MULTISOURCE (GENERIC) PHARMACEUTICAL PRODUCTS: GUIDELINES ON REGISTRATION
REQUIREMENTS TO ESTABLISH INTERCHANGEABILITY. REVISION
(JULY 2014)

REVISED DRAFT FOR COMMENT

Should you have any comments on the attached text, please send these to:
Dr Sabine Kopp, Group Lead, Medicines Quality Assurance, Technologies, Standards and Norms, World
Health Organization, 1211 Geneva 27, Switzerland; email: kopps@who.int; fax: (+41 22) 791 4730
(kopps@who.int) and to Ms Marie Gaspard (gaspardm@who.int), by 15 September 2014.
Working documents are sent out electronically and they will also be placed on the Medicines website
for comment. If you do not already receive directly our draft guidelines please let us have your email
address (to bonnyw@who.int) and we will add it to our electronic mailing list.

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Please send any request for permission to:
Dr Sabine Kopp, Group Lead, Medicines Quality Assurance, Technologies, Standards and Norms, Department of
Essential Medicines and Health Products, World Health Organization, CH-1211 Geneva 27, Switzerland. Fax: (41-22)
791 4730; email: kopps@who.int.
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SCHEDULE FOR THE PROPOSED ADOPTION PROCESS OF DOCUMENT QAS/14.583:
MULTISOURCE (GENERIC) PHARMACEUTICAL PRODUCTS: GUIDELINES ON
REGISTRATION REQUIREMENTS TO ESTABLISH INTERCHANGEABILITY.
REVISION

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<td>July 2013</td>
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<tr>
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<td>Dressman, WHO Collaborating Centre on Bioequivalence Testing of</td>
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BACKGROUND

Over the course of time and especially in view of the implementation of the existing guidelines the users have indicated that there was a need to review and update certain requirements. The forty-eighth meeting of the World Health Organization (WHO) Expert Committee on Specifications for Pharmaceutical Products discussed this and included the following passage in its report (extract from the forty-eighth report):

“WHO published Multisource (generic) pharmaceutical products: Guidelines on registration requirements to establish interchangeability in 2006. In preparation for the revision of the WHO document, the Expert Committee received a report that compared the WHO guidance on interchangeability with that of the European Medicines Agency (EMA) and the United States Food and Drug Administration (US-FDA). Guidance issues proposed for revision were highlighted. It was stated that a first draft of the revision would be available in due course. The Committee was also asked to consider to what extent WHO guidance should extend to non-biological complex drugs (NBCDs). The Committee agreed that guidance for this new group of products might be relevant if they were included in the EML and asked the Secretariat to look into it.”

In connection with the revision of these guidelines, the following related guidance texts are also under review and update:

• Proposal to waive in vivo bioequivalence requirements for WHO Model List of Essential Medicines immediate-release, solid oral dosage forms

Annex 8, WHO Technical Report Series 937, 2006;

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- Additional guidance for organizations performing in vivo bioequivalence studies
  Annex 9, WHO Technical Report Series 937, 2006;

- Guidance on the selection of comparator pharmaceutical products for equivalence assessment of interchangeable multisource (generic) products

- Guidance on the selection of pharmaceutical products for assessment of interchangeable multisource (generic) products (working document QAS/14.594);

- List of international comparator products (working document QAS/14.595).
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1. INTRODUCTION

These guidelines are intended to 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 (NRA) should ensure that all pharmaceutical products subject to their control conform to acceptable standards of safety, efficacy and quality, and that all premises and practices employed in the manufacture, storage and distribution of these products comply with good manufacturing practice (GMP) standards so as to ensure the continued conformity of the products with these requirements until they are delivered to the end-user.
All pharmaceutical products, including multisource products, should be used in a country only after approval by the national or regional authority. Regulatory authorities should require the documentation of a multisource pharmaceutical product to meet the following:

- GMP;
- quality control specifications;
- pharmaceutical product interchangeability.

Multisource pharmaceutical products need to conform to the same appropriate standards of quality, efficacy and safety as those required of the innovator’s (comparator) product. In addition, reasonable assurance must be provided that the multisource product is therapeutically equivalent and interchangeable with the comparator product. For some classes of products, including – most evidently – aqueous parenteral solutions, interchangeability is adequately assured by assessment of the composition, implementation of GMP and evidence of conformity with appropriate specifications including relevant pharmacopoeial specifications. For a wide range of pharmaceutical products, the concepts and approaches covered by these guidelines will enable the national regulatory authority to decide whether a given multisource product can be approved. This guidance is generally applicable to orally-administered multisource products, as well as to non-orally-administered pharmaceutical products for which systemic exposure measures are suitable for documenting bioequivalence (e.g. transdermal delivery systems and certain parenteral, rectal and nasal pharmaceutical products). Some information applicable for locally acting products is also provided in this document. For yet other classes of products, 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 formulation differences and usually require a very large number of patients. Further, such studies in humans can be financially daunting, are often unnecessary and may be unethical. For these reasons, the science of bioequivalence testing has been developed over the last 50 years. According to the tenets of this science, therapeutic equivalence can be assured when the multisource product is both pharmaceutically equivalent/alternative and bioequivalent.

Assuming that, in the same subject, an essentially similar plasma concentration-time course will result in essentially similar concentrations at the site(s) of action and thus 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 existing international agreement on trade-related aspects of intellectual property rights (1).

2. GLOSSARY

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

The rate and extent to which the active moiety is absorbed from a pharmaceutical dosage form and becomes available at the site(s) of action. Reliable measurements of active pharmaceutical ingredient (API) concentrations at the site(s) of action are usually not possible. The substance in the systemic circulation, however, is considered to be in equilibrium with the substance at the site(s) of action. Bioavailability can be therefore defined as the rate and extent to which the API or active moiety is absorbed from a pharmaceutical dosage form and becomes available in the systemic circulation. Based on pharmacokinetic and clinical considerations it is generally accepted that in the same subject an essentially similar plasma concentration time course will result in an essentially similar concentration time course at the site(s) of action.

bioequivalence

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

biological medicinal product (ECBS)

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

In small print: Developers of such medicinal products that do not fit the definition of biological medicinal
products provided in this document should consult the relevant NRAs for product classification and the
licensing application pathway.

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

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

comparator product
The comparator product is a pharmaceutical product with which the multisource product
is intended to be interchangeable in clinical practice. The comparator product will
normally be the innovator product for which efficacy, safety and quality have been
established. If the innovator product is no longer marketed in the jurisdiction, the
selection principle as described in “Guidance on the selection of comparator
pharmaceutical products for equivalence assessment of interchangeable multisource
(generic) products” should be used to identify a suitable alternative comparator product.
dosage form

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

equivalence requirements

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

equivalence test

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

fixed-dose combination (FDC)

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

fixed-dose combination finished pharmaceutical product (FDC-FPP)

A finished pharmaceutical product that contains two or more active pharmaceutical ingredients.

generic product

See multisource pharmaceutical products.

innovator pharmaceutical product

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

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

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

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

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

**non-biological (Oxford)**
Not involving or derived from biology or living organisms.

**pharmaceutical alternatives**
Products are pharmaceutical alternative(s) if they contain the same active pharmaceutical moiety(s) but differ in dosage form (e.g. tablets versus capsules), strength, and/or chemical form (e.g. different salts, 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 ingredient(s) (API) in the same dosage form, if they meet comparable standards, and if they are intended to be administered by the same route. Pharmaceutical equivalence does not necessarily imply therapeutic equivalence, as differences in the API solid state properties, the excipients and/or the manufacturing process and some other variables can lead to differences in product performance.

**quantitatively similar amounts (concentrations) of excipients**

The relative amount of excipient present in two solid oral FPPs is considered to be quantitatively similar if the differences in amount fall within the limits described in the following table.

<table>
<thead>
<tr>
<th>Excipient type</th>
<th>Percentage difference (w/w) out of total product (core) weight</th>
</tr>
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<tbody>
<tr>
<td>Filler</td>
<td>5.0%</td>
</tr>
<tr>
<td>Disintegrant</td>
<td></td>
</tr>
<tr>
<td>Starch</td>
<td>3.0%</td>
</tr>
<tr>
<td>Other</td>
<td>1.0%</td>
</tr>
<tr>
<td>Binder</td>
<td>0.5%</td>
</tr>
<tr>
<td>Lubricant</td>
<td></td>
</tr>
<tr>
<td>Ca or Mg stearate</td>
<td>0.25%</td>
</tr>
<tr>
<td>Other</td>
<td>1.0%</td>
</tr>
<tr>
<td>Glidant</td>
<td></td>
</tr>
<tr>
<td>Talc</td>
<td>1.0%</td>
</tr>
<tr>
<td>Other</td>
<td>0.1%</td>
</tr>
</tbody>
</table>

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

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

The applicability of each of these four methods is discussed below. Detailed information is provided on conducting an assessment of equivalence studies using pharmacokinetic measurements and in vitro methods, which are currently the methods most often used to document equivalence for most orally-administered pharmaceutical products for systemic exposure.
Acceptance of any test procedure in the documentation of equivalence between two pharmaceutical products by a NRA depends on many factors, including the characteristics of the API and the pharmaceutical product. Where an API produces measurable concentrations in an accessible biological fluid such as plasma, comparative pharmacokinetic studies can be performed. This type of study is considered to be the gold standard in equivalence testing; however, where appropriate, in vitro testing, e.g. BCS-based biowaivers for immediate-release pharmaceutical products can also assure equivalence between the multisource product and the comparator product (see sections 5 and 10). Where an API does not produce measurable concentrations in an accessible biological fluid and a BCS-based biowaiver is not an option, comparative pharmacodynamics studies may be an alternative method for documenting equivalence. Further, in certain cases when it is not possible to assess equivalence through other methods, comparative clinical trials may be considered appropriate.

The criteria that indicate when equivalence studies are necessary are discussed in the following two sections of the guideline.

4. WHEN EQUIVALENCE STUDIES ARE NOT NECESSARY

The following types of multisource pharmaceutical product are considered to be equivalent without the need for further documentation:

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

(b) when pharmaceutically-equivalent products are solutions for oral use (e.g. syrups, elixirs and tinctures), contain the API in the same molar concentration as the comparator product, and contain the same excipients in similar concentrations.

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

d) when pharmaceutically-equivalent products are gases;

e) when pharmaceutically-equivalent products are otic or ophthalmic products prepared as aqueous solutions and contain the same API(s) in the same molar concentration and the same excipients in similar concentrations. Certain excipients (e.g. preservative, buffer, substance to adjust tonicity or thickening agent) may be different provided their use is not expected to affect bioavailability, safety and/or efficacy of the product;

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

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

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

5. WHEN IN VIVO EQUIVALENCE STUDIES ARE NECESSARY AND TYPES OF STUDY REQUIRED

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

5.1 In vivo studies

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

Examples are listed below.

(a) Oral, immediate-release pharmaceutical products with systemic action, except for the conditions outlined in section 10.

(b) Non-oral, non-parenteral pharmaceutical products designed to act systemically (such as transdermal patches, suppositories, nicotine chewing gum, testosterone gel and skin-inserted contraceptives).

(c) Modified-release pharmaceutical products designed to act systemically, except for the conditions outlined in section 10.

(d) Fixed-dose combination (FDC) products with systemic action, where at least one of the APIs requires an in vivo study (3).

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

5.2 In vitro studies

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

6. IN VIVO EQUIVALENCE STUDIES IN HUMANS
6.1 General considerations

6.1.1 Provisions for studies in humans

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

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

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

6.1.2 Justification of human bioequivalence studies

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

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

6.1.3 Selection of investigators

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

6.1.4 Study protocol

A bioequivalence study should be carried out in accordance with a protocol agreed upon and signed by the investigator and the sponsor. The protocol and its attachments and/or appendices should state the aim of the study and the procedures to be used, the reasons for proposing the study to be undertaken in humans, the nature and degree of any known risks, assessment methodology, criteria for acceptance of bioequivalence, the groups from which it is proposed that trial subjects be selected and the means for ensuring that they are adequately informed before they give their consent. The investigator is responsible for ensuring that the protocol is strictly followed. Any change(s) required must be agreed on and signed by the investigator and sponsor and appended as amendments, except when necessary to eliminate an apparent immediate hazard or danger to a trial subject.

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

The signed and dated study protocol should be approved by the NRA before commencing the study, if required by national and regional laws and regulations. The study report forms the 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 difference between products, minimize the variability that is not caused by formulation effects and eliminate bias as far as possible. Test conditions should reduce variability within and between subjects. In general, for a bioequivalence study involving a multisource product and a comparator product, a randomized, two-period, two-sequence, single-dose, cross-over study conducted with healthy volunteers is the preferred study design. In this design each subject is given the multisource and the comparator product in randomized order. An adequate wash-out period should follow the administration of each product.
It should be noted however that under certain circumstances an alternative, well-established and statistically-appropriate study design may be more appropriate.

7.1.1 Alternative study designs for studies in patients

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

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

A single-dose, cross-over bioequivalence study of an orally-administered product with a long elimination half-life is preferred, provided an adequate wash-out period between administrations of the treatments is possible. The interval between study days should be long enough to permit elimination of essentially all of the previous dose from the body. Ideally the interval should not be less than five terminal elimination half-lives of the active compound or metabolite, if the latter is measured. If the cross-over study is problematic due to a very long elimination half-life, a bioequivalence study with a parallel design may be more appropriate. A parallel design may also be necessary when comparing some depot formulations.
For both cross-over and parallel-design studies of oral products, sample collection time should be adequate to ensure completion of GI transit (approximately 2–3 days) of the pharmaceutical product and absorption of the API. Blood sampling should be conducted for up to 72 hours following administration but sampling beyond this time is not generally necessary.

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

7.1.3 Considerations for multiple-dose studies

In certain situations multiple-dose studies may be considered appropriate. Multiple-dose studies in patients are most useful in cases where the API being studied is considered to be too potent and/or too toxic to be administered to healthy volunteers, even in single doses (see also 7.1.1). In this case, a multiple-dose, cross-over study in patients may be performed without interrupting therapy.

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

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

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

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

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

Unless single-dose studies are not possible for reasons such as those discussed above in section 7.1.1 single-dose, cross-over bioequivalence studies conducted under both fasted and fed conditions comparing the highest strength of the multisource product and the comparator product must be performed to demonstrate bioequivalence. Single-dose studies are preferred to multiple-dose studies as single-dose studies are considered to provide more sensitive measurement of the release of API from the pharmaceutical product into the systemic circulation. In addition to single-dose studies, multiple-dose studies may be considered for extended-release dosage forms with a tendency to
accumulate, e.g. after a single dose of the highest strength the AUC for the dosing
interval covers <90% of AUC extrapolated to infinity.

The comparator product in these studies should be a pharmaceutically-equivalent,
modified-release product. The bioequivalence criteria for modified-release products are
essentially the same as for conventional-release dosage forms except acceptance criteria
should also be applied to $C_{\min}$ ($C_{\text{im}}$) in case of multiple dose studies. As release
mechanisms of pharmaceutical products become more complex, e.g. products with an
immediate-release and modified-release component, additional parameters such as partial
AUC measures may be necessary to ensure the bioequivalence of two products.

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

7.2 Subjects

7.2.1 Number of subjects

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

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

The number of subjects to be recruited for the study should be estimated by considering
the standards that must be met using an appropriate method (see, for example, Julious
2004 (6)). In addition, an extra number of subjects should be recruited, dosed and their
samples analysed based on the expected rate of drop-outs and/or withdrawals, which
depends on the safety and tolerability profile of the API. The number of subjects
recruited should always be justified by the sample-size calculation provided in the study
protocol. A minimum of 12 subjects is required.

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

7.2.2 Drop-outs and withdrawals

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

The concentration-time profiles of subjects who exhibit pre-dose concentrations higher
than 5% of the corresponding Cmax should be excluded from the statistical analysis. The
concentration-time profiles of subjects who exhibit pre-dose concentrations equal to or
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 a priori 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 foetus if they should become pregnant. The investigators should ensure that female volunteers are not pregnant or likely to become pregnant during the study. Confirmation should be obtained by urine tests just before administration of the first and last doses of the product under study.

Generally subjects should be between the ages of 18 and 55 years, and their weight should be within the normal range with a Body Mass Index (BMI) between 18 and 30 kg/m². The subjects should have no history of alcohol or drug-abuse problems and should preferably be non-smokers.

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

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

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

7.2.5 Monitoring the health of subjects during the study

In keeping with GCP (4), the health of volunteers should be monitored during the study so that onset of side-effects, toxicity or any intercurrent disease may be recorded and appropriate measures taken. The incidence, severity and duration of any adverse reactions and side-effects observed during the study must be reported. The probability that an adverse effect is API-induced is to be judged by the investigator.

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

7.2.6 Considerations for genetic phenotyping

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

7.3 Investigational product

7.3.1 Multisource pharmaceutical product

The multisource pharmaceutical product used in the bioequivalence studies for registration purposes should be identical to the projected commercial pharmaceutical product. Therefore, not only the composition and quality characteristics (including stability), but also the manufacturing methods (including equipment and procedures) should be the same as those to be used in the future routine production runs. Test products must be manufactured under GMP regulations. Batch-control results, lot number, manufacturing date and, if possible, expiry date for the multisource product should be stated.

Samples should ideally be taken from batches of industrial scale. When this is not feasible pilot or small-scale production batches may be used, provided that they are not smaller than 10% of expected full production batches, or 100 000 units, whichever is larger, and are produced with the same formulation and similar equipment and process as that planned for commercial production batches. A biobatch of less than 100 000 units may be accepted on provision that this is the proposed production batch size, with the understanding that future scale up for production batches will not be accepted.
It is recommended that potency and in vitro dissolution characteristics of the multisource and the comparator pharmaceutical products be ascertained prior to performance of an equivalence study. Content of the API(s) of the comparator product should be close to the label claim and the difference between two products being compared should not be more than ± 5%. If, because of the lack of availability of different batches of the comparator product, it is not possible to study batches with potencies within ± 5%, potency correction may be required on the statistical results from the bioequivalence study.

### 7.3.2 Choice of comparator product

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

### 7.4 Study conduct

#### 7.4.1 Selection of strength

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

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

7.4.1.1 Non-linear pharmacokinetics

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

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

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

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

7.4.2 Study standardization

Standardization of study conditions is important to minimize the magnitude of variability other than in the pharmaceutical products. Standardization between study periods is critical to a successful study. Standardization should cover exercise, diet, fluid intake, posture, as well as the restriction of the intake of alcohol, caffeine, certain fruit juices and concomitant medicines for a specified time period before and during the study.
Volunteers should not take any other medicine, alcoholic beverages or over-the-counter (OTC) medicines and supplements for an appropriate interval before or during the study. In the event of emergency the use of any non-study medicine must be reported (dose and time of administration).

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

7.4.3 Co-administration of food and fluid with the dose

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

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

7.4.3.1 Immediate-release formulations

Fasted-state studies are generally preferred. However, when the product is known to cause GI disturbances if given to subjects in the fasted state, or if the labelling of the comparator product restricts administration to subjects in the fed state, then the fed-state study becomes the preferred approach.
For products with specific formulation characteristics (e.g. micro-emulsions, solid dispersions), bioequivalence studies performed under both fasted and fed conditions are required, unless the product taken is only in a fasted or fed state.

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

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

7.4.3.2 Modified-release formulations

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

In these cases the objective is to select a meal that will challenge the robustness of the new multisource formulation to prandial effects on bioavailability. To achieve this a meal that will provide a maximal perturbation to the GI tract relative to the fasted state should be employed, e.g. a high-fat (approximately 50% of the total caloric content of the meal), high-calorie (approximately 800 to 1000 kilocalories) test meal has been recommended (FDA, EMA guidelines referenced). 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 test meal should be consumed within a 30-minute interval prior to administration of the FPP.

7.4.4 Wash-out interval

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

7.4.5 Sampling times

Blood samples should be taken at a frequency sufficient for assessing Cmax, AUC and other parameters. Sampling points should include a pre-dose sample, at least 1–2 points before Cmax, 2 points around Cmax and 3–4 points during the elimination phase. Consequently at least seven sampling points will be necessary for estimation of the required pharmacokinetic parameters.

For most 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 (Cmax) and
terminal elimination rate constant in all subjects. Generally sampling should continue for
long enough to ensure that 80% of the AUC ($0 \rightarrow \infty$) can be accrued, but it is not
necessary to sample for more than 72 hours. The exact duration of sample collection
depends on the nature of the API and the input function from the administered dosage
form.

7.4.6 Sample fluids and their collection

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

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

7.4.7 Parameters to be assessed

In bioavailability studies the shape and area under the plasma concentration versus time
curves are mostly used to assess rate ($C_{\text{max}}, t_{\text{max}}$) and extent ($\text{AUC})$ 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/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;

• \( C_{\text{max}} \) 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 \( C_{\text{max}} \) 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/blood concentration–time curve from time zero to time infinity (AUC\(_{0-\infty}\)) representing total exposure, where AUC\(_{0-\infty}\) = AUC\(_{0-t}\) + Clast/Ke; Clast is the last measurable analyte concentration and Ke is the terminal or elimination rate constant calculated according to an appropriate method;

• \( t_{\text{max}} \) is the time after administration of the FPP at which \( C_{\text{max}} \) is observed.

For additional information the elimination parameters can be calculated:

• \( T1/2 \) is the plasma (serum, whole blood) half-life.

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

• AUC\(_{\tau}\) is AUC over one dosing interval (\( \tau \)) at steady state;

• \( C_{\text{max}} \);

• \( C_{\text{min}} \) (\( C_{\text{tau}} \)) is concentration at the end of a dosing interval;

• peak trough fluctuation is percentage difference between \( C_{\text{max}} \) and \( C_{\text{min}} \).
As release mechanisms of pharmaceutical products become more complex, e.g. products with an immediate-release and a modified-release component, additional parameters such as partial AUC measures may be necessary to ensure the bioequivalence of two products.

When urine samples are used cumulative urinary recovery (Ae) and maximum urinary excretion rate are employed instead of AUC and $C_{\text{max}}$.

### 7.4.8 Studies of metabolites

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

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

In rare cases it may be necessary to measure concentrations of a primary active metabolite rather than those of the active parent moiety if concentrations of the active parent moiety 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 note that measurement of one analyte, API or metabolite, carries the risk of making a type-I error (the consumer risk) to remain at the 5% level. However, if more than one of several analytes is selected retrospectively as the bioequivalence determinant, then both the consumer and producer risks change (8). The analyte whose data will be used to assess bioequivalence cannot be changed retrospectively.
When measuring active metabolites, wash-out period and sampling times may need to be adjusted to enable adequate characterization of the pharmacokinetic profile of the metabolite.

7.4.9 *Measurement of individual enantiomers*

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

7.5 *Quantification of active pharmaceutical ingredient*

For the measurement of concentrations of the active compound and/or metabolites in biological matrices, such as serum, plasma, blood, urine and saliva, 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 practices (GCP).

State of the art principles and procedures for bioanalytical method validation and analysis of study samples should be employed.

The main characteristics of a bioanalytical method that are essential to ensure the acceptability of the performance and the reliability of analytical results are: selectivity; lower limit of quantification; the response function and calibration range (calibration curve performance); accuracy; precision; matrix effects; stability of the analyte(s) in the biological matrix; stability of the analyte(s) and of the internal standard in the stock and
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1142 working solutions, and in extracts under the entire period of storage and processing
1143 conditions.
1144
1145 In general:
1146
1147 • the analytical method should be able to differentiate the analyte(s) of interest, and
1148   if employed, the internal standard (IS) from endogenous components in the matrix
1149   or other components in the sample;
1150 • the lower limit of quantification (LLOQ), being the lowest concentration of
1151   analyte in a sample, should be estimated to prove that the analyte at this
1152   concentration can be quantified reliably, with an acceptable accuracy and
1153   precision;
1154 • the response of the instrument with regard to the concentration of analyte should
1155   be known and should be evaluated over a specified concentration range. The
1156   calibration curve should be prepared in the same matrix as the matrix of the
1157   intended subject samples by spiking the blank matrix with known concentrations
1158   of the analyte. A calibration curve should consist of a blank sample, a zero sample
1159   and 6–8 non-zero samples covering the expected range;
1160 • within-run and between-run accuracy and precision should be assessed on
1161   samples spiked with known amounts of the analyte, the quality control (QC)
1162   samples, at a minimum of three different concentrations;
1163 • matrix effects should be investigated when using mass spectrometric methods;
1164 • stability of the analyte in the stock solution and in the matrix should be proven
1165   covering every step taken during sample preparation and sample analysis, as well
1166   as the storage conditions used;
1167 • when more than one analyte is present in subject samples, it is recommended to
1168   demonstrate the stability of the analytes in the matrix in the presence of the other
1169   analytes;
in case changes are made to an analytical method that has already been validated, a full validation may not be necessary, depending on the nature of the changes implemented. A partial validation may be acceptable;

- a cross-validation is needed in cases where data are obtained from different 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;

- reasons for reanalysis, reinjection and reintegration of subject samples should be predefined in the protocol, study plan or SOP. Re-injection of a full analytical run or of individual calibration standard samples or QC samples, simply because the calibration or QC failed, without any identified analytical cause, is considered not acceptable. 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;

- it is recommended to evaluate accuracy of incurred samples by reanalysis of subject samples in separate runs at different days;

- it is recommended that all the samples from one subject (all periods) should be analysed in the same analytical run, if possible.

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

The results of subject sample determination should be given in the analytical report together with calibration and 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 around the ratio of the log-transformed population means (multisource/comparator) for the pharmacokinetic parameters under consideration and by carrying out two one-sided tests at the 5% level of significance. To establish bioequivalence, the calculated confidence interval should fall within a preset bioequivalence limit. The procedures should lead to a decision scheme which is symmetrical with respect to the formulations being compared (i.e. leading to the same decision whether the multisource formulation is compared to the comparator product or the comparator product to the multisource formulation).

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

Logarithmically transformed, concentration-dependent pharmacokinetic parameters should be analysed using analysis of variance (ANOVA). Normally the ANOVA model should include formulation, period, sequence and subject factors.

Parametric methods, i.e. those based on normal distribution theory, are recommended for the analysis of log-transformed bioequivalence measures.
The general approach is to construct a 90% confidence interval for the quantity $\mu_T - \mu_R$ and to reach a conclusion of pharmacokinetic equivalence if this confidence interval is within the stated limits. The nature of parametric confidence intervals means that this is equivalent to carrying out two one-sided tests of the hypothesis at the 5% level of significance (9, 10). 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 $t_{\text{max}}$ descriptive statistics should be given. In those cases where $t_{\text{max}}$ is considered clinically relevant, median and range of $t_{\text{max}}$ 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 $t_{\text{max}}$. However, if $t_{\text{max}}$ 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 $t_{\text{max}}$ estimate. For parameters describing the elimination phase (T1/2), only descriptive statistics should be given.

See section 7.2.3 for information on the handling of extreme data. Exclusion of data for statistical or pharmacokinetic reasons alone is not acceptable.

**7.6.1 Two-stage sequential design**

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

It is recommended that the same alpha for both stages be employed which gives an alpha
of 0.0294 for this case (11), however, the amount of alpha to be spent at the time of the
interim analysis can be set at the study designer’s discretion. For example, the first stage
may be planned as an analysis where no alpha is spent in the interim analysis since the
objective of the interim analysis is to obtain information on the point estimate difference
and variability and where all the alpha is spent in the final analysis with the conventional
90% confidence interval. In this case no test against the acceptance criteria is made
during the interim analysis and bioequivalence cannot be proven at that point. The
proposed statistical plan must be clearly defined in the study protocol, including the
adjusted significance level that is to be employed during each analysis.

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

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

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

**C\textsubscript{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 \(C_{\text{max}}\)-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. Refer to section 7.9.3 for information on an approach for proving bioequivalence when the \(C_{\text{max}}\) parameter intra-subject variability is high. If the API is determined to possess a narrow therapeutic index (NTI) the bioequivalence acceptance range may need to be restricted to 90.00–111.11%, if appropriate.

**\(t_{\text{max}}\)-difference**
Statistical evaluation of \(t_{\text{max}}\) 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 complying with GCP rules (4). The relevant ICH guideline (12) can be used in the preparation of the study report. The responsible investigator(s) should sign the respective sections of the report. Names and affiliations of
the responsible investigator(s), site of the study and period of its execution should be stated.

The names and batch numbers of the pharmaceutical products used in the study as well as the composition(s) of the tests product(s) should be given. Results of in vitro dissolution tests conducted in pH 1.2, 4.5 and 6.8 media and the QC media, if different, should be provided. In addition the applicant should submit a signed statement confirming that the test product is identical to the pharmaceutical product that is submitted for registration.

The bioanalytical validation report should be attached. The bioanalytical report should include the information recommended in the SRA guidance chosen as a guide for the bioanalytical portion of a study (see section 7.5). All results should be presented clearly. All concentrations measured in each subject and the sampling time should be tabulated for each formulation. Tabulated results showing API concentration analyses according to analytical run (including runs excluded from further calculations, including 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 (e.g. AUC) from the raw data should be stated. Any deletion of data should be documented and justified.

Individual blood concentration/time curves should be plotted on a linear/linear and log/linear scale. All individual data and results should be given, including information on those subjects who dropped out. The drop-outs and/or withdrawn subjects should be reported and accounted for. All adverse events that occurred during the study should be reported along with the study physician’s classification of the events. Further, any treatments employed to address adverse events should be reported.
Results of all measured and calculated pharmacokinetic parameters should be tabulated for each subject–formulation combination together with descriptive statistics. The statistical report should be sufficiently detailed to enable the statistical analyses to be repeated if necessary. If the statistical methods applied deviate from those specified in the study protocol the reasons for the deviations should be stated.

7.9 Special considerations

7.9.1 Fixed-dose combination products

If the bioequivalence of FDC products is assessed by in vivo studies the study design should follow the same general principles as described in previous sections. The multisource FDC product should be compared with the pharmaceutically-equivalent comparator FDC product. In certain cases (e.g. when no comparator FDC product is available on the market) separate products administered in free combination can be used as a comparator (3). Sampling times should be chosen to enable the pharmacokinetic parameters of all APIs to be adequately assessed. The bioanalytical method should be validated with respect to all analytes measured in the presence of the other analytes. Statistical analyses should be performed with pharmacokinetic data collected on all active ingredients; the 90% confidence intervals of test/comparator ratio of all active ingredients should be within acceptance limits.

7.9.2 Clinically important variations in bioavailability

Innovators should make all efforts to provide formulations with good bioavailability characteristics. If a better formulation is developed over time 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 a within-subject variability of > 30% in terms of the ANOVA-CV (13). 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 within-subject standard deviation observed in the relevant parameters for the comparator product (14-16). Of the two most common assessment parameters Cmax is the parameter that is subject to the highest variability and hence, 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 four-way fully replicated cross-over bioequivalence study be conducted and reference-scaled average bioequivalence be employed to widen the acceptance interval for the $C_{\text{max}}$ parameter, if the within-subject variability for $C_{\text{max}}$ following replicate administrations of the comparator product is >30%. If this is the case, the acceptance criteria for $C_{\text{max}}$ can be widened to a maximum of 69.84 – 143.19%. The applicant should justify that the calculated intra-subject variability is a reliable estimate and that it is not the result of outliers.

The extent of the widening of the acceptance interval for $C_{\text{max}}$ is defined based upon the within-subject variability seen in the bioequivalence study using scaled-average-bioequivalence according to $[U, L] = \exp [\pm k \cdot s_{WR}]$, where $U$ is the upper limit of the acceptance range, $L$ is the lower limit of the acceptance range, $k$ is the regulatory constant set to 0.760 and $s_{WR}$ is the within-subject standard deviation of the log-transformed values of $C_{\text{max}}$ of the reference product. The table below gives examples of
how different levels of variability lead to different acceptance limits using this methodology.

<table>
<thead>
<tr>
<th>Within-subject CV (%)</th>
<th>Lower limit</th>
<th>Upper limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>80.00</td>
<td>125.00</td>
</tr>
<tr>
<td>35</td>
<td>77.23</td>
<td>129.48</td>
</tr>
<tr>
<td>40</td>
<td>74.62</td>
<td>134.02</td>
</tr>
<tr>
<td>45</td>
<td>72.15</td>
<td>138.59</td>
</tr>
<tr>
<td>≥50</td>
<td>69.84</td>
<td>143.19</td>
</tr>
</tbody>
</table>

\[
CV(\%) = \sqrt{e^{s_{wr}^2} - 1}
\]

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

The standard bioequivalence acceptance criterion for AUC should be maintained without scaling. If the within-subject variability for \( C_{\text{max}} \), following replicate administration of the comparator, is found to <30%, standard bioequivalence acceptance criteria should be applied to both AUC and \( C_{\text{max}} \) 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 prior to study conduct to confirm that the proposed approach is acceptable for that jurisdiction.

8. PHARMACODYNAMIC STUDIES

Studies in healthy volunteers or patients using pharmacodynamic measurements may be used for establishing equivalence between two pharmaceutical products when the pharmacokinetic approach is not feasible. Pharmacodynamic equivalence studies may become necessary if quantitative analysis of the API and/or metabolite(s) in plasma or urine cannot be made with sufficient accuracy and sensitivity; however, this is extremely unlikely given current technology. Furthermore, pharmacodynamic equivalence studies in humans are required if measurements of API concentrations cannot be used as surrogate
end-points for the demonstration of efficacy and safety of the particular pharmaceutical product such as pharmaceutical products designed to act locally. However, local availability studies based on pharmacokinetic studies alone or in combination with in vitro dissolution studies, are being considered as surrogate end-points for the demonstration of equivalent biopharmaceutical quality and release at the site of action for some products acting locally. In addition, 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 PK parameters, it is essential to ensure the internal validity of the study by showing assay sensitivity, i.e. the ability to distinguish the response obtained by adjacent doses (two-fold or even four-fold difference in dose). It is essential to perform the comparison at the dose level where the dose response is steepest, which may require a previous pilot study for its identification. Furthermore, variability in pharmacodynamic measures is usually greater than that in pharmacokinetic measures. In addition, pharmacodynamic measures are often subject to significant placebo effects, which add to the variability and complicate experimental design. The result is often that huge numbers of patients would have to be enrolled in pharmacodynamic studies to achieve adequate statistical power.

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

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

- the response measured should be a pharmacological or therapeutic effect which
is relevant to the claims of efficacy and/or safety;
- the methodology must be validated for precision, accuracy, reproducibility and specificity;
- neither the test product nor the comparator product should produce a maximal response in the course of the study, since it may be impossible to detect differences between formulations given in doses which give maximum or near maximum effects. Investigation of dose–response relationships may be a necessary part of the design;
- the response should be measured quantitatively, preferably under double-blind conditions, and be recordable by an instrument that produces and records the results of repeated measurements to provide a record of the pharmacodynamic events, which are substitutes for measurements of plasma concentrations. Where such measurements are not possible, recordings on visual analogue scales may be used. Where the data are limited to qualitative (categorized) measurements appropriate special statistical analysis will be required;
- participants should be screened prior to the study to exclude non-responders. The criteria by which responders are distinguished from non-responders must be stated in the protocol;
- in instances where an important placebo effect can occur, comparison between pharmaceutical products can only be made by a priori consideration of the potential placebo effect in the study design. This may be achieved by adding a third phase with placebo treatment in the design of the study;
- the underlying pathology and natural history of the condition must be considered in the study design. There should be knowledge of the reproducibility of baseline conditions;
- a cross-over design can be used. Where this is not appropriate a parallel group study design should be chosen.

The selection basis for the multisource and comparator products should be the same as described in section 7.3.
In studies in which continuous variables can be recorded, the time-course of the intensity of the action can be described in the same way as in a study in which plasma concentrations are measured and parameters can be derived which describe the area under the effect–time curve, the maximum response and the time at which the maximum response occurred.

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

(a) dose-scale analysis or relative potency, which is defined as the ratio of the potency of the test product to that of the reference product. It is a way of summarising the relationship between the dose-response curves of the test and comparator product;

(b) response-scale analysis, which consist of demonstration of equivalence (for at least two dose levels) on the pharmacodynamic end-point.

For either approach to be acceptable a minimum requirement is that the study has assay sensitivity. For a study to have assay sensitivity 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 test and reference products are studied. However, it is essential that doses on the steep part of the dose-response curve are studied. If a dose too low on the dose response curve is chosen then demonstrating equivalence between two products is not convincing, as this dose could be sub-therapeutic. 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 test and reference products should lie within the chosen equivalence margins to provide convincing evidence of equivalence. Like in
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 pre-specified and appropriately justified in the protocol.

9. CLINICAL TRIALS

In some instances (see example (e) in section 5.1, “In vivo studies”) plasma concentration
time–profile data may be not suitable for assessing equivalence between two
formulations. Although in some cases pharmacodynamic equivalence studies can be an
appropriate tool for establishing equivalence, in others this type of study cannot be
performed because of a lack of meaningful pharmacodynamic parameters that can be
measured; a comparative clinical trial then has to be performed to demonstrate
equivalence between two formulations. However, it is preferable to assess equivalence by
performing a pharmacokinetic equivalence study rather than a clinical trial that is less
sensitive and would require a huge number of subjects to achieve adequate statistical
power. For example, it has been calculated that 8600 patients would be required to give
adequate statistical power to detect a 20% improvement in response to the study API
compared with placebo (18). 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 in patients with a therapeutic end-point has not yet evolved as extensively as for bioequivalence studies. However, some important items that need to be defined in the protocol can be identified as follows:

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

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

10. IN VITRO EQUIVALENCE TESTING

Over the past three decades dissolution testing has evolved into a powerful tool for characterizing the quality of oral pharmaceutical products. The dissolution test, at first exclusively a 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 profile, in addition to excipient comparisons and a risk benefit analysis, can be used to document equivalence of a multisource product with a comparator product.

It should be noted that, although the dissolution tests recommended in *The International Pharmacopoeia* (Ph.Int.) (20) for quality control have been designed to be compatible with the biowaiver dissolution tests, they do not fulfill all the requirements for evaluating equivalence of multisource products with comparator products. Dissolution tests for quality control purposes do not generally correspond to the test conditions required for evaluating equivalence of multisource products and should not be applied for this purpose.

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

#### 10.1.1 Biopharmaceutics Classification System

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

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

Combining the dissolution results and a critical examination of the excipients of the pharmaceutical product with these two properties of the API takes the four major factors that govern the rate and extent of API absorption from immediate-release, solid dosage forms into account (21). On the basis of their dissolution properties, immediate-release dosage forms can be categorized as having “very rapid”, “rapid”, or “not rapid” dissolution characteristics.
On the basis of solubility and permeability of the API, excipient nature, excipient content and dissolution characteristics of the dosage form, the BCS approach provides an opportunity to waive in vivo bioequivalence testing for certain categories of immediate-release FPPs. Oral FPPS containing an API possessing a narrow therapeutic index are not eligible for a so-called “biowaiver” based on the BCS approach.

10.1.1.1 High solubility

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

[Note from the Secretariat:]

Major discussion took place on whether or not to use highest dosage strength or highest single therapeutic dose, or both. A number of regulatory authorities favour the highest single therapeutic dose approach; however, some favour the highest dosage strength. In view of this discussion during the informal consultation there was a proposal to have both options in the text. Your feedback is sought. The approach used will have repercussions on the biowaiver guidance document, which is based on the essential medicines list.]

10.1.1.2 High permeability

An API is considered highly permeable when the extent of absorption in humans is 85% or more based on a mass balance determination or in comparison with an intravenous comparator dose. Ideally the mass balance study /i.v. comparison would be conducted at
the same dose as that used for the solubility classification. If this is not possible, dose linearity of pharmacokinetics should be used to justify the use of other doses.

An acceptable alternative test method for permeability determination of the API could be:

(i) in vivo intestinal perfusion in humans.

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

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

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

(iii) in vitro permeation across a monolayer of cultured epithelial cells (e.g. Caco-2) using a method validated using APIs with known permeabilities, although data from neither method (ii) nor (iii) would be considered acceptable on a stand-alone basis. In these experiments high permeability is assessed with respect to the high permeability of a series of reference compounds with documented permeabilities and fraction absorbed values, including some for which fraction of dose absorbed is at least 85% (22).

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

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

10.1.2.1 Very rapidly dissolving

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

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

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

(See also section 10.2, dissolution profile comparison.)

10.1.2.2 Rapidly dissolving

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

- pH 1.2 HCl solution or buffer;
- pH 4.5 acetate buffer;
- pH 6.8 phosphate buffer.
Surfactants should not be employed.

**10.2 Qualification for a biowaiver based on the Biopharmaceutics Classification System**

A biowaiver based on the BCS considers:

(a) the solubility and permeability of the API (see section 10.1);

(b) the similarity of the dissolution profiles of the multisource and comparator products in pH 1.2, 4.5 and 6.8 media (see below);

(c) the excipients used in the formulation (see below);

(d) the risks of an incorrect biowaiver decision in terms of the therapeutic index of, and clinical indications for, the API (see section 5.1 for cases where an in vivo study would be required to demonstrate bioequivalence). Only when there is an acceptable benefit–risk balance in terms of public health and risk to the individual patient should bioequivalence testing be waived and the in vitro methods described in this section applied as a test of product equivalence.

**Risk reduction and assessment of excipients**

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

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

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

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

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

Sub- and supra-bioavailable products

A further consideration is the potential risk to public health and to the individual patient, should an inappropriate decision with respect to bioequivalence be reached. Essentially there are two possible negative outcomes.
The first arises when the multisource product is sub-bioavailable. In this case substitution of the comparator with the multisource product could lead to reduced therapeutic efficacy. APIs which must reach a certain concentration to be effective (e.g. antibiotics) are most susceptible to problems of sub-bioavailability.

The second negative outcome arises when the multisource product is supra-bioavailable. In this case substitution of the comparator with the multisource product could lead to toxicity. APIs which exhibit toxic effects at concentrations close to the therapeutic range are most susceptible to problems of supra-bioavailability. For these reasons therapeutic index is an important consideration in determining whether the biowaiver based on BCS can be applied or not.

Dissolution profile comparison

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

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

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

- BCS Class 1 APIs, if the multisource and comparator product are very rapidly dissolving or similarly rapidly dissolving;
- BCS Class 3 APIs, if the multisource and comparator product are very rapidly dissolving.
In summary, biowaivers for solid oral dosage forms based on BCS can be considered under the following conditions:

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

   (i) the dosage form is rapidly dissolving (as defined in section 10.1.2.2) and the dissolution profile of the multisource product is similar to that of the comparator product at pH 1.2, pH 4.5 and pH 6.8 buffer using the paddle method at 75 rpm or the basket method at 100 rpm (as described in section 10.2) and meets the criteria of dissolution profile similarity, $f_2 \geq 50$ (or equivalent statistical criterion);

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

2. Dosage forms of APIs that are highly soluble and have low permeability (BCS Class 3) are eligible for biowaivers provided all the criteria (a–d) listed in section 10.2 are met and the risk–benefit is additionally addressed in terms of extent, site and mechanism of absorption.

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

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

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

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

10.3.1 Proportional formulations

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

(i) All active and inactive ingredients are exactly in the same proportions in the
different strengths (e.g. a tablet of 50 mg strength has all the active and inactive
ingredients exactly half that of a tablet of 100 mg strength and twice that of a
tablet of 25 mg strength).

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

A waiver is considered:

- if the amounts of the different excipients or capsule contents are the same for the
  concerned strengths 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
  concerned strengths.
10.3.2 Qualification for biowaivers based on dose-proportionality of formulations

10.3.2.1 Immediate-release tablets

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

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

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

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

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

10.3.2.2  Delayed-release tablets and capsules

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

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

10.3.2.3  Extended-release tablets and capsules

(a) For extended-release tablets, when there is a series of strengths of a multisource product that are proportionally similar in their active and inactive ingredients and have the same API-release mechanism, in vivo bioequivalence studies should be conducted with the lowest and highest strengths; however, an intermediate strength can be granted a biowaiver if it exhibits similar dissolution profiles, $f_2 \geq 50$, in three different pH buffers (between pH 1.2 and 7.5) and the QC media by the recommended test method.
For extended release tablets, with an osmotic pump release mechanism the dissolution profile comparison ($f_2 \geq 50$) under one recommended test condition is sufficient for a biowaiver based on dose-proportionality of formulation.

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

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

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

The dissolution sampling times for both reference strength and additional strength profiles should be the same, for example:

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

For the application of the $f_2$ value, please refer to the Annex.

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2 The reference strength is the strength of the FPP that was compared to the comparator product in an in vivo equivalence study.
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 in 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 (26).

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

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

(b) Suspensions for nebulization with the different qualitative and quantitative composition might be waived if, in addition to the requirements defined above under (a), the difference in excipient composition does not alter the nebulizer efficiency (e.g. by the presence or absence of a different surfactant/preservative) and the aerodynamic particle size distribution (e.g. altering product hygroscopicity
by the presence of a different amount of salt as isotonic agent). To this end the
appropriate state-of-the-art in vitro test should be conducted to ensure product
equivalence. Any excipient difference should be critically reviewed because
certain excipients that are considered irrelevant in other dosage forms (e.g.
preservative, substance to adjust tonicity or thickening agent) may affect safety
and/or efficacy of the product.

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

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

(e) Nasal sprays in solution with the same qualitative and quantitative composition in
excipients can be waived based on a battery of in vitro tests as defined by SRAs
\((17, 29)\).

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

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

(i) In 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 of stringent regulatory authorities (27).

(j) When pharmaceutically-equivalent products are topical prepared gels where the
API is in solution, contain the same API(s) in the same molar concentration and
essentially the same excipients in comparable concentrations, equivalence can be
demonstrated by means of in vitro membrane diffusion studies (28).

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

(l) Locally-acting products acting in the GI tract containing highly soluble APIs (as
defined by the BCS) in immediate release dosage forms might be waived from in
vivo equivalence studies based on the same dissolution requirements applied for
the BCS based biowaiver.
10.5 In vitro equivalence testing for scale-up and post-approval changes

Although these guidelines comment primarily on registration requirements for multisource pharmaceutical products, it should be noted that under certain conditions, following permissible formulation or manufacturing changes 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 guidances on variations in pharmaceutical products.

[Note from the Secretariat: references will be updated when the document is finalized.]

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ANNEX

RECOMMENDATIONS FOR CONDUCTING AND ASSESSING
COMPARATIVE DISSOLUTION PROFILES

The dissolution measurements of the two FPPs (e.g. test and comparator or two different strengths) should be made under the same test conditions. A minimum of three time-points (zero excluded) should be included, the time-points for both reference (comparator) and test product being the same. The sampling intervals should be short for a scientifically sound comparison of the profiles (e.g. 5, 10, 15, 20, 30, 45 (60, 90, 120) minutes). The 15-minute time-point is critical to determine whether a product is very rapidly dissolving and to determine whether f2 must be calculated. For extended release FPPs, the time-points should be set to cover the entire duration of expected release, e.g. 1, 2, 3, 5 and 8 hours for a 12-hour release and additional test intervals 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. *International Pharmacopoeia* buffers are recommended; other pharmacopoeial buffers with the same pH and buffer capacity are also accepted. 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):

\[ f_2 = 50 \times \log \left\{ \left[ 1 + \frac{1}{n} \sum_{t=1}^{n} (R_t - T_t)^2 \right]^{-0.5} \times 100 \right\} \]
where $R_t$ and $T_t$ are the mean per cent API dissolved in reference (comparator) and test product, respectively, at each time-point. An $f_2$ value between 50 and 100 suggests that the two dissolution profiles are similar;

- a maximum of one time-point should be considered after 85% dissolution of the reference (comparator) product has been reached;

- in the case where 85% dissolution cannot be reached due to poor solubility of the API, the dissolution should be conducted until an asymptote (plateau) has been reached;

- at least 12 units should be used for determination of each profile. Mean dissolution values can be used to estimate the similarity factor, $f_2$. To use mean data, the percentage coefficient of variation at time points up to 10 minutes should be not more than 20% and at other time points should be not more than 10%;

- when delayed-release products (e.g. enteric coated) are being compared, the recommended conditions are acid medium (pH 1.2) for 2 hours and buffer pH 6.8 medium;

- when comparing extended-release beaded capsules, where different strengths have been achieved solely by means of adjusting the number of beads containing the API, one condition (normally the release condition) will suffice;

- surfactants should be avoided in comparative dissolution testing.

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

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