Annex 8

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. 1003, Annex 6.

Background

Following the publication of the *WHO guideline on Biopharmaceutics Classification System-based biowaivers*, the relevant sections from this guideline have been removed, including the appendix *Equilibrium solubility experiments for the purpose of classification of active pharmaceutical ingredients according to the Biopharmaceutics Classification System*.

Contents

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 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 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:

- good manufacturing practices
- quality control 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 to 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 good manufacturing practices, 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 national regulatory authorities 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 (for example, 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 sites 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 at and extent to which the active moiety is absorbed from a pharmaceutical dosage form and becomes available at the sites of action. Reliable measurements of active pharmaceutical ingredient concentrations at the sites of action are usually not possible. The substance in the systemic circulation, however, is considered to be in equilibrium with the substance at the sites of action. Bioavailability can therefore be defined as the rate at and extent to which the active pharmaceutical ingredient 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 sites 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), 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, and 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 method of analysis, source or method of production. Nevertheless, many biologicals are produced using in vitro culture systems.

Biopharmaceutics Classification System. The Biopharmaceutics Classification System is a scientific framework for classifying active pharmaceutical ingredients based on 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 Biopharmaceutics Classification System takes into account the major factors that govern the rate and extent of active pharmaceutical ingredient absorption (exposure) from immediate-release oral solid dosage forms: excipient composition, dissolution, solubility and intestinal permeability.

biowaiver. The regulatory pharmaceutical product approval process whereby the dossier (application) is approved based on evidence of equivalence rather 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*, [10] should be used to identify a suitable alternative comparator product.

dosage form. The form of the finished pharmaceutical product (for example, tablet, capsule, suspension or suppository).

equivalence requirements. In vivo 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 or in vitro approaches.

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

fixed-dose combination finished pharmaceutical product. A finished pharmaceutical product that contains two or more active pharmaceutical ingredients.

generic product. See "multisource pharmaceutical product".

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. A product that is therapeutically equivalent to a comparator product and can be interchanged with the comparator in clinical practice.

in vitro equivalence dissolution test. A dissolution test that includes comparison of the dissolution profile between the multisource product and the

[10] Guidance on the selection of comparator pharmaceutical products for equivalence assessment of interchangeable multisource (generic) products. In: WHO Expert Committee on Specifications for Pharmaceutical Preparations: forty-ninth report. WHO Technical Report Series No. 992, Annex 8. Geneva: World Health Organization; 2015.

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 quality control of product batches, generally a single time point dissolution test for immediate-release products and a three or more time points dissolution test for modified release products.

multisource pharmaceutical product. A pharmaceutically equivalent or pharmaceutically alternative product 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 alternative. A products is a pharmaceutical alternative if it contains the same active pharmaceutical moiety or moieties but differs in dosage form (for example, tablets versus capsules), strength, or chemical form (for example, 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 active pharmaceutical ingredients 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 active pharmaceutical ingredient solid-state properties, the excipients, the manufacturing process or other variables can lead to differences in product performance.

If an excipient serves multiple functions (for example, microcrystalline cellulose as a filler and as a disintegrant), then the most conservative recommended range should be applied (for example, ± 1.0% for microcrystalline cellulose should be applied in this example). The relative concentration of an excipient present in two aqueous solution finished pharmaceutical products 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 active pharmaceutical ingredient (API) or its metabolites 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 area under the curve (AUC) and Cmax, 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 a national regulatory authority 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, for example Biopharmaceutics Classification System (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 or are not 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 (for example, 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 (such as buffer, preservative and antioxidant) may be different provided it can be shown that the changes in these excipients would not affect the safety 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. The change of other excipients should be critically reviewed.

(b) When pharmaceutically equivalent products are solutions for oral use (for example, syrups, suspensions and tinctures), contain the API in the same molar concentration as the comparator product, and 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 APIs in the same molar concentration and the same excipients in similar concentrations. Certain excipients (such as preservative, buffer, substance to adjust tonicity or thickening agent) may be different provided their use is not expected to affect bioavailability, safety or efficacy of the product.

(f) When pharmaceutically equivalent products are topical products prepared as aqueous solutions and contain the same APIs in the same molar concentration and the same excipients in similar concentrations (note that a waiver would not apply to other

topical dosage forms such as 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 APIs in the same concentration, and contain the same excipients in similar concentrations (note that this waiver does not apply to other dosage forms such as 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 suspension). The pharmaceutical product may include different excipients provided their use is not expected to affect bioavailability, safety 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 (that is, (a), (e) and (g)), that their use is not expected to affect the bioavailability, safety or efficacy of the product. In the event that the applicant cannot provide this information and the national regulatory authority 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 subsection 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 as follows:

(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 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 (for example, 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, for example, comparative clinical or pharmacodynamic studies, local availability studies or in vitro studies. In certain cases, measurement of the concentration of the API may still be required for safety reasons, that is, 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: general considerations

6.1 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 on Ethical Principles for Medical Research Involving Human Subjects, 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, or laws and regulations of the country in which the research is conducted, whichever represents the greater protection for study subjects.

6.2 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.

6.3 Selection of investigators

The investigators should have the appropriate expertise, qualifications and competence to undertake the proposed study. Prior to the trial, the investigators and the sponsor should draw up an agreement on the protocol, monitoring, auditing, standard operating procedures, and 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.

6.4 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 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 changes 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 (for example, institutional review board, peer review committee, ethics committee or national regulatory authority) constituted appropriately for these purposes and independent of the investigators and sponsor.

The signed and dated study protocol should be approved by the national regulatory authority 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, crossover 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 washout 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 (for example, 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 crossover 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, crossover bioequivalence study for an orally administered product with a long elimination half-life is preferred, provided an adequate washout period between administrations of the treatment 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 crossover 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 crossover and parallel design studies of oral products, sample collection time should be adequate to ensure completion of gastrointestinal transit (approximately two to three 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 crossover 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 or too toxic to be administered to healthy volunteers, even in single doses (see also subsection 7.1.1). In this case a multiple-dose, crossover 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 washout 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 coadministration 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 gastrointestinal 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 subsection 7.1.1, single-dose, crossover 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; for example, after a single dose of the highest strength the AUC for the dosing interval covers < 90% of the 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, as in the case of 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 gastrointestinal tract conditions relative to the fasted state should be given (see subsection 7.4.4 for more recommendations on 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 for 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 (*7*). In addition, a number of extra subjects should be recruited, dosed appropriately, and their samples analysed based on the expected rate of dropout or withdrawal, 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 subsection 7.6.1 for more information).

7.2.2 Dropout and withdrawal

Sponsors should select a sufficient number of study subjects to allow for possible dropout or withdrawal. Because replacement of subjects during the study could complicate the statistical model and analysis, dropouts generally should not be replaced. Reasons for withdrawal (for example, 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 or serum concentration data should be provided.

The concentration–time profiles of subjects who exhibit predose concentrations higher than 5% of the corresponding C_{max} should be excluded from the statistical analysis. The concentration–time profiles of subjects who exhibit predose 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 aged between 18 and 55 years and their weight should be within the normal range, with a body mass index between 8 and 30 kilograms per square metre (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, for example, 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 gastrointestinal 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 subsection 7.2.6).

If the aim of the bioequivalence study is to address specific questions (such as 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 guidelines for good clinical practice (*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 finished pharmaceutical product (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, such as 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 and for determination of sampling times and washout periods in crossover 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 good manufacturing practice 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 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 pre-marketing studies and post-marketing 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 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 subsection 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 – that is, 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.

7.4.2 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.

7.4.3 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 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 gastrointestinal blood flow and motility. The same pattern of posture and activity should be maintained for each day of the study. The time of day at which the study product is to be administered should be specified.

7.4.4 Coadministration 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 millilitres). 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.

Immediate-release formulations

Fasted-state studies are generally preferred. However, when the product is known to cause gastrointestinal 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 (such as microemulsions or 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 the following subsection on "Modified-release formulations" should be employed in fed-state studies. The exact composition of the meal may depend on local diet and customs, as determined by the national regulatory authority. For studies conducted with immediate-release products there may be situations where it is appropriate to employ a predose meal with a different caloric or fat content from a meal meeting the composition recommendations identified in the following subsection.

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

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 gastrointestinal 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 gastrointestinal 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 gastrointestinal tract relative to the fasted state should be employed; for example, 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.

7.4.5 Washout interval

The interval (washout 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 washout 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, for example 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 washout 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 washout period should be at least seven days unless a shorter period is justified by a short half-life. The adequacy of the washout period can be estimated from the predose concentrations of the API in the second study period and should be less than 5% of the observed C_{max}.

7.4.6 Sampling times

Blood samples should be taken at a frequency sufficient for assessing C_{max}, AUC and other parameters. Sampling points should include a predose sample, at least

one or two points before C_{max}, two points around C_{max} and three or four 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.

7.4.7 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 t_{max} 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 subsection 7.5).

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

7.4.8 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 – for example, 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}.

7.4.9 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, washout period and sampling times may need to be adjusted to enable adequate characterization of the pharmacokinetic profile of the metabolite.

7.4.10 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 or 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 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, good laboratory practice and the most up-to-date guidelines from stringent regulatory authorities 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 analytes in the biological matrix;
- stability of the analytes 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 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, 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 six to eight 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 and the quality control 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 that the stability of the analytes in the matrix be demonstrated 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 quality control standards should be processed in an identical manner and at the same time as the subject's samples from the same run.
- Reasons for reanalysis, reinjection and reintegration of subject samples should be predefined in the protocol, study plan or standard operating procedures. Reinjection of a full analytical run or of individual calibration standard samples or quality control samples, simply because the calibration or quality controls 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). Incurred samples reanalysis 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 or the standard operating procedures.

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 quality control 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 for the ratio of the log-transformed population means (multisource or 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 that is symmetrical with respect to the formulations being compared (that is, 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 (for example, 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, that is, 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, the median and range of t_{max} 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 subsection 7.2.3 for information on the handling of extreme data.

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

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 intrasubject variance estimate, with some added subjects to compensate for dropout. 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 crossover 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, the bioequivalence acceptance range should be restricted to 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 subsection 7.9.3 for information on an approach for proving bioequivalence when the intrasubject 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 t_{max} 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 good clinical practice and good laboratory practice 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 investigators should sign the respective sections of the report. Names and affiliations of the responsible investigators, 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 of the test products, should be given. Results of in vitro dissolution tests conducted in media with pHs of 1.2, 4.5 and 6.8 and the quality control 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 stringent regulatory authority guidance chosen as a guide for the bioanalytical portion of a study (see subsection 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 quality control samples from the respective run) should also be presented. The tabulated results should present the date of run, subject, study period, product administered (multisource or comparator) and time elapsed between FPP administration and blood sampling, in a clear format. The procedure for calculating the parameters used (for example, 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 dropouts 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 fixed-dose combination products is assessed by in vivo studies, the study design should follow the same general principles as described

in previous sections. The multisource fixed-dose combination product should be compared with the pharmaceutically equivalent comparator fixed-dose combination product. In certain cases (for example, when no comparator fixed-dose combination 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 the 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 coefficient of variation (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 crossover bioequivalence study be conducted and reference-scaled average bioequivalence be employed to widen the acceptance interval for the C_{max} 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 A8.2 gives examples of how different levels of variability lead to different acceptance limits using this methodology.

Table A8.2
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

$$CV\ (\%) = \sqrt{(e\ \text{^}(\{S\_WR\}\text{^}2\ ) -1)}$$

The geometric mean ratio 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 AUC_{τ} 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 or metabolites 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, that is, 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 first 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 good clinical practice 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 that is relevant to the claims of efficacy and 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.
- 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 crossover 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 subsection 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 product and the comparator product can be performed in two different ways:

- **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 products.
- **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 product and the comparator product 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 subsection 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 a 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 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 indications.
- 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 subsection 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 quality control 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* (*20*) for quality control 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 quality control 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

The Biopharmaceutics Classification System (BCS)-based biowaiver approach is intended to reduce the need for in vivo bioequivalence studies, as it can provide a surrogate for in vivo bioequivalence. In vivo bioequivalence studies may be exempted if an assumption of equivalence in in vivo performance can be justified by satisfactory in vitro data. The BCS is a scientific approach based on the aqueous solubility and intestinal permeability characteristics of the APIs.

The BCS categorizes APIs into one of four BCS classes, as follows:

- class I: high solubility, high permeability
- class II: low solubility, high permeability
- class III: high solubility, low permeability
- class IV: low solubility, low permeability.

Guidance providing recommendations to support the biopharmaceutics classification of APIs and the BCS-based biowaiver of bioequivalence studies for FPPs can be found in the *WHO guideline on Biopharmaceutics Classification System-based biowaivers*.

10.2 Qualification for a biowaiver based on the Biopharmaceutics Classification System

Guidance providing recommendations to support the biopharmaceutics classification of APIs and the BCS-based biowaiver of bioequivalence studies for FPPs can be found in the *WHO guideline on Biopharmaceutics Classification System-based biowaivers*.

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.

(a) All active and inactive ingredients are exactly in the same proportions in the different strengths (for example, a tablet of 50 milligram (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.

(b) 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 (b) 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

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:

- an in vivo equivalence study has been performed on at least one of the strengths of the formulation (as described in subsection 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);
- all strengths are proportionally similar in formulation to that of the strength studied;
- the dissolution profiles for the different strengths are similar at pH 1.2, 4.5, 6.8 and for the quality control 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 (for example, 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 the *WHO guideline on Biopharmaceutics Classification System-based biowaivers*, the profile comparison with an f2 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.

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, $f2 \geq 50$, in the recommended test condition for delayed-release product, for example, 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 (f2 > 50) under the test conditions recommended for delayed-release products (see above) is sufficient for a biowaiver.

Extended-release tablets and capsules

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, f2 ≥ 50, in three different pH buffers (between pH 1.2 and 7.5) and the quality control media by the recommended test method.

For extended-release tablets with an osmotic pump release mechanism, the dissolution profile comparison (f2 ≥ 50) under one recommended test condition is sufficient for a biowaiver based on dose proportionality of formulation.

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 (f2 ≥ 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 (for example, f2 test) can be used for comparing the dissolution profiles of two products. The dissolution profiles 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 f2 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 (*21*).

Locally applied, locally acting products in the form of aqueous suspensions containing the same API 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, for example 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 stringent regulatory authorities 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, for example 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 (for example, by the presence or absence of a different surfactant or preservative) or the aerodynamic particle size distribution (for example, 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 (for example, preservative, substance to adjust tonicity or thickening agent) may affect safety 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, such as 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 (for example, 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 stringent regulatory authorities (*22*).

(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, such as 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 stringent regulatory authorities (*23*). A waiver of in vivo studies

for a dry powder inhaler is not considered feasible unless the device for the dry powder inhaler 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 APIs in the product are in solution (*24*).

(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, such as dissolution.

(l) Products acting locally in the gastrointestinal 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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Appendix 1

Recommendations for conducting and assessing comparative dissolution profiles

The dissolution measurements of the two FPPs (for example, 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 (for example, 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 f2 must be calculated. For extended-release FPPs, the time points should be set to cover the entire duration of expected release, for example, 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. *The International Pharmacopoeia* 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 (f2):

  $$f2 = 50 \text{ LOG } \{[1+1/n \sum nt =1 (Rt- Tt)2] -0.5 \times 100\},$$

  where Rt and Tt are the mean per cent API dissolved in reference (comparator) and test product, respectively, at each time point. An f2 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, f2. 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 (for example, 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.