and certain parenteral, rectal and nasal pharmaceutical products). Some information applicable to locally acting products is also provided in this document. For other classes of product, including many biologicals such as vaccines, animal sera, products derived from human blood and plasma and products manufactured

by biotechnology, as well as non-biological complex products, the concept of interchangeability raises issues that are beyond the scope of this document and these products are consequently excluded from consideration.

To ensure interchangeability, the multisource product must be therapeutically equivalent to the comparator product. Types of in vivo equivalence studies include comparative pharmacokinetic studies, comparative pharmacodynamic studies and comparative clinical studies.

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

Assuming that, in the same subject, an essentially similar plasma concentration time course will result in essentially similar concentrations at the site(s) of action and thus in an essentially similar therapeutic outcome, pharmacokinetic data may be used instead of therapeutic results. Further, in selected cases, in vitro comparison of the dissolution profiles of the multisource product with those of the comparator product may be sufficient to provide an indication of equivalence.

It should be noted that interchangeability includes the equivalence of the dosage form as well as of the indications and instructions for use. Alternative approaches to the principles and practices described in this document may be acceptable provided they are supported by adequate scientific justification. These guidelines should be interpreted and applied without prejudice to obligations incurred through the existing international Agreement on Trade-Related Aspects of Intellectual Property Rights (*1*).

2. Glossary

Some important terms used in these guidelines are defined below. They may have different meanings in other contexts.

bioavailability. The rate and extent to which the active moiety is absorbed from a pharmaceutical dosage form and becomes available at the site(s) of action. Reliable measurements of active pharmaceutical ingredient (API) concentrations at the site(s) of action are usually not possible. The substance in the systemic circulation, however, is considered to be in equilibrium with the substance at the site(s) of action. Bioavailability can therefore be defined as the rate and extent to which the API or active moiety is absorbed from a pharmaceutical dosage form and becomes available in the systemic circulation.

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

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

biological pharmaceutical product. A biological pharmaceutical product is a synonym for biological product or biological (as described in the reports of the Expert Committee on Biological Standardization in the World Health Organization (WHO) Technical Report Series). The definition of a pharmaceutical substance used in treatment, prevention or diagnosis as a "biological" has been variously based on criteria related to its source, its amenability to characterization by physicochemical means alone, the requirement for biological assays or arbitrary systems of classification applied by regulatory authorities. For the purposes of WHO, including the current document, the list of substances considered to be biologicals is derived from their earlier definition as "substances which cannot be fully characterized by physicochemical means alone and which therefore require the use of some form of bioassay". However, developments in the utility and applicability of physicochemical analytical methods, improved control of biological and biotechnology based production methods and an increased applicability of chemical synthesis to larger molecules, have made it effectively impossible to base a definition of a biological on any single criterion related to methods of analysis, source or method of production. Nevertheless many biologicals are produced using in vitro culture systems.

Biopharmaceutics Classification System. The Biopharmaceutics Classification System (BCS) is a scientific framework for classifying APIs based upon their aqueous solubility and intestinal permeability. When combined with the dissolution of the pharmaceutical product and the critical examination of the excipients of the pharmaceutical product, the BCS takes into account the major factors that govern the rate and extent of API absorption (exposure) from immediate-release oral solid dosage forms: excipient composition, dissolution, solubility and intestinal permeability.

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

comparator product. The comparator product is a pharmaceutical product with which the multisource product is intended to be interchangeable in clinical practice. The comparator product will normally be the innovator product

for which efficacy, safety and quality have been established. If the innovator product is no longer marketed in the jurisdiction, the selection principle as described in *Guidance on the selection of comparator pharmaceutical products for equivalence assessment of interchangeable multisource (generic) products* (WHO Technical Report Series, No. 992, Annex 8 (2015)) should be used to identify a suitable alternative comparator product.

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

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

equivalence test. A test that determines the equivalence between the multisource product and the comparator product using in vivo and/or in vitro approaches.

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

fixed-dose combination finished pharmaceutical product. An FPP that contains two or more APIs.

generic product. See multisource pharmaceutical products.

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

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

in vitro equivalence dissolution test. An in vitro equivalence test is a dissolution test that includes comparison of the dissolution profile between the multisource product and the comparator product, typically in at least three media: pH 1.2, pH 4.5 and pH 6.8 buffer solutions.

in vitro quality control dissolution test. A dissolution test procedure identified in the pharmacopoeia for routine QC of product batches, generally a one time-point dissolution test for immediate release products and a three or more time-points dissolution test for modified release products.

multisource pharmaceutical products. Pharmaceutically equivalent or pharmaceutically alternative products that may or may not be therapeutically equivalent. Multisource pharmaceutical products that are therapeutically equivalent are interchangeable.

non-biological. Not involving or derived from biology or living organisms.

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

pharmaceutical equivalence. Products are pharmaceutical equivalents if they contain the same molar amount of the same APIs in the same dosage form, if they meet comparable standards and if they are intended to be administered by the same route. Pharmaceutical equivalence does not necessarily imply therapeutic equivalence, as differences in the API solid-state properties, the excipients and/or the manufacturing process and other variables can lead to differences in product performance.

quantitatively similar amounts (concentrations) of excipients. The relative amount of excipient present in two solid oral FPPs is considered to be quantitatively similar if the differences in amount fall within the limits shown in Table A6.1.

Table A6.1

Limits on the relative difference in the amount of excipient in two solid oral finished pharmaceutical products for the products to be considered quantitatively similar in that excipient

Excipient type	Percentage difference (w/w) out of total product (core) weight
Filler	5.0
Disintegrant	
Starch	3.0
Other	1.0
Binder	0.5
Lubricant	
Calcium or magnesium stearate	0.25
Other	1.0
Glidant	
Talc	1.0
Other	0.1

If an excipient serves multiple functions (e.g. microcrystalline cellulose as a filler and as a disintegrant) then the most conservative recommended range should be applied (e.g. ± 1.0% for microcrystalline cellulose should be applied in this example). The relative concentration of an excipient present in two aqueous solution FPPs is considered to be similar if the difference is ≤ 10%.

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

3. Documentation of equivalence for marketing authorization

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

- comparative pharmacokinetic studies in humans, in which the API and/or its metabolite(s) are measured as a function of time in an accessible biological fluid such as blood, plasma, serum or urine to obtain pharmacokinetic measures, such as AUC and C_{max} that reflect the systemic exposure;
- comparative pharmacodynamic studies in humans;
- comparative clinical trials;
- comparative in vitro tests.

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

Acceptance of any test procedure in the documentation of equivalence between two pharmaceutical products by an NRA depends on many factors, including the characteristics of the API and the pharmaceutical product. Where an API produces measurable concentrations in an accessible biological fluid, such as plasma, comparative pharmacokinetic studies can be performed. This type of study is considered to be the gold standard in equivalence testing; however, where appropriate, in vitro testing, e.g. BCS based biowaivers for immediate release

pharmaceutical products, can also assure equivalence between the multisource product and the comparator product (see sections 5 and 10). Where an API does not produce measurable concentrations in an accessible biological fluid and a BCS based biowaiver is not an option, comparative pharmacodynamics studies may be an alternative method for documenting equivalence. Further, in certain cases when it is not possible to assess equivalence through other methods, comparative clinical trials may be considered appropriate.

The criteria that indicate when equivalence studies are necessary are discussed in sections 4 and 5 of these guidelines.

4. When equivalence studies are not necessary

In the following circumstances, multisource pharmaceutical products are considered to be equivalent without the need for further documentation:

(a) when the pharmaceutical product is to be administered parenterally (e.g. intravenously, subcutaneously or intramuscularly) as an aqueous solution containing the same API in the same molar concentration as the comparator product and the same or similar excipients in comparable concentrations to those in the comparator product. Certain excipients (e.g. buffer, preservative and antioxidant) may be different provided it can be shown that the change(s) in these excipients would not affect the safety and/or efficacy of the pharmaceutical product. The same principles are applicable for parenteral oily solutions but, in this case, the use of the same oily vehicle is essential. Similarly, for micellar solutions, solutions containing complexing agents or solutions containing co solvents of the same qualitative and quantitative composition of the functional excipients are necessary in order to waive equivalence studies and the change of other excipients should be critically reviewed;

(b) when pharmaceutically equivalent products are solutions for oral use (e.g. syrups, elixirs and tinctures), contain the API in the same molar concentration as the comparator product, contain the same functional excipients in similar concentrations (if the API is BCS Class I) and the same excipients in similar concentrations (for APIs from other BCS classes);

(c) when pharmaceutically equivalent products are in the form of powders for reconstitution as an aqueous solution and the resultant solution meets either criterion (a) or criterion (b) above;

(d) when pharmaceutically equivalent products are gases;

(e) when pharmaceutically equivalent products are otic or ophthalmic products prepared as aqueous solutions and contain the same

API(s) in the same molar concentration and the same excipients in similar concentrations. Certain excipients (e.g. preservative, buffer, substance to adjust tonicity or thickening agent) may be different provided their use is not expected to affect bioavailability, safety and/or efficacy of the product;

(f) when pharmaceutically equivalent products are topical products prepared as aqueous solutions and contain the same API(s) in the same molar concentration and the same excipients in similar concentrations (note that a waiver would not apply to other topical dosage forms like gels, emulsions or suspensions, but might be applicable to oily solutions if the vehicle composition is sufficiently similar);

(g) when pharmaceutically equivalent products are aqueous solutions for nebulization or nasal drops, intended to be administered with essentially the same device, contain the same API(s) in the same concentration and contain the same excipients in similar concentrations (note that this waiver does not apply to other dosage forms like suspensions for nebulization, nasal drops where the API is in suspension, nasal sprays in solution or suspension, dry powder inhalers or pressurized metered dose inhalers in solution or suspensions). The pharmaceutical product may include different excipients provided their use is not expected to affect bioavailability, safety and/or efficacy of the product.

For situations (b), (c), (e), (f) and (g) above it is incumbent upon the applicant to demonstrate that the excipients in the pharmaceutically equivalent product are the same and that they are in concentrations similar to those in the comparator product or, where applicable (i.e. (a), (e) and (g)), that their use is not expected to affect the bioavailability, safety and/or efficacy of the product. In the event that the applicant cannot provide this information and the NRA does not have access to the relevant data, it is incumbent upon the applicant to perform appropriate studies to demonstrate that differences in excipients or devices do not affect product performance.

5. When equivalence studies are necessary and types of study required

Except for the cases discussed in section 4, these guidelines recommend that documentation of equivalence with the comparator product be required by registration authorities for a multisource pharmaceutical product. Studies must be carried out using the product intended for marketing (see also section 7.3).

5.1 In vivo studies

For certain APIs and dosage forms, in vivo documentation of equivalence, through either a pharmacokinetic comparative bioavailability (bioequivalence) study, a comparative pharmacodynamic study or a comparative clinical trial, is regarded as especially important. In vivo documentation of equivalence is necessary when there is a risk that possible differences in bioavailability may result in therapeutic inequivalence (*2*). Examples are listed below:

(a) oral, immediate-release pharmaceutical products with systemic action, except for the conditions outlined in section 10;
(b) non-oral, non-parenteral pharmaceutical products designed to act systemically (such as transdermal patches, suppositories, nicotine chewing gum, testosterone gel and skin inserted contraceptives);
(c) modified-release pharmaceutical products designed to act systemically, except for the conditions outlined in section 10;
(d) fixed-dose combination (FDC) products with systemic action, where at least one of the APIs requires an in vivo study (*3*);
(e) non-solution pharmaceutical products, which are for non-systemic use (e.g. for oral, nasal, ocular, dermal, rectal or vaginal application) and are intended to act without systemic absorption.

In the case of non-solution pharmaceutical products for non-systemic use, the equivalence is established through, e.g. comparative clinical or pharmacodynamic studies, local availability studies and/or in vitro studies. In certain cases, measurement of the concentration of the API may still be required for safety reasons, i.e. in order to assess unintended systemic absorption.

5.2 In vitro studies

For certain APIs and dosage forms, in vitro documentation of equivalence may be appropriate. In vitro approaches for systemically acting oral products are discussed in section 10.

6. In vivo equivalence studies in humans

6.1 General considerations

6.1.1 Provisions for studies in humans

Pharmacokinetic, pharmacodynamic and comparative clinical trials are clinical studies and should therefore be carried out in accordance with the provision and prerequisites for a clinical study, as outlined in the WHO *Guidelines for good clinical practice for trials on pharmaceutical products* (*4*) and with WHO good

laboratory practices (*5*). Additional guidance for organizations performing in vivo equivalence studies is available from WHO (*6*).

All research involving human subjects should be conducted in accordance with the ethical principles contained in the current version of the Declaration of Helsinki, including respect for persons, beneficence ("maximize benefits and minimize harms and wrongs") and non-maleficence ("do no harm"), as defined by the International Ethical Guidelines for Biomedical Research Involving Human Subjects issued by the Council for International Organizations of Medical Sciences (CIOMS), or laws and regulations of the country in which the research is conducted, whichever represents the greater protection for study subjects.

6.1.2 Justification of human bioequivalence studies

Most pharmacokinetic and pharmacodynamic equivalence studies are non-therapeutic studies in which no direct clinical benefit accrues to the subject.

It is important for anyone preparing a trial of a medicinal product in humans that the specific aims, problems and risks or benefits of the proposed human study be thoroughly considered and that the chosen design be scientifically sound and ethically justified. It is assumed that people involved in the planning of a study are familiar with the pharmacokinetic theories underlying bioavailability and bioequivalence studies. The overall design of the bioequivalence study should be based on the knowledge of the pharmacokinetics, pharmacodynamics and therapeutics of the API. Information about manufacturing procedures and data from tests performed on the product batch to be used in the study should establish that the product under investigation is of suitable quality.

6.1.3 Selection of investigators

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

6.1.4 Study protocol

A bioequivalence study should be carried out in accordance with a protocol agreed upon and signed by the investigator and the sponsor. The protocol and its attachments and/or appendices should state the aim of the study and the procedures to be used, the reasons for proposing the study to be undertaken in humans, the nature and degree of any known risks, assessment methodology,

criteria for acceptance of bioequivalence, the groups from which it is proposed that trial subjects be selected and the means for ensuring that they are adequately informed before they give their consent. The investigator is responsible for ensuring that the protocol is strictly followed. Any change(s) required must be agreed on and signed by the investigator and sponsor and appended as amendments, except when necessary to eliminate an apparent immediate hazard or danger to a trial subject.

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

The signed and dated study protocol should be approved by the NRA before commencing the study, if required by national and regional laws and regulations. The study report forms an integral part of the registration dossier of the multisource product in order to obtain the marketing authorization for the multisource product.

7. Pharmacokinetic comparative bioavailability (bioequivalence) studies in humans

7.1 Design of pharmacokinetic studies

Bioequivalence studies are designed to compare the in vivo performance of a multisource product with that of a comparator product. Such studies on products designed to deliver the API for systemic exposure serve two purposes:

- as a surrogate for clinical evidence of the safety and efficacy of the multisource product;
- as an in vivo measure of pharmaceutical quality.

The design of the study should maximize the sensitivity to detect any difference between products, minimize the variability that is not caused by formulation effects and eliminate bias as far as possible. Test conditions should reduce variability within and between subjects. In general, for a bioequivalence study involving a multisource product and a comparator product, a randomized, two-period, two-sequence, single-dose, cross-over study conducted with healthy volunteers is the preferred study design. In this design each subject is given the multisource product and the comparator product in randomized order. An adequate wash-out period should follow the administration of each product.

It should be noted, however, that under certain circumstances an alternative, well-established and statistically appropriate study design may be more suitable.

7.1.1 Alternative study designs for studies in patients

For APIs that are very potent or too toxic to administer in the highest strength to healthy volunteers (e.g. because of the potential for serious adverse events or because the trial necessitates a high dose), it is recommended that the study be conducted using the API at a lower strength in healthy volunteers. For APIs that show unacceptable pharmacological effects in healthy volunteers, even at lower strengths, a study conducted in patients may be required. Depending on the dosing posology this may be a multiple-dose, steady-state study. As above, such studies should employ a cross-over design if possible; however, a parallel group design study in patients may be required in some situations. The use of such an alternative study design should be fully justified by the sponsor and should include patients whose disease process is stable for the duration of the bioequivalence study if possible.

7.1.2 Considerations for active pharmaceutical ingredients with long elimination half-lives

A single-dose, cross-over bioequivalence study for an orally administered product with a long elimination half-life is preferred, provided an adequate wash-out period between administrations of the treatments is possible. The interval between study days should be long enough to permit elimination of essentially all of the previous dose from the body. Ideally the interval should not be less than five terminal elimination half-lives of the active compound or metabolite, if the latter is measured. If the cross-over study is problematic owing to a very long elimination half-life, a bioequivalence study with a parallel design may be more appropriate. A parallel design may also be necessary when comparing some depot formulations.

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

The number of subjects should be derived from statistical calculations, but generally more subjects are needed for a parallel study design than for a cross-over study design.

7.1.3 Considerations for multiple-dose studies

In certain situations multiple dose studies may be considered appropriate. Multiple dose studies in patients are most useful in cases where the API being studied is considered to be too potent and/or too toxic to be administered to healthy volunteers, even in single doses (see also section 7.1.1). In this case

a multiple-dose, cross-over study in patients may be performed without interrupting therapy.

The dosage regimen used in multiple dose studies should follow the usual dosage recommendations.

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

- cases where the analytical sensitivity is too low to adequately characterize the pharmacokinetic profile after a single dose;
- for extended-release dosage forms with a tendency to accumulate (in addition to single-dose studies).

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

7.1.4 Considerations for modified-release products

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

Owing to the more complex nature of modified-release products relative to immediate-release products, additional data are required to ensure the bioequivalence of two modified-release products. Factors such as the co-administration of food, which influences API bioavailability and also, in certain cases, bioequivalence, must be taken into consideration. The presence of food can affect product performance both by influencing the release of the API from the formulation and by causing physiological changes in the GI tract. In this regard a significant concern with regard to modified-release products is the possibility that food may trigger a sudden and abrupt release of the API leading to "dose dumping". This would most likely be manifested as a premature and abrupt rise in the plasma concentration time profile. Therefore, bioequivalence studies conducted under both fasted and fed conditions are required for orally administered, modified-release pharmaceutical products. Unless single-dose studies are not possible for reasons such as those discussed in section 7.1.1, single-dose, cross-over bioequivalence studies conducted under both fasted and fed conditions comparing the highest strength of the multisource product and the comparator product must be performed to demonstrate bioequivalence. Single-dose studies are preferred to multiple-dose studies as single-dose studies are considered to provide more sensitive measurement of the release of API from

the pharmaceutical product into the systemic circulation. In addition to single-dose studies, multiple-dose studies may be considered for extended release dosage forms with a tendency to accumulate, e.g. after a single dose of the highest strength the AUC for the dosing interval covers < 90% of AUC extrapolated to infinity. The comparator product in these studies should be a pharmaceutically equivalent, modified-release product. The bioequivalence criteria for modified-release products are essentially the same as for conventional release dosage forms except that acceptance criteria should also be applied to C_{min} (C_{tau}) in the case of multiple-dose studies. As release mechanisms of pharmaceutical products become more complex, e.g. products with an immediate-release and modified-release component, additional parameters such as partial AUC measures may be necessary to ensure the bioequivalence of two products.

The fed-state bioequivalence study should be conducted after the administration of an appropriate standardized meal at a specified time (usually not more than 30 minutes) before taking the pharmaceutical product. A meal that will promote the greatest change in GI tract conditions relative to the fasted state should be given. See section 7.4.3 for more recommendations for the content of the meal. The composition of the meal should take local diet and customs into consideration. The composition and caloric breakdown of the test meal should be provided in the study protocol and report.

7.2 Subjects

7.2.1 Number of subjects

The number of subjects required for a bioequivalence study is determined by:

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

The number of subjects to be recruited for the study should be estimated by considering the standards that must be met using an appropriate method (see, for example, Julious 2004 (*7*)). In addition, a number of extra subjects should be recruited, dosed appropriately, and their samples analysed based on

the expected rate of drop-outs and/or withdrawals, which depends on the safety and tolerability profile of the API. The number of subjects recruited should always be justified by the sample size calculation provided in the study protocol. A minimum of 12 subjects is required.

In some situations, reliable information concerning the expected variability in the parameters to be estimated may not be available. In such situations a two-stage sequential study design can be employed as an alternative to conducting a pilot study (see section 7.6.1 for more information).

7.2.2 Drop-outs and withdrawals

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

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

7.2.3 Exclusion of subject data

Extreme values can have a significant impact on bioequivalence study data because of the relatively small number of subjects typically involved; however, it is rarely acceptable to exclude data. Potential reasons for excluding subject data and the procedure to be followed should be included in the study protocol. Exclusion of data for statistical or pharmacokinetic reasons alone is not acceptable. Retesting of subjects is not recommended.

7.2.4 Selection of subjects

Bioequivalence studies should generally be performed with healthy volunteers. Clear criteria for inclusion and exclusion should be stated in the study protocol. If the pharmaceutical product is intended for use in both sexes, the sponsor should include both males and females in the study. The potential risk to women will need to be considered on an individual basis and, if necessary, they should be warned of any possible dangers to the fetus if they should become pregnant. The investigators should ensure that female volunteers are not pregnant or likely to become pregnant during the study. Confirmation should be obtained

by urine tests just before administration of the first and last doses of the product under study.

Generally subjects should be between the ages of 18 and 55 years and their weight should be within the normal range with a body mass index between 18 and 30 kg/m^2. The subjects should have no history of alcohol or drug abuse problems and should preferably be non-smokers.

The volunteers should be screened for their suitability using standard laboratory tests, a medical history and a physical examination. If necessary, special medical investigations may be carried out before and during studies, depending on the pharmacology of the individual API being investigated, e.g. an electrocardiogram if the API has a cardiac effect. The ability of the volunteers to understand and comply with the study protocol has to be assessed. Subjects who are being or have previously been treated for any GI problems or convulsive, depressive or hepatic disorders, and in whom there is a risk of a recurrence during the study period, should be excluded.

If a parallel design study is planned, standardization of the two groups of subjects is important in order to minimize variation not attributable to the investigational products (see section 7.2.6).

If the aim of the bioequivalence study is to address specific questions (e.g. bioequivalence in a special population) the selection criteria should be adjusted accordingly.

7.2.5 Monitoring the health of subjects during the study

In keeping with GCP (*4*) the health of volunteers should be monitored during the study so that the onset of side-effects, toxicity or any intercurrent disease may be recorded and appropriate measures taken. The incidence, severity, seriousness and duration of any adverse event observed during the study must be reported. The probability that an adverse event is due to the FPP should be judged by the investigator. Health monitoring before, during and after the study must be carried out under the supervision of a qualified medical practitioner licensed in the jurisdiction in which the study is conducted.

7.2.6 Considerations for genetic phenotyping

Phenotyping for metabolizing activity can be important for studies with high-clearance APIs that are metabolized by enzymes that are subject to genetic polymorphism, e.g. propranolol. In such cases slow metabolizers will have a higher bioavailability of the API while the bioavailability of possible active metabolites will be lower. Phenotyping of subjects can be considered for studies of APIs that show phenotype-linked metabolism and for which a parallel group design is to be used, because it allows fast and slow metabolizers to be evenly distributed between the two groups of subjects. Phenotyping could also be

important for safety reasons, determination of sampling times and wash-out periods in cross-over design studies.

7.3 Investigational product

7.3.1 Multisource pharmaceutical product

The multisource pharmaceutical product used in the bioequivalence studies for registration purposes should be identical to the planned commercial pharmaceutical product. Therefore, not only the composition and quality characteristics (including stability), but also the manufacturing methods (including equipment and procedures) should be the same as those to be used in the future routine production runs. Test products must be manufactured under GMP regulations. Batch control results, lot number, manufacturing date and, if possible, expiry date for the multisource product should be stated. Samples should ideally be taken from batches of industrial scale. When this is not feasible, pilot or small-scale production batches may be used, provided that they are not smaller than 10% of expected full production batches, or 100 000 units, whichever is larger, and are produced with the same formulation and similar equipment and process to that planned for commercial production batches. A biobatch of less than 100 000 units may be accepted provided that this is the proposed production batch size, with the understanding that future scale-up for production batches will not be accepted unless supported by in vitro and/or in vivo data as applicable.

7.3.2 Choice of comparator product

The innovator pharmaceutical product is usually the most logical comparator product for a multisource pharmaceutical product because its quality, safety and efficacy should have been well assessed and documented in premarketing studies and postmarketing monitoring schemes. Preferably this will mean employing the innovator product available on the market when studying multisource products for national and regional approval. There will be situations, however, where this is not feasible. Detailed guidance for the selection of comparator products for use in national and regional applications is provided in the comparator guidance (*8*).

It is recommended that potency and in vitro dissolution characteristics of the multisource and the comparator pharmaceutical products be ascertained prior to the performance of an equivalence study. Content of the API(s) of the comparator product should be close to the label claim and the difference between two products being compared should not be more than ± 5%. If, because of the lack of availability of different batches of the comparator product, it is not possible to study batches with potencies within ± 5%, potency correction may be required on the statistical results from the bioequivalence study.

7.4 Study conduct

7.4.1 Selection of strength

In bioequivalence studies the molar equivalent dose of multisource and comparator product must be used. For a series of strengths that can be considered proportionally formulated (see section 10.3) the strength with the greatest sensitivity for bioequivalence assessment should be administered as a single unit. This will usually be the highest marketed strength. A higher dose, i.e. more than one dosage unit, may be employed when analytical difficulties exist. In this case, the total single dose should not exceed the maximal daily dose of the dosage regimen. In certain cases a study performed with a lower strength can be considered acceptable if this lower strength is chosen for reasons of safety or if the API is highly soluble and its pharmacokinetics are linear over the therapeutic range.

7.4.1.1 Non-linear pharmacokinetics

When the API in a series of strengths, which are considered proportionally formulated, exhibits non-linear pharmacokinetics over the range of strengths, special consideration is necessary when selecting the strength for study.

For APIs exhibiting non-linear pharmacokinetics within the range of strengths resulting in greater than proportional increases in AUC with increasing dose, the comparative bioavailability study should be conducted on at least the highest marketed strength.

For APIs with non-linear pharmacokinetics within the range of strengths due to saturable absorption and resulting in less than proportional increases in AUC with increasing dose, the bioequivalence study should be conducted on at least the lowest strength (or a strength in the linear range).

For APIs with non-linear pharmacokinetics within the range of strengths due to limited solubility of the API and resulting in less than proportional increases in AUC with increasing dose, bioequivalence studies should be conducted on at least the lowest strength (or a strength in the linear range) and the highest strength.

7.4.2 Study standardization

Standardization of study conditions is important to minimize variability other than in the pharmaceutical products. Standardization between study periods is critical to a successful study. Standardization should cover exercise, diet, fluid intake and posture, as well as the restriction of the intake of alcohol, caffeine, certain fruit juices and concomitant medicines for a specified period before and during the study.

Volunteers should not take any other medicine, alcoholic beverages or over-the-counter medicines and supplements for an appropriate interval before,

or during, the study. In the event of emergency the use of any non-study medicine must be reported (dose and time of administration).

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

7.4.3 Co-administration of food and fluid with the dose

FPPs are usually given after an overnight fast of at least 10 hours and participants are allowed free access to water. On the morning of the study no water is allowed during the hour prior to FPP administration. The dose should be taken with a standard volume of water (usually 150–250 mL). Two hours after FPP administration, water is again permitted as often as desired. A standard meal is usually provided four hours after FPP administration. All meals should be standardized and the composition stated in the study protocol and report.

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

7.4.3.1 Immediate-release formulations

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

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 is only taken in a fasted or fed state.

Typically a meal meeting the composition recommendations identified in section 7.4.3.2 should be employed in fed state studies. The exact composition of the meal may depend on local diet and customs as determined by the NRA. For studies conducted with immediate-release products there may be situations where it is appropriate to employ a pre-dose meal with a different caloric/fat content from a meal meeting the composition recommendations identified in section 7.4.3.2.

The test meal should be consumed beginning 30 minutes prior to administration of the FPP.

7.4.3.2 Modified-release formulations

In addition to a study conducted under fasted conditions, food effect studies are necessary for all multisource, modified-release formulations to ensure that the interaction between the varying conditions in the GI tract and the product

formulations does not differentially impact the performance of the multisource and comparator products. The presence of food can affect product performance both by influencing the release of the API from the formulation and by causing physiological changes in the GI tract. A significant concern with regard to modified-release products is the possibility that food may trigger a sudden and abrupt release of the API leading to "dose dumping". In these cases the objective is to select a meal that will challenge the robustness of the new multisource formulation to prandial effects on bioavailability. To achieve this, a meal that will provide a maximal perturbation to the GI tract relative to the fasted state should be employed, e.g. a high-fat (approximately 50% of the total caloric content of the meal), high-calorie (approximately 800 to 1000 kilocalories) test meal has been recommended (*2*). The meal selected should take into account local customs and diet. The caloric breakdown of the test meal should be provided in the study report.

The subject should start eating the meal 30 minutes before the FPP is administered and complete eating the meal prior to FPP administration.

7.4.4 Wash-out interval

The interval (wash-out period) between doses of each formulation should be long enough to permit the elimination of essentially all of the previous dose from the body. The wash-out period should be the same for all subjects and should normally be more than five times the median terminal half-life of the API. Consideration should be given to extending this period in some situations, e.g. if active metabolites with longer half-lives are produced or if the elimination rate of the API has high variability between subjects. In this second case a longer wash-out period should be considered to allow for the slower elimination in subjects with lower elimination rates. Just prior to administration of the treatment during the second study period, blood samples should be collected and assayed to determine the concentration of the API or metabolites. The minimum wash-out period should be at least seven days unless a shorter period is justified by a short half-life. The adequacy of the wash-out period can be estimated from the pre-dose concentrations of the API in the second study period and should be less than 5% of the observed C_{max}.

7.4.5 Sampling times

Blood samples should be taken at a frequency sufficient for assessing C_{max}, AUC and other parameters. Sampling points should include a pre-dose sample, at least 1–2 points before C_{max}, 2 points around C_{max} and 3–4 points during the elimination phase. Consequently at least seven sampling points will be necessary for estimation of the required pharmacokinetic parameters. For most APIs the number of samples necessary will be higher to compensate for between-

subject differences in absorption and elimination rate and thus enable accurate determination of the maximum concentration of the API in the blood (C_{max}) and terminal elimination rate constant in all subjects. Generally, sampling should continue for long enough to ensure that 80% of the $AUC_{0-\infty}$ can be accrued but it is not necessary to sample for more than 72 hours. The exact duration of sample collection depends on the nature of the API and the input function from the administered dosage form.

7.4.6 Sample fluids and their collection

Under normal circumstances blood should be the biological fluid sampled to measure the concentrations of the API. In most cases the API or its metabolites are measured in serum or plasma. If it is not possible to measure the API in blood, plasma or serum, the API is excreted unchanged in the urine and there is a proportional relationship between plasma and urine concentrations; urine can be sampled for the purpose of estimating exposure. The volume of each urine sample must be measured at the study centre, where possible immediately after collection, and the measurements included in the report. The number of samples should be sufficient to allow the estimation of pharmacokinetic parameters. However, in most cases the exclusive use of urine excretion data should be avoided as this does not allow estimation of the tmax and the maximum concentration. Blood, plasma, serum and urine samples should be processed and stored under conditions that have been shown not to cause degradation of the analytes. Details of these conditions should be included in the analytical validation report (see section 7.5).

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

7.4.7 Parameters to be assessed

In bioavailability studies, the shape and area under the plasma concentration versus time curves are mostly used to assess rate (C_{max}, t_{max}) and extent (AUC) of exposure. Sampling points or periods should be chosen such that the concentration versus time profile is sufficiently defined to allow calculation of relevant parameters. For single-dose studies, the following parameters should be measured or calculated:

- area under the plasma, serum or blood concentration–time curve from time zero to time *t* (AUC_{0-t}), where t is the last sampling time-point with a measurable concentration of the API in the individual formulation tested. The method of calculating AUC values should be specified. Non-compartmental methods should be used for pharmacokinetic calculations in bioequivalence studies;

– C_{max} is the maximum or peak concentration observed representing peak exposure of API (or metabolite) in plasma, serum or whole blood.

Usually AUC_{0-t} and C_{max} are considered to be the most relevant parameters for assessment of bioequivalence. In addition it is recommended that the following parameters be estimated:

- area under the plasma, serum or blood concentration–time curve from time zero to time infinity ($AUC_{0-\infty}$) representing total exposure, where $AUC_{0-\infty} = AUC_{0-t} + C_{last}/K_e$; C_{last} is the last measurable analyte concentration and Ke is the terminal or elimination rate constant calculated according to an appropriate method;
- t_{max} is the time after administration of the FPP at which C_{max} is observed.

For additional information the elimination parameters can be calculated:

- $t_{1/2}$ is the plasma (serum, whole blood) half-life.

For multiple-dose studies conducted with modified-release products, the following parameters should be calculated:

- AUC_τ is AUC over one dosing interval (τ) at steady state;
- C_{max};
- C_{min} (C_{tau}) is concentration at the end of a dosing interval;
- peak trough fluctuation is percentage difference between C_{max} and C_{min}.

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

When urine samples are used, cumulative urinary recovery (A_e) and maximum urinary excretion rate are employed instead of AUC and C_{max}.

7.4.8 Studies of metabolites

Generally evaluation of bioequivalence will be based on the measured concentrations of the API released from the dosage form rather than the metabolite. The concentration–time profile of the API is more sensitive to changes in formulation performance than a metabolite which is more reflective of metabolite formation, distribution and elimination.

In rare cases it may be necessary to measure concentrations of a primary active metabolite rather than those of the API if concentrations of the API are too low to allow reliable analytical measurement in blood, plasma or serum for an adequate length of time, or when the parent compound is unstable in the biological matrix.

It is important to decide beforehand and state in the study protocol, which chemical entities (API or metabolite) will be analysed in the samples and to identify the analyte whose data will be used to assess bioequivalence.

It is also important to note that measurement of one analyte, API or metabolite carries the risk of making a type 1 error (the consumer's risk) to remain at the 5% level. However, if more than one of several analytes is selected retrospectively as the bioequivalence determinant, then both the consumer and producer risks change (*9*). The analyte whose data will be used to assess bioequivalence cannot be changed retrospectively.

When measuring active metabolites, wash-out period and sampling times may need to be adjusted to enable adequate characterization of the pharmacokinetic profile of the metabolite.

7.4.9 Measurement of individual enantiomers

A non-stereoselective assay is acceptable for most bioequivalence studies. A stereospecific assay measuring the individual enantiomers should be employed when the enantiomers exhibit different pharmacokinetic properties, different pharmacodynamic properties and the exposure of the enantiomers, as estimated by their AUC ratio or C_{max} ratio, changes when there is a change in the rate of absorption.

7.5 Quantification of active pharmaceutical ingredient

For the measurement of concentrations of the active compound and/or metabolites in biological matrices, such as serum, plasma, blood and urine, the applied bioanalytical method should be well characterized, fully validated and documented to a satisfactory standard in order to yield reliable results.

The validation of bioanalytical methods and the analysis of subject samples for clinical trials in humans should be performed following the principles of good clinical practice (GCP), good laboratory practice (GLP) and the most up-to-date guidelines from stringent regulatory authorities (SRAs) on the topic of bioanalytical method validation.

State-of-the-art principles and procedures for bioanalytical method validation and analysis of study samples should be employed. The main characteristics of a bioanalytical method that are essential to ensure the acceptability of the performance and the reliability of analytical results are:

- selectivity;

- lower limit of quantification;
- the response function and calibration range (calibration curve performance);
- accuracy;
- precision;
- matrix effects;
- stability of the analyte(s) in the biological matrix;
- stability of the analyte(s) and of the internal standard in the stock and working solutions, and in extracts throughout the entire period of storage and processing conditions.

In general:

- the analytical method should be able to differentiate the analyte(s) of interest and, if employed, the internal standard from endogenous components in the matrix or other components in the sample;
- the lower limit of quantification (LLOQ), being the lowest concentration of analyte in a sample, should be estimated to prove that the analyte at this concentration can be quantified reliably, with an acceptable accuracy and precision;
- the response of the instrument with regard to the concentration of analyte should be known and should be evaluated over a specified concentration range. The calibration curve should be prepared in the same matrix as the matrix of the intended subject samples by spiking the blank matrix with known concentrations of the analyte. A calibration curve should consist of a blank sample, a zero sample and 6–8 non-zero samples covering the expected range;
- within-run and between-run accuracy and precision should be assessed on samples spiked with known amounts of the analyte, the QC samples, at a minimum of three different concentrations;
- matrix effects should be investigated when using mass spectrometric methods;
- stability of the analyte in the stock solution and in the matrix should be proven covering every step taken during sample preparation and sample analysis, as well as the storage conditions used;
- when more than one analyte is present in subject samples, it is recommended to demonstrate the stability of the analytes in the matrix in the presence of the other analytes under standard conditions such as freeze–thaw testing, short-term room temperature storage and long-term freezer storage;

- where changes are made to an analytical method that has already been validated, a full validation may not be necessary depending on the nature of the changes implemented. A partial validation may be acceptable;
- a cross-validation is needed in cases where data are obtained from different methods within and across studies or when data are obtained within a study from different laboratories applying the same method;
- analysis of subject samples should be carried out after validation of the analytical method. Before the start of the analysis of the subject samples, the performance of the bioanalytical method should have been verified;
- calibration and QC standards should be processed in an identical manner and at the same time as the subjects' samples from the same run;
- reasons for reanalysis, reinjection and reintegration of subject samples should be predefined in the protocol, study plan or SOP. Reinjection of a full analytical run or of individual calibration standard samples or QC samples, simply because the calibration or QCs failed, without any identified analytical cause, is considered unacceptable. For bioequivalence studies, reanalysis, reinjection or reintegration of subject samples for reasons related to pharmacokinetic fit is normally not acceptable as this may affect and bias the outcome of such a study;
- when analysing subject samples, the precision and accuracy of the method should be confirmed by reanalysing subject samples in a separate analytical run on a different day (incurred samples reanalysis (ISR)). ISR should be performed for each bioequivalence trial. The extent of testing done should be based on an in-depth understanding of the analytical method and analyte used;
- the samples from one subject (all periods) should be analysed in the same analytical run if possible.

Validation procedures, methodology and acceptance criteria should be specified in the analytical protocol and/or the SOP. All experiments used to support claims or draw conclusions about the validity of the method should be described in a report (method validation report).

The results of subject sample determination should be given in the analytical report together with calibration and QC sample results, repeat analyses, reinjections and reintegrations (if any) and a representative number of sample chromatograms.

7.6 Statistical analysis

The primary concern in bioequivalence assessment is to limit the risk of a false declaration of equivalence. Statistical analysis of the bioequivalence trial should demonstrate that a clinically significant difference in bioavailability between the multisource product and the comparator product is unlikely. The statistical procedures should be specified in the protocol before the data collection starts.

The statistical method for testing bioequivalence is based on the determination of the 90% confidence interval around the ratio of the log-transformed population means (multisource/comparator) for the pharmacokinetic parameters under consideration and by carrying out two one-sided tests at the 5% level of significance (*10*). To establish bioequivalence, the calculated confidence interval should fall within a preset bioequivalence limit. The procedures should lead to a decision scheme which is symmetrical with respect to the formulations being compared (i.e. leading to the same decision whether the multisource formulation is compared to the comparator product or the comparator product to the multisource formulation).

All concentration-dependent pharmacokinetic parameters (e.g. AUC and C_{max}) should be log-transformed using either common logarithms to the base 10 or natural logarithms. The choice of either common or natural logs should be consistent and should be stated in the study report.

Logarithmically transformed, concentration-dependent pharmacokinetic parameters should be analysed using analysis of variance (ANOVA). Normally the ANOVA model should include formulation, period, sequence and subject factors. Parametric methods, i.e. those based on normal distribution theory, are recommended for the analysis of log-transformed bioequivalence measures.

The general approach is to construct a 90% confidence interval for the quantity $\mu T - \mu R$ and to reach a conclusion of pharmacokinetic equivalence if this confidence interval is within the stated limits. The nature of parametric confidence intervals means that this is equivalent to carrying out two one-sided tests of the hypothesis at the 5% level of significance (*10*, *11*). The antilogs of the confidence limits obtained constitute the 90% confidence interval for the ratio of the geometric means between the multisource and comparator products. The same procedure should be used for analysing parameters from steady-state trials or cumulative urinary recovery if required.

For t_{max} descriptive statistics should be given. Where t_{max} is considered clinically relevant, median and range of tmax should be compared between test and comparator to exclude numerical differences with clinical importance. A formal statistical comparison is rarely necessary. Generally the sample size is not calculated to have enough statistical power for t_{max}. However, if t_{max} is to be subjected to a statistical analysis, this should be based on non-parametric methods and should be applied to untransformed data. A sufficient number

of samples around predicted maximal concentrations should have been taken to improve the accuracy of the t_{max} estimate. For parameters describing the elimination phase ($t_{1/2}$) only descriptive statistics should be given. See section 7.2.3 for information on the handling of extreme data.

Exclusion of data for statistical or pharmacokinetic reasons alone is not acceptable.

7.6.1 Two-stage sequential design

In some situations reliable information concerning the expected variability in the parameters to be estimated may not be available. In such situations a two-stage sequential study design can be employed such that an accurate estimate of the variability can be determined in the first stage of the study. The number of subjects employed in the first stage is generally based on the most likely intra- subject variance estimate with some added subjects to compensate for drop-outs. The analysis undertaken at the end of the first stage is treated as an interim analysis. If bioequivalence is proven at this point the study can be terminated. If bioequivalence is not proven at the end of the first stage, the second stage is conducted employing an appropriate number of additional subjects as determined based on the variance estimates and point estimate calculated from the stage 1 data. At the end of the second stage, the results from both groups combined are used in the final analysis. In order to use a two-stage design, adjustments must be made to protect the overall Type 1 error rate and maintain it at 5%. To do this, both the interim and final analyses must be conducted at adjusted levels of significance with the confidence intervals calculated using the adjusted values.

It is recommended that the same alpha for both stages be employed. This gives an alpha of 0.0294 for this case (*12*), however, the amount of alpha to be spent at the time of the interim analysis can be set at the study designer's discretion. For example, the first stage may be planned as an analysis where no alpha is spent in the interim analysis since the objective of the interim analysis is to obtain information on the point estimate difference and variability and where all the alpha is spent in the final analysis with the conventional 90% confidence interval. In this case no test against the acceptance criteria is made during the interim analysis and bioequivalence cannot be proven at that point. The proposed statistical plan must be clearly defined in the study protocol, including the adjusted significance level that is to be employed during each analysis.

A factor for stage should be included in the ANOVA model for the final analysis of the combined data from the two stages.

This approach can be employed in both cross-over and parallel study designs.

7.7 Acceptance ranges

AUC_{0-t} - ratio

The 90% confidence interval for this measure of relative bioavailability should lie within a bioequivalence range of 80.00–125.00%. If the API is determined to possess a narrow therapeutic index (NTI) the bioequivalence acceptance range should be restricted 90.00–111.11%.

The same criterion applies to the parameter AUCτ in multiple-dose studies and for partial AUCs if they are necessary for comparative testing of a modified-release product.

C_{max} - ratio

For maximal concentration data, the acceptance limit of 80.00–125.00% should be applied to the 90% confidence interval for the mean C_{max} ratio. However, this measure of relative bioavailability is inherently more variable than, for example, the AUC ratio, and in certain cases this variability can make proving bioequivalence challenging. See section 7.9.3 for information on an approach for proving bioequivalence when the intra-subject variability for the C_{max} parameter is high. If the API is determined to possess a narrow therapeutic index, the bioequivalence acceptance range may need to be restricted to 90.00–111.11%, if appropriate. The same criterion applies to the parameters C_{max} and C_{tau} in multiple-dose studies.

t_{max} - difference

Statistical evaluation of tmax makes sense only if there is a clinically relevant claim for rapid onset of action or concerns about adverse effects. In such a case, comparison of the median and range data for each product should be undertaken. For other pharmacokinetic parameters the same considerations as outlined above apply.

7.8 Reporting of results

The report of a bioequivalence study should give the complete documentation of its protocol, conduct and evaluation in compliance with GCP and GLP rules. The relevant International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guideline (*13*) can be used in the preparation of the study report. The responsible investigator(s) should sign the respective sections of the report. Names and affiliations of the responsible investigator(s), site of the study and period of its execution should be stated.

The names and batch numbers of the pharmaceutical products used in the study as well as the composition(s) of the tests product(s) should be given. Results of in vitro dissolution tests conducted in media with pHs of 1.2, 4.5

and 6.8 and the QC media, if different, should be provided. In addition, the applicant should submit a signed statement confirming that the test product is identical to the pharmaceutical product that is submitted for registration.

The bioanalytical validation report should be attached. This report should include the information recommended in the SRA guidance chosen as a guide for the bioanalytical portion of a study (see section 7.5).

All results should be presented clearly. All concentrations measured in each subject and the sampling time should be tabulated for each formulation. Tabulated results showing API concentration analyses according to analytical run (including runs excluded from further calculations, together with all calibration standards and QC samples from the respective run) should also be presented. The tabulated results should present the date of run, subject, study period, product administered (multisource or comparator) and time elapsed between FPP administration and blood sampling, in a clear format. The procedure for calculating the parameters used (e.g. AUC) from the raw data should be stated. Any deletion of data should be documented and justified.

Individual blood concentration/time curves should be plotted on a linear/linear and log/linear scale. All individual data and results should be given, including information on subjects who dropped out. The drop-outs and/or withdrawn subjects should be reported and accounted for. All adverse events that occurred during the study should be reported together with the study physician's classification of the events. Further, any treatments given to address adverse events should be reported.

Results of all measured and calculated pharmacokinetic parameters should be tabulated for each subject–formulation combination together with descriptive statistics. The statistical report should be sufficiently detailed to enable the statistical analyses to be repeated if necessary. If the statistical methods applied deviate from those specified in the study protocol the reasons for the deviations should be stated.

7.9 Special considerations

7.9.1 Fixed-dose combination products

If the bioequivalence of FDC products is assessed by in vivo studies, the study design should follow the same general principles as described in previous sections. The multisource FDC product should be compared with the pharmaceutically equivalent comparator FDC product. In certain cases (e.g. when no comparator FDC product is available on the market) separate products administered in free combination can be used as a comparator (*3*). Sampling times should be chosen to enable the pharmacokinetic parameters of all APIs to be adequately assessed. The bioanalytical method should be validated with respect to all analytes measured in the presence of the other analytes. Statistical analyses should be

performed with pharmacokinetic data collected on all active ingredients; the 90% confidence intervals of test/comparator ratio of all active ingredients should be within acceptance limits.

7.9.2 Clinically important variations in bioavailability

Innovators should make every effort to provide formulations with good bioavailability characteristics. If a better formulation is later developed by the innovator, this should then serve as the comparator product. A new formulation with a bioavailability outside the acceptance range for an existing pharmaceutical product is not interchangeable by definition.

7.9.3 "Highly variable active pharmaceutical ingredients"

A "highly variable API" has been defined as an API with an intrasubject variability of > 30% in terms of the ANOVA CV (*14*). Proving the bioequivalence of FPPs containing highly variable APIs can be problematic because the higher the ANOVA CV, the wider the 90% confidence interval. Thus large numbers of subjects must be enrolled in studies involving highly variable APIs to achieve adequate statistical power.

Although there is variability in how regulatory authorities deal with the issue of highly variable APIs, the most rigorous of the current approaches involve the scaling of bioequivalence acceptance criteria based on the intrasubject standard deviation observed in the relevant parameters for the comparator product (*15–17*). Of the two most common assessment parameters C_{max} is subject to the highest variability and hence is the parameter for which a modified approach is most needed.

For highly variable FPPs it is recommended that a three-way partial replicate (where the comparator product is administered twice) or a four-way fully replicated cross-over bioequivalence study be conducted and reference-scaled average bioequivalence be employed to widen the acceptance interval for the Cmax parameter, if the intrasubject variability for C_{max} following replicate administrations of the comparator product is > 30%. If this is the case the acceptance criteria for C_{max} can be widened to a maximum of 69.84–143.19%. The applicant should justify that the calculated intrasubject variability is a reliable estimate and that it is not the result of outliers.

The extent of the widening of the acceptance interval for C_{max} is defined based upon the intrasubject variability seen in the bioequivalence study using scaled average bioequivalence according to [U, L] = exp [± k·sWR], 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 sWR is the intrasubject standard deviation of the log-transformed values of C_{max} of the reference product. Table A6.2 gives examples of how different levels of variability lead to different acceptance limits using this methodology.

Table A6.2
Acceptance limits for different levels of variability

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

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

The geometric mean ratio (GMR) for C_{max} should lie within the conventional acceptance range of 80.00–125.00%.

The standard bioequivalence acceptance criterion for AUC should be maintained without scaling. If the intrasubject variability for C_{max}, following replicate administration of the comparator, is found to be < 30%, standard bioequivalence acceptance criteria should be applied to both AUC and C_{max} without scaling.

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

The approach to be employed should be clearly defined prospectively in the study protocol. The regulatory authority of the country to which the study data will be submitted should be consulted before commencing the study to confirm that the proposed approach is acceptable for that jurisdiction.

8. Pharmacodynamic equivalence studies

Studies in healthy volunteers or patients using pharmacodynamic measurements may be used for establishing equivalence between two pharmaceutical products when the pharmacokinetic approach is not feasible. Pharmacodynamic equivalence studies may become necessary if quantitative analysis of the API and/or metabolite(s) in blood, serum, plasma or urine cannot be made with sufficient accuracy and sensitivity; however, this is extremely unlikely given current technology. Furthermore, pharmacodynamic equivalence studies in humans are required if measurements of API concentrations cannot be used as

surrogate end-points for the demonstration of efficacy and safety of the particular pharmaceutical product as is the case with pharmaceutical products designed to act locally. However, local availability studies based on pharmacokinetic studies alone or in combination with in vitro dissolution studies are being considered as surrogate end-points for the demonstration of equivalent biopharmaceutical quality and release at the site of action for some products acting locally. In addition, bioequivalence studies are also required in order to demonstrate equivalent systemic exposure for systemic safety purposes.

Pharmacodynamic studies are not recommended for orally administered pharmaceutical products for systemic action when the API is absorbed into the systemic circulation and a pharmacokinetic approach can be used to assess systemic exposure and establish bioequivalence. This is because the sensitivity to detect differences between products in their biopharmaceutical quality, release and absorption is lower with pharmacodynamic or clinical end-points. As the dose–response curve for pharmacodynamics or clinical end-points is usually flatter than the relationship between dose and pharmacokinetic parameters, it is essential to ensure the internal validity of the study by showing assay sensitivity, i.e. the ability to distinguish the response obtained by adjacent doses (twofold or even fourfold difference in dose). It is essential to perform the comparison at the dose level at which the dose-response is steepest, which may require firstly doing a pilot study for its identification. Furthermore, variability in pharmacodynamic measures is usually greater than that in pharmacokinetic measures. In addition, pharmacodynamic measures are often subject to significant placebo effects, which add to the variability and complicate experimental design. The result is often that huge numbers of patients would have to be enrolled in pharmacodynamic studies to achieve adequate statistical power.

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

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

- The response measured should be a pharmacological or therapeutic effect which is relevant to the claims of efficacy and/or safety.
- The methodology must be validated for precision, accuracy, reproducibility and specificity.
- Neither the multisource product nor the comparator product should produce a maximal response during the course of the study since it may be impossible to detect differences between formulations given in doses which give maximum or near maximum effects.Investigation of dose–response relationships may be a necessary part of the design.

- The response should be measured quantitatively, preferably under double blind conditions, and be recordable by an instrument that produces and records the results of repeated measurements to provide a record of the pharmacodynamic events, which are substitutes for measurements of plasma concentrations. Where such measurements are not possible, recordings on visual analogue scales may be used. Where the data are limited to qualitative (categorized) measurements, appropriate special statistical analysis will be required.
- Participants should be screened prior to the study to exclude non-responders. The criteria by which responders are distinguished from non-responders must be stated in the protocol.
- In situations where an important placebo effect can occur, comparison between pharmaceutical products can only be made by *a priori* consideration of the potential placebo effect in the study design. This may be achieved by adding a third phase with placebo treatment during the design of the study.
- The underlying pathology and natural history of the condition must be considered in the study design. There should be confirmation that the baseline conditions are reproducible.
- A cross-over design can be used. Where this is not appropriate, a parallel-group study design should be chosen.

The basis for the selection of the multisource and comparator products should be the same as described in section 7.3.

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

The comparison between the multisource and the comparator product can be performed in two different ways:

(a) *dose-scale analysis or relative potency*: this is defined as the ratio of the potency of the multisource product to that of the comparator product. It is a way of summarizing the relationship between the dose–response curves of the multisource and comparator product;

(b) *response-scale analysis*: this consists of demonstration of equivalence (for at least two dose levels) at the pharmacodynamic end-point.

For either approach to be acceptable a minimum requirement is that the study has assay sensitivity. To meet this requirement, at least two non-zero levels need to be studied and one dose level needs to be shown to be superior to the other.

Therefore, it is recommended that unless otherwise justified more than one dose of both the multisource and comparator products are studied. However, it is essential that doses on the steep part of the dose–response curve are studied. If the chosen dose is too low on the dose–response curve, then demonstrating equivalence between two products is not convincing, as this dose could be subtherapeutic. Equally if a dose at the top of the dose–response curve is included, similar effects will be seen for doses much higher than that studied and hence demonstrating equivalence at this dose level would also not be convincing.

The results using both approaches should be provided. In both cases the observed confidence intervals comparing multisource and comparator products should lie within the chosen equivalence margins to provide convincing evidence of equivalence. As for bioequivalence studies, 90% confidence intervals should be calculated for relative potency whereas 95% confidence intervals should be calculated for the response-scale analysis. It should be noted that the acceptance range as applied for bioequivalence assessment may not be appropriate. For both approaches the chosen equivalence ranges should be prespecified and appropriately justified in the protocol.

9. Clinical equivalence studies

In some instances (see example (e) in section 5.1, In vivo studies) plasma concentration time–profile data may not be suitable for assessing equivalence between two formulations. Although in some cases pharmacodynamic equivalence studies can be an appropriate tool for establishing equivalence, in others this type of study cannot be performed because of a lack of meaningful pharmacodynamic parameters that can be measured; a comparative clinical trial then has to be performed to demonstrate equivalence between two formulations. However, it is preferable to assess equivalence by performing a pharmacokinetic equivalence study rather than a clinical trial that is less sensitive and would require a huge number of subjects to achieve adequate statistical power. For example, it has been calculated that 8600 patients would be required to give adequate statistical power to detect a 20% improvement in response to the study API compared with placebo (*18*, *19*). Similarly it was calculated that 2600 myocardial infarct patients would be required to show a 16% reduction in risk. A comparison of two formulations of the same API based on such end-points would require even greater numbers of subjects (*19*).

If a clinical equivalence study is considered as being undertaken to prove equivalence, the same statistical principles apply as for the bioequivalence studies, although a 95% confidence interval might be necessary for pharmacodynamic and clinical end-points in contrast to the 90% confidence level employed conventionally for pharmacokinetic studies. The number of patients to be included in the study will depend on the variability of the target parameters and

the acceptance range and is usually much higher than the number of subjects needed in bioequivalence studies.

The methodology for establishing equivalence between pharmaceutical products by means of a clinical trial with a therapeutic end-point conducted in patients is not yet as far advanced as that for bioequivalence studies. However, some important items that need to be defined in the protocol can be identified as follows:

- the target parameters that usually represent relevant clinical end-points from which the onset, if applicable and relevant, and intensity of the response are to be derived;
- the size of the acceptance range has to be defined case by case, taking into consideration the specific clinical conditions. These include, among others, the natural course of the disease, the efficacy of available treatments and the chosen target parameter. In contrast to bioequivalence studies (where a conventional acceptance range is applied) the size of the acceptance range in clinical trials should be set individually according to the therapeutic class and indication(s);
- the currently used statistical method is the confidence interval approach;
- the confidence intervals can be derived from either parametric or non-parametric methods;
- where appropriate a placebo arm should be included in the design;
- in some cases it is relevant to include safety end-points in the final comparative assessments.

The selection basis for the multisource and comparator products should be the same as described in section 7.3.

10. In vitro equivalence testing

Over the past three decades dissolution testing has evolved into a powerful tool for characterizing the quality of oral pharmaceutical products. The dissolution test, at first exclusively a QC test, is now emerging as a surrogate equivalence test for certain categories of orally administered, pharmaceutical products. For these products (typically solid oral dosage forms containing APIs with suitable properties) similarity in in vitro dissolution profiles, in addition to excipient comparisons and a risk–benefit analysis, can be used to document equivalence of a multisource product with a comparator product.

It should be noted that although the dissolution tests recommended in *The International Pharmacopoeia* (Ph.Int.) (*20*) for QC have been designed to be compatible with the biowaiver dissolution tests, they do not fulfil all the

requirements for evaluating equivalence of multisource products with comparator products. Dissolution tests for QC purposes, including those described in other pharmacopoeias, do not address all test conditions required for evaluating equivalence of multisource products and should not be applied for this purpose.

10.1 In vitro equivalence testing in the context of the Biopharmaceutics Classification System

10.1.1 Biopharmaceutics Classification System

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

- Class 1: high solubility, high permeability;
- Class 2: low solubility, high permeability;
- Class 3: high solubility, low permeability;
- Class 4: low solubility, low permeability.

Combining the dissolution results and a critical examination of the excipients of the pharmaceutical product with these two properties of the API takes the four major factors that govern the rate and extent of API absorption from immediate release, solid dosage forms into account (*21*). On the basis of their dissolution properties, immediate-release dosage forms can be categorized as having “very rapid”, “rapid”, or “not rapid” dissolution characteristics.

On the basis of solubility and permeability of the API, excipient nature, excipient content and dissolution characteristics of the dosage form, the BCS approach provides an opportunity to waive in vivo bioequivalence testing for certain categories of immediate release FPPs. Oral FPPs containing an API possessing a narrow therapeutic index are not eligible for a so-called biowaiver based on the BCS approach.

10.1.1.1 High solubility

An API is considered highly soluble when the highest single therapeutic dose as determined by the relevant regulatory authority, typically defined by the labelling for the innovator product, is soluble in 250 mL or less of aqueous media over the pH range of 1.2–6.8. The pH solubility profile of the API should be determined at 37 ± 1 °C in aqueous media. A minimum of three replicate determinations of solubility at each pH condition is recommended.

10.1.1.2 High permeability

An API is considered highly permeable when the extent of absorption in humans is 85% or more based on a mass balance determination or in comparison with an intravenous comparator dose. Ideally the mass balance study or comparison

with an intravenous comparator dose would be conducted at the same dose as that used for the solubility classification. If this is not possible, dose linearity of pharmacokinetics should be used to justify the use of other doses.

Absolute bioavailability or mass balance study data obtained from published literature may be accepted as evidence if it can be clearly established that the data were derived from appropriately designed studies.

In vivo intestinal perfusion in humans is an acceptable alternative test method.

When this method is used for permeation studies, suitability of the methodology should be demonstrated, including determination of permeability relative to that of a reference compound whose fraction of dose absorbed has been documented to be at least 85%, as well as use of a negative control.

Supportive data can be provided by the following additional test methods:

(i) in vivo or in situ intestinal perfusion using animal models;

(ii) in vitro permeation across a monolayer of cultured epithelial cells (e.g. Caco 2) using a method validated using APIs with known permeabilities, although data from neither method (i) nor (ii) would be considered acceptable on a stand-alone basis.

In these experiments, high permeability is assessed with respect to the high permeability of a series of reference compounds with documented permeabilities and values of the absorbed fraction, including some for which fraction of dose absorbed is at least 85% (*22*).

10.1.2 Determination of dissolution characteristics of multisource products in consideration of a biowaiver based on the Biopharmaceutics Classification System

For exemption from an in vivo bioequivalence study, an immediate release, multisource product should exhibit very rapid or rapid in vitro dissolution characteristics (see sections 10.1.2.1 and 10.1.2.2), depending on the BCS properties of the API. In vitro data should also demonstrate the similarity of dissolution profiles between the multisource and comparator products.

10.1.2.1 Very rapidly dissolving

A multisource product is considered to be very rapidly dissolving when no less than 85% of the labelled amount of the API dissolves in 15 minutes at 37 ± 1 °C using a paddle apparatus at 75 rpm or a basket apparatus at 100 rpm in a volume of 900 mL or less in each of the following media:

– pH 1.2 HCl solution or buffer;

- a pH 4.5 acetate buffer;
- a pH 6.8 phosphate buffer.

Pharmacopoeial buffers (e.g. Ph.Int.) are recommended for use at these three pH values. Surfactants should not be used in the dissolution media. Enzymes (pepsin at pH 1.2 and pancreatin at pH 6.8) may be used if the pharmaceutical product contains gelatin (e.g. capsules or caplets) due to the possibility of cross-linking.

(See also section 10.2, Dissolution profile comparison.)

10.1.2.2 Rapidly dissolving

A multisource product is considered to be rapidly dissolving when no less than 85% of the labelled amount of the API dissolves in 30 minutes at 37 ± 1 °C using a paddle apparatus at 75 rpm or a basket apparatus at 100 rpm in a volume of 900 mL or less in each of the following media:

- pH 1.2 HCl solution or buffer;
- pH 4.5 acetate buffer;
- pH 6.8 phosphate buffer.

Surfactants should not be used in the dissolution media. Enzymes (pepsin at pH 1.2 and pancreatin at pH 6.8) may be used if the pharmaceutical product contains gelatin (e.g. capsules or caplets) due to the possibility of cross-linking.

10.2 Qualification for a biowaiver based on the Biopharmaceutics Classification System

A biowaiver based on the BCS considers:

(a) the solubility and intestinal permeability of the API (see section 10.1);
(b) the similarity of the dissolution profiles of the multisource and comparator products in pH 1.2, 4.5 and 6.8 media (see below);
(c) the excipients used in the formulation (see below);
(d) the risks of an incorrect biowaiver decision in terms of the therapeutic index of and clinical indications for the API (see section 5.1 for cases where an in vivo study would be required to demonstrate bioequivalence).

Only when there is an acceptable risk–benefit balance in terms of public health and risk to the individual patient should bioequivalence testing be waived and the in vitro methods described in this section applied as a test of product equivalence.

Risk reduction and assessment of excipients

The risk of reaching an incorrect decision that the multisource product is equivalent to the comparator product can be reduced by correct classification of the API and by following the recommendations for dissolution testing and comparison of the dissolution profiles. In all cases it should be further demonstrated that the excipients included in the formulation of the multisource product are well established for use in products containing that API and that the excipients used will not lead to differences between the comparator and multisource product with respect to processes affecting absorption (e.g. by effects on GI motility or interactions with transport processes) or which might lead to interactions that alter the pharmacokinetics of the API.

In all cases, well established excipients in usual amounts should be used in multisource products. Excipients that might affect the bioavailability of the API, e.g. mannitol, sorbitol or surfactants, should be identified and an assessment of their impact provided. These critical excipients should not differ qualitatively and must be quantitatively similar between the test product and comparator product.

For biowaivers for products containing Class 1 APIs there is some flexibility in the excipients employed, with the exception of critical excipients as discussed above. It is recommended that the excipients employed be present in the comparator product or be present in other products which contain the same API as the multisource product and which have marketing authorizations in ICH associated countries.

For biowaivers for products containing Class 3 APIs all excipients in the proposed product formulation should be qualitatively the same and quantitatively similar to that of the comparator product, as defined by the WHO quality limits on allowable quantitative changes in excipients for a variation (*23*).

As a general rule, the closer the composition of the multisource product to that of the comparator product with regard to excipients, the lower the risk of an inappropriate decision on equivalence using a biowaiver based on the BCS.

Sub- and supra-bioavailable products

A further consideration is the potential risk to public health and to the individual patient, should an inappropriate decision with respect to bioequivalence be reached. Essentially there are two possible negative outcomes.

The first arises when the multisource product is sub bioavailable. In this case substitution of the comparator with the multisource product could lead to reduced therapeutic efficacy. APIs which must reach a certain concentration to be effective (e.g. antibiotics) are most susceptible to problems of sub bioavailability.

The second negative outcome arises when the multisource product is supra bioavailable. In this case substitution of the comparator with the

multisource product could lead to toxicity. APIs which exhibit toxic effects at concentrations close to the therapeutic range are most susceptible to problems of supra bioavailability. For these reasons therapeutic index is an important consideration in determining whether the biowaiver based on BCS can be applied or not.

Dissolution profile comparison

Approval of multisource formulations using comparative in vitro dissolution studies should be based on the generation of comparative dissolution profiles rather than a single point dissolution test. For details refer to Appendix 1.

10.2.1 Dissolution criteria for biowaivers based on the Biopharmaceutics Classification System according to the properties of active pharmaceutical ingredients

The major application of BCS is to provide criteria for biowaiver of multisource products. It is recommended that products containing the following BCS classes of APIs be eligible for a biowaiver:

- BCS Class 1 APIs, if the multisource and comparator product are *very rapidly dissolving or similarly rapidly dissolving*;
- BCS Class 3 APIs, if the multisource and comparator product are *very rapidly dissolving*.

In summary, biowaivers for solid oral dosage forms based on BCS can be considered under the following conditions.

1. Dosage forms of APIs that are highly soluble, highly permeable (BCS Class 1) with acceptable excipient content and favourable risk–benefit analysis and which are rapidly dissolving, are eligible for a biowaiver based on the BCS provided:

   (i) the dosage form is rapidly dissolving (as defined in section 10.1.2.2) and the dissolution profile of the multisource product is similar to that of the comparator product in aqueous buffers at pH 1.2, pH 4.5 and pH 6.8 using the paddle method at 75 rpm or the basket method at 100 rpm and meets the criteria of dissolution profile similarity, $f_2 \geq 50$ (or equivalent statistical criterion);

   (ii) if both the comparator and the multisource dosage forms are very rapidly dissolving (as defined in section 10.1.2.1) the two products are deemed equivalent and a profile comparison is not necessary.

2. Dosage forms of APIs that are highly soluble and have low permeability (BCS Class 3) are eligible for biowaivers provided all the criteria (a–d) listed

in section 10.2 are met and the risk–benefit is additionally addressed in terms of extent, site and mechanism of absorption.

In general, the risks of reaching an inappropriate biowaiver decision need to be more critically evaluated when the extent of absorption is lower (especially if absolute bioavailability < 50%); therefore it is essential that the excipients in the proposed product formulation be scrutinized carefully. In order to minimize the risk of an inappropriate decision, excipients in the proposed product formulation should be qualitatively the same and quantitatively similar to that of the comparator.

If it is deemed that the risk of reaching an inappropriate biowaiver decision and its associated risks to public health and for individual patients is acceptable, the multisource product is eligible for a biowaiver based on BCS when both the comparator and the multisource dosage forms are very rapidly dissolving (85% dissolution in 15 minutes as described in section 10.1.2.1).

10.3 In vitro equivalence testing based on dose-proportionality of formulations

Under certain conditions, approval of different strengths of a multisource product can be considered on the basis of dissolution profiles if the formulations have proportionally similar compositions.

10.3.1 Proportional formulations

For the purpose of this guidance proportional formulations can be defined in two ways, based on the strength of dosage forms.

(i) All active and inactive ingredients are exactly in the same proportions in the different strengths (e.g. a tablet of 50 mg strength has exactly half of all the active and inactive ingredients contained in a tablet of 100 mg strength and twice what would be contained in a tablet of 25 mg strength). For immediate release products, coating components, capsule shell, colour agents and flavours are not generally required to meet this requirement.

(ii) For an FPP, where the amount of the API in the dosage form is relatively low (up to 10 mg per dosage unit or not more than 5% of the weight of the dosage form), the total weight of the dosage form remains similar for all strengths.

For (ii) a waiver is considered:

- if the amounts of the different excipients or capsule contents are the same for the strengths concerned and only the amount of the API has changed;

- if the amount of filler is changed to account for the change in amount of API: the amounts of other core excipients or capsule content should be the same for the strengths concerned.

10.3.2 Qualification for biowaivers based on dose-proportionality of formulations

10.3.2.1 Immediate-release tablets

A biowaiver based on dose proportionality of formulations for a series of strengths of a multisource product, when the pharmaceutical products are manufactured with the same manufacturing process, may be granted when:

(i) an in vivo equivalence study has been performed on at least one of the strengths of the formulation. As described in section 7.4.1, the strength studied will usually be the highest strength, unless a lower strength is chosen for reasons of safety or the API is highly soluble and displays linear pharmacokinetics);

(ii) all strengths are proportionally similar in formulation to that of the strength studied;

(iii) the dissolution profiles for the different strengths are similar at pH 1.2, 4.5, 6.8 and for the QC media, unless justified by the absence of sink conditions. If the different strengths of the test product do not show similar dissolution profiles owing to the absence of sink conditions in any of the above media, this should be substantiated by showing similar dissolution profiles when testing the same dose per vessel (e.g. two tablets of 5 mg versus one tablet of 10 mg) or by showing the same behaviour in the comparator product.

As for the BCS based biowaiver, if both strengths release 85% or more of the label amount of the API in 15 minutes, using all three dissolution media as recommended in section 10.2, the profile comparison with an f_2 test is unnecessary.

In the case where an immediate release dosage form with several strengths deviates from proportionality a bracketing approach is possible, so that only two strengths representing the extremes need to be studied in vivo.

If approval of one strength of a product is based on a BCS based biowaiver instead of an in vivo equivalence study, other strengths in the series of strengths should also be assessed based on BCS based biowaivers as opposed to a biowaiver based on dose-proportionality.

10.3.2.2 Delayed-release tablets and capsules

For delayed release tablets, for a series of strengths of a multisource product where the strengths are proportionally similar in formulation to that of the

strength studied in an in vivo equivalence study, a lower strength can be granted a biowaiver if it exhibits similar dissolution profiles, $f_2 \geq 50$, in the recommended test condition for delayed release product, e.g. dissolution test in acid medium (pH 1.2) for 2 hours followed by dissolution in pH 6.8. When evaluating proportionality in composition, it is recommended to consider the proportionality of gastro resistant coating with respect to the surface area (not to core weight) to have the same gastro resistance (mg/cm2).

For delayed release capsules where different strengths have been achieved solely by means of adjusting the number of beads containing the API, similarity in the dissolution profile of the new (lower) strength to that of the approved strength ($f_2 > 50$) under the test conditions recommended for delayed release products (see above) is sufficient for a biowaiver.

10.3.2.3 Extended-release tablets and capsules

(a) For extended-release tablets, when there is a series of strengths of a multisource product that are proportionally similar in their active and inactive ingredients and have the same API release mechanism, in vivo bioequivalence studies should be conducted with the highest proposed strength. Subsequently, lower strengths in the series can be granted a biowaiver if they exhibit similar dissolution profiles to the highest strength, $f_2 \geq 50$, in three different pH buffers (between pH 1.2 and 7.5) and the QC media by the recommended test method.

(b) For extended-release tablets with an osmotic pump release mechanism, the dissolution profile comparison ($f_2 \geq 50$) under one recommended test condition is sufficient for a biowaiver based on dose proportionality of formulation.

(c) For extended-release, beaded capsules where different strengths have been achieved solely by means of adjusting the number of beads containing the API, a dissolution profile comparison ($f_2 \geq 50$) under one recommended test condition is sufficient for a biowaiver based on dose proportionality of formulation.

10.3.3 Dissolution profile comparison for biowaivers based on dose-proportionality of formulations

As for biowaivers based on the BCS, a model-independent mathematical approach (e.g. f_2 test) can be used for comparing the dissolution profiles of two products.The dissolution profile of the two products (reference strength and additional strength) should be measured under the same test conditions. The dissolution sampling times for both reference strength and additional strength profiles should be the same. For example:

- for immediate release products 5, 10, 15, 20, 30, 45 and 60 minutes;
- for 12-hour extended-release products 1, 2, 4, 6, 8 and 12 hours;
- for 24-hour extended-release products 1, 2, 4, 6, 8, 16 and 24 hours.

For the application of the f_2 value see Appendix 1.

10.4 In vitro equivalence testing for non-oral dosage forms

In the case of intravenous micellar solutions with the same qualitative and quantitative composition of the surfactant, but significant changes to other excipients, an in vitro comparison might avoid the need for in vivo studies if a similar micellar system and API release from the micelle after dilution of the FPP or API administration into the blood system is ensured (*24*).

Locally applied, locally acting products in the form of aqueous suspensions containing the same API(s) in the same molar concentration and essentially the same excipients in comparable concentrations might be waived from the demonstration of equivalence by means of local availability, pharmacodynamic or clinical studies if in vitro characterization is able to ensure a similar crystallographic structure and particle size distribution as well as any other in vitro test specific for each dosage form, e.g. dissolution. The methodological details for the techniques mentioned below are not covered in these guidelines. Additional information regarding these techniques should be sought from guidelines produced by SRAs or from state-of-the-art literature.

(a) Suspensions for nebulization with the same qualitative and quantitative composition as the comparator product might be waived from in vivo studies if the particles in the suspensions are shown to have the same crystallographic structure and particle size distribution as those from the comparator product, as well as comparability in any other appropriate in vitro test, e.g. dissolution. In addition, the nebulized droplets should exhibit a similar aerodynamic particle size distribution to that of the comparator product.

(b) Suspensions for nebulization with different qualitative and quantitative composition might be granted a waiver if, in addition to the requirements defined above under (a), the difference in excipient composition does not alter the nebulizer efficiency (e.g. by the presence or absence of a different surfactant or preservative) and the aerodynamic particle size distribution (e.g. altering product hygroscopicity by the presence of a different amount of salt as isotonic agent). To this end the appropriate state-of-the-art in vitro test should be conducted to ensure product equivalence.

Any difference in excipients should be critically reviewed because certain excipients that are considered irrelevant in other dosage forms (e.g. preservative, substance to adjust tonicity or thickening agent) may affect safety and/or efficacy of the product.

(c) Nasal drops where the API is in suspension with the same qualitative and quantitative composition as the comparator product might be waived from in vivo studies if the particles in suspension are shown to have the same crystallographic structure and similar particle size distribution to that of the comparator product, as well as comparability in any other appropriate in vitro test, e.g. dissolution.

(d) Nasal drops where the API is in suspension, with qualitative or quantitative differences in excipient composition with respect to the comparator product, might be waived from in vivo studies if, in addition to the requirements defined above under (c), the difference in excipient composition does not affect efficacy and safety (e.g. a different preservative may affect the safety profile due to greater irritation of the nasal passages and a different viscosity or thixotropy may affect the residence time in the site of action). Therefore any difference in excipients should be critically reviewed.

(e) Nasal sprays in solution with the same qualitative and quantitative composition in excipients can be granted waivers based on a battery of in vitro tests as defined by SRAs (*18*, *25*).

(f) Nasal sprays in solution with qualitative and quantitative differences in the excipient composition might be waived if, in addition to showing similarity in the battery of in vitro tests referenced under (e), differences in excipients are critically reviewed as described above under (d).

(g) Nasal sprays in suspension with the same qualitative and quantitative composition in excipients might be waived if, in addition to the battery of in vitro tests referenced above under (e), the particles in suspension are shown to have the same crystallographic structure and similar particle size distribution, as well as comparability in any other appropriate in vitro test, e.g. dissolution.

(h) Nasal sprays in suspension with qualitative and quantitative differences in excipient composition might be waived if, in addition to the battery of in vitro tests referenced above under (e) and (g), differences in excipients are critically reviewed as described above under (d).

(i) In the case of pressurized metered dose inhalers in solution or suspension, in vivo studies might be waived if similarity is shown in a battery of in vitro tests as described in specific guidelines produced by SRAs (*26*). A waiver of in vivo studies for a dry powder inhaler (DPI) is not considered feasible unless the device for the DPI is identical to the comparator.

(j) For pharmaceutically equivalent topical gel products, equivalence can be demonstrated by means of in vitro membrane diffusion studies when the products contain essentially the same excipients in comparable concentrations and the API(s) in the product are in solution (*27*).

(k) Otic and ophthalmic suspensions with the same qualitative and quantitative composition in excipients might be granted a waiver if the particles in suspension are shown to have the same crystallographic structure and similar particle size distribution, as well as comparability in any other appropriate in vitro test, e.g. dissolution.

(l) Products acting locally in the GI tract containing highly soluble APIs (as defined by the BCS) in immediate release dosage forms might be waived from in vivo equivalence studies based on the same dissolution requirements as are applied for the BCS-based biowaiver.

10.5 In vitro equivalence testing for scale-up and post-approval changes

Although these guidelines refer primarily to registration requirements for multisource pharmaceutical products, it should be noted that under certain conditions, following permissible changes to formulation or manufacturing after FPP approval, in vitro dissolution testing may also be suitable to confirm similarity of product quality and performance characteristics. More information on when dissolution testing may be used to support product variations is provided in WHO guidance on variations in pharmaceutical products.

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Further reading

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Appendix 1

Recommendations for conducting and assessing comparative dissolution profiles

The dissolution measurements of the two finished pharmaceutical product (FPPs) (e.g. test and comparator or two different strengths) should be made under the same test conditions. A minimum of three time points (zero excluded) should be included, the time points for both reference (comparator) and test product being the same. The sampling intervals should be short for a scientifically sound comparison of the profiles (e.g. 5, 10, 15, 20, 30, 45 and 60 minutes for an immediate-release dosage form). The 15-minute time-point is critical to determine whether a product is very rapidly dissolving and to determine whether f_2 must be calculated. For extended-release FPPs the time-points should be set to cover the entire duration of expected release, e.g. in addition to earlier time-points: samples at 1, 2, 3, 5 and 8 hours should be collected for a 12-hour release and additional test intervals would be necessary for longer duration of release.

Studies should be performed in at least three media covering the physiological range, including pH 1.2 hydrochloric acid, pH 4.5 buffer and pH 6.8 buffer. Ph. Int. buffers are recommended; other pharmacopoeial buffers with the same pH and buffer capacity are also acceptable. Water may be considered as an additional medium, especially when the API is unstable in the buffered media to the extent that the data are unusable.

If both the test and reference (comparator) products show more than 85% dissolution in 15 minutes the profiles are considered similar (no calculations required). Otherwise:

- similarity of the resulting comparative dissolution profiles should be calculated using the following equation that defines a similarity factor (f_2)

  f_2 = 50 LOG {[1+1/n ∑nt=1 (Rt– Tt)2] -0.5 × 100}

  where Rt and Tt are the mean per cent API dissolved in reference (comparator) and test product, respectively, at each time-point.

  An f_2 value between 50 and 100 suggests that the two dissolution profiles are similar;
- a maximum of one time point should be considered after 85% dissolution of the reference (comparator) product has been reached;

- in the case where 85% dissolution cannot be reached owing to poor solubility of the API or the release mechanism of the dosage form, the dissolution should be conducted until an asymptote (plateau) has been reached;
- at least 12 units should be used for determination of each profile. Mean dissolution values can be used to estimate the similarity factor, f_2. To use mean data the percentage coefficient of variation at time-points up to 10 minutes should be not more than 20% and at other time-points should be not more than 10%;
- when delayed-release products (e.g. enteric coated) are being compared, the recommended conditions are acid medium (pH 1.2) for 2 hours and buffer pH 6.8 medium;
- when comparing extended-release beaded capsules, where different strengths have been achieved solely by means of adjusting the number of beads containing the API, one condition (normally the release condition) will suffice;
- surfactants should be avoided in comparative dissolution testing.

A statement that the API is not soluble in any of the media is not sufficient, and profiles in the absence of surfactant should be provided. The rationale for the choice and concentration of surfactant should be provided. The concentration of the surfactant should be such that the discriminatory power of the test will not be compromised.

Appendix 2

Equilibrium solubility experiments for the purpose of classification of active pharmaceutical ingredients according to the biopharmaceutics classification system

Introduction

The BCS was proposed in 1995 by Amidon et al. (*1*). It is a scientific framework that divides active pharmaceutical ingredients (APIs) into four groups according to their solubility and permeability. The recommended method for determination of the solubility is described below. Please refer to the *Multisource (generic) pharmaceutical products: guidelines on registration requirements to establish interchangeability* for further explanation of BCS classification and qualification of multisource products for a biowaiver based on the BCS (*2*).

This text was drafted based on the *Proposal to waive in vivo bioequivalence requirements for WHO Model List of Essential Medicines immediate-release, solid oral dosage forms* (*3*), the *Multisource (generic) pharmaceutical products: guidelines on registration requirements to establish interchangeability* (*2*) and the *Classification of orally administered drugs on the World Health Organization Model list of Essential Medicines according to the biopharmaceutics classification system* (*4*).

Recommendations for conducting experiments for assessing solubility of APIs

Prior to the experiment, a solubility study protocol should be prepared describing the equipment and procedures in detail. The protocol should include, for example, methods of sample preparation, experimental conditions such as temperature, method and rate of agitation, method of solid/solution separation of the API, and method of sample analysis. The source and purity of the API to be used in the study should also be recorded in the protocol, as well as the methods that will be used to characterize the material.

Characterization of the solid API should be completed prior to the investigation. The depth of the characterization will depend on the existing knowledge of the solid-state properties of the API in question. For example, if it has been established that the API exists as a single polymorphic form, then less solid-state characterization is needed. In some cases, it may be necessary to characterize the solid starting material as well as the solid residue remaining after equilibrium has been reached and sampling has been completed. For a

discussion of the factors that should be considered when planning the solid-state characterization studies, see Avdeef et al. (*5*).

Solubility experiments should preferably be carried out with the shake-flask method, which is used to determine equilibrium solubility, although other methods are possible if justified. A discussion of the factors that should be considered when designing the study can be found in Avdeef et al. (*5*). The conditions employed should be fully described in the study protocol.

The pH-solubility profile of the API should be determined over the pH range of 1.2–6.8 at 37 ± 1 °C. Measurements should be made in triplicate under at least three pH conditions, pH 1.2, 4.5 and 6.8, as well as at the pH of any known solubility minima in aqueous media within that pH range. Pharmacopoeial buffer solutions are recommended for use in solubility experiments (see, e.g. chapter 5.5 Dissolution test for solid oral dosage forms in *The International Pharmacopoeia* (*6*)). Factors such as common ion effects and ionic strength should be considered when selecting buffers for the study. The pH should be verified after addition of the API and at the end of the experiment with a calibrated pH meter. Samples should be taken at several time-points to ensure that the equilibrium solubility has been reached. Strong agitation followed by a period of sedimentation is suggested, to achieve solubility equilibrium.

A description of the method(s) of solid/solution separation employed, including details such as filter type and pore size or centrifugation speed, should be provided in the study protocol. Sedimentation, centrifugation and filtration are the standard methods of separation. The factors described by Avdeef et al. (*5*) should be considered when selecting the most appropriate approach for the API under study.

A validated, stability-indicating analytical method should be employed for determination of the solubility of APIs, e.g. high-performance liquid chromatographic analysis (see chapter 1.14.4 High-performance liquid chromatography in *The International Pharmacopoeia* (*6*)) or an alternative, validated stability-indicating assay.

A study report should be created after the experiment detailing the actual experimental conditions, results (raw data plus mean values with standard deviations), and any observations, for example, the degradation of an API as a result of pH or buffer composition. The section describing the experimental conditions should include initial and equilibrium pH of solutions and de facto buffer concentrations. If applicable, filter adsorption studies should be documented. Any deviations from the protocol should be noted and justified.

The dose/solubility ratio is calculated as follows: highest single therapeutic dose (mg) divided by solubility (mg/mL). An API is considered highly soluble when the highest single therapeutic dose is soluble in 250 mL or less of aqueous media over the pH range of 1.2–6.8, i.e. the dose/solubility ratio is ≤ 250 (*2*).

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