

INVESTIGATION OF BIOAVAILABILITY AND BIOEQUIVALENCE

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INVESTIGATION OF BIOAVAILABILITY AND BIOEQUIVALENCE

1 INTRODUCTION

To exert an optimal pharmacotherapeutic action, an active substance should be delivered at the site of its action in an effective concentration during the desired period. To allow prediction of the therapeutic effect, the performance of the pharmaceutical form containing the active substance should be reproducible. Several therapeutic misadventures in the past (digoxin, phenytoin, primidone) testify the necessity of this reproducibility as a quality requirement. Thus the bioavailability (definition: par. 2.3) of an active substance from a pharmaceutical product should be known and be reproducible. This is especially the case if one product is substituted for another. In that case the product should show the same therapeutic effect in the clinical situation. It is generally cumbersome to assess this by clinical studies.

Assuming that in the same subject an essentially similar plasma concentration time course will result in essentially similar concentrations at the site of action and thus in an essentially similar effect, pharmacokinetic data instead of therapeutic results may be used to establish equivalence: bioequivalence (definition: par. 2.4).

It is the objective of this recommendation to define, for immediate release products with a systemic effect, when bioavailability or bioequivalence studies are necessary and to formulate requirements for their design, conduct and evaluation.

For medicinal products not intended to be delivered into the general circulation, the common systemic bioavailability approach cannot be applied. Under these conditions, the (local) availability may be assessed, where necessary, by measurements quantitatively reflecting the presence of the active substance at the site of action, by methods specially chosen for that combination of active substance and localisation.

2. DEFINITIONS

Before defining bioavailability and related terminology some definitions pertaining to pharmaceutical forms are given:

2.1 Pharmaceutical equivalents

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

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

2.2 Pharmaceutical alternatives

Medicinal products are pharmaceutical alternatives if they contain the same therapeutic moiety but differ in chemical form of that moiety or in the dosage form or strength.

The therapeutic moiety may be used in the form of salts, esters, etc.

2.3 Bioavailability

Bioavailability means the rate and extent to which the active substance or therapeutic moiety is absorbed from a pharmaceutical form and becomes available at the site of action.

In the majority of cases substances are intended to exhibit a systemic therapeutic effect, and a more practical definition can then be given, taking into consideration that the substance in the general circulation is in exchange with the substance at the site of action:

- bioavailability is understood to be the extent and the rate to which a substance or its therapeutic moiety is delivered from a pharmaceutical form into the general circulation.

It may be useful to distinguish between the “absolute bioavailability” of a given pharmaceutical form as compared with that (100%) following intravenous administration, and the “relative bioavailability” as compared with another form administered by any route other than intravenous (e.g. tablets v. oral solution).

2.4 Bioequivalents

Two medicinal products are bioequivalents if they are pharmaceutical equivalents or alternatives and if their bioavailabilities (rate and extent) after administration in the same molar dose are similar to such degree that their effects, with respect to both efficacy and safety, will be essentially the same.

2.5 Essentially similar products

“A proprietary medicinal product will be regarded as essentially similar to another product if it has the same qualitative and quantitative composition in terms of active principles (substances), and the pharmaceutical form is the same and, where necessary, bioequivalence with the first product has been demonstrated by appropriate bioavailability studies carried out.” (*The Rules Governing Medicinal Products In The European Union*, Vol. II, Notice to Applicants).

Pharmaceutical products essentially similar to an “innovator” product are usually designated as “generics” or “branded generics”. A product is an “innovator” product if its marketing authorisation has been obtained on the basis of a dossier with full documentation.

2.6 Therapeutic equivalents

A medicinal product is therapeutically equivalent with an other product if it contains the same active substance or therapeutic moiety and, clinically shows the same efficacy and safety as that product, whose efficacy and safety has been established.

In practice evidence of bioequivalence is generally the most appropriate proof to substantiate therapeutic equivalence between medicinal products which are pharmaceutical equivalents or alternatives, provided they contain excipients generally recognised as safe and carry the

same labelling for use. However, in some cases where different rates of absorption are observed the products – though not bioequivalent – can be judged therapeutically equivalent since the differences in absorption rate are not of therapeutic relevance.

It is however important to point out that bioequivalence may not necessarily dictate therapeutic equivalence since excipients may raise safety questions; therefore excipients should be well known and safe.

For other considerations on this subject reference is made to an annex to this recommendation (Appendix I).

3. DESIGN AND CONDUCT OF STUDIES

In the following sections, requirements for the design and conduct of comparative bioavailability studies are formulated. It is assumed that the applicant is familiar with pharmacokinetic theories underlying bioavailability studies. The design should be based on a reasonable knowledge of the pharmacodynamics and/or the pharmacokinetics of the active substance in question. For the pharmacokinetic basis of these studies reference is made to the recommendation “Pharmacokinetic studies in man”. The design and conduct of the study should follow EC-rules for Good Clinical Practice, including reference to an Ethics Committee.

3.1 Design

The study should be designed in such a way that the treatment effect (formulation effect) can be distinguished from other effects. In order to reduce variability a cross-over design usually is the first choice. Other designs or methods may be chosen in specific situations, but should be fully justified in the protocol and study report provided. The allocation of the subjects to the treatment sequences should be randomised. In general, single dose studies will suffice, but there are situations in which steady-state studies may be required:

- a) if problems of sensitivity preclude sufficiently precise plasma concentration measurement after single dose;
- b) if the intra-individual variability in the plasma concentrations or disposition rate is inherently large;
- c) in the case of dose- or time-dependent pharmacokinetics;
- d) in the case of extended release products (in addition to single dose studies).

In such steady-state studies the administration scheme should follow the usual dosage recommendations.

The number of subjects required is determined by the error variance associated with the primary characteristic to be studied (as estimated from a pilot experiment, from previous studies or from published data), by the significance level desired, and by the deviation from the reference product compatible with bioequivalence and with safety and efficacy. It should be calculated by appropriate methods and should not be smaller than 12. The deviation allowable usually is $\pm 20\%$ (cf. Appendix III). The number of recruited subjects should always be justified.

Subsequent treatments should be separated by periods long enough to eliminate the previous dose before the next one (wash-out period). In steady-state studies, wash-out of the last dose of

the previous treatment can overlap with the build-up of the second treatment, provided the build-up period is sufficiently long (at least three times the dominating half-life).

Sampling should be done long enough to cover at least 80% of the area under the plasma concentration curve as extrapolated to infinity. The extrapolation should be based on knowledge of the dominating elimination half-life.

In steady-state study, sampling should be carried out over a full 24 hours cycle, enabling the detection of circadian rhythms in bioavailability, unless these rhythms can be argued not to have practical importance.

3.2 Subjects

Bioavailability studies generally will be performed with healthy volunteers. If feasible, taking into account reproduction toxicology, they should belong to both sexes and be between 18 and 55 years old. In the case of genetic polymorphism in clearance it is wise to take this into consideration in selecting subjects. In some cases the toxic character of the active substance studied may be such that only patients – under suitable precautions and supervision – can be studied. In that case the applicant will have to justify his alternative.

To minimise intra- and inter-individual variation subjects should be standardised as much as possible and acceptable. They should preferably be fasting at least during the night before administration of the products or they should take a standard meal at a specified time before the treatment. Time and preferably composition of meals taken after the treatment should be standardised. Because fluid intake may profoundly influence gastric passage, it should be strictly standardised and specified. The subjects should not take other medicines during a suitable period before and during the study. They should preferably abstain from food and drinks which may interact with circulatory, gastro-intestinal, liver or renal function (e.g. alcoholic or xanthine-containing beverages). Preferably they should be non-smokers. If smokers are included they should be identified as such. In some cases (e.g. study of high clearance substances) even posture or physical activity may have to be standardised.

3.3 Characteristics to be investigated

In bioavailability studies the form of, and the area under the plasma concentration curve or the cumulative renal excretion and excretion rate are mostly used to assess extent and rate of absorption. Sampling points or periods should be thus chosen, that a sufficiently detailed time course of the characteristics to be measured will be produced. From the primary results the bioavailability characteristics desired are calculated. AUC_t , AUC , C_{max} , A_e , $A_{e\infty}$, dA_e/dt , or any other justifiable characteristics (cf. Appendix II). The method of calculating AUC -values should be specified. For additional information $t_{1/2}$ and MRT can be calculated. During studies in steady-state AUC , and fluctuation can be calculated. The exclusive use of modelled characteristics is not recommended unless the pharmacokinetic model has been validated for the active substance and the products.

If pharmacodynamic effects are used as characteristics the measurements should provide a sufficiently detailed time course and the initial values should be the same. Specificity, precision and reproducibility of the measurements should be sufficient. The non-linear character of the dose/response relationship should be taken into account.

3.4 Chemical analysis

The bioanalytical methods used to determine the active principle and/or its biotransformation products in plasma, serum, blood or urine or any other suitable matrix should meet requirements of specificity, accuracy, sensitivity and precision. For this item reference is made to the note for guidance on *Tests On Samples Of Biological Origin* in Volume III, part A. Results of validation should be reported.

Knowledge of the stability of the active substance and/or its biotransformation product in the sample material is a prerequisite for obtaining reliable results.

Generally evaluation of bioequivalence will be based upon the measured concentrations of the active substance. If this is impossible a major biotransformation product should be used. The measurement of concentrations of biotransformation product is essential if the substance studied is a prodrug. If urinary excretion (rate) is measured the product determined should represent a major fraction of the dose, and the excretion rate should be considered to parallel plasma concentrations of the active substance.

The requirements of the guideline on *Investigation of Chiral Active Substances* as far as relevant for bioavailability and bioequivalence studies should be taken into account for products containing chiral active substances.

3.5 Reference and test product

All investigated products must have been prepared in accordance with GMP-rules. Batch control results of the test product should be reported.

Generic products, being pharmaceutical equivalents or alternatives are normally compared with the corresponding form of a well established "Innovator" medicinal product (reference product). The choice of reference product should be justified by the applicant.

For an abridged application of an essentially similar medicinal product, demonstration of bioequivalence with the same reference product in any Member State should be accepted in other concerned Member States where the reference product (same manufacturer including subsidiaries) has been authorised. In case of doubt, a Member State may request additional information from the authorities in the first Member State.

The test product will mostly originate from a test batch. After scale-up samples of the product the production batches should be compared with those of the test batch, and they should show the same dissolution rate "in vitro" in a discriminatory test. The study sponsor will have to retain a sufficient number of product samples for the accepted shelf life plus one year to allow repetition of "in vitro" and "in vivo" studies at the request of the authority.

3.6 Data analysis

The aim of a bioequivalence study is to demonstrate equivalence within the acceptance range regarded as clinically relevant.

The primary concern in bioequivalence assessment is to limit the risk of erroneously accepting bioequivalence. Only statistical procedures which do not exceed the nominal risk of 5% can be approved, and among them the one with the smallest risk of erroneously rejecting bioequivalence should be selected.

In case of a parametric approach the inclusion of the classical 90% confidence interval for the chosen measure of relative bioavailability within the acceptance range (bioequivalence

range) is the procedure of choice. This procedure is equivalent to the rejection of two one-sided hypotheses concerning bioinequivalence at the nominal 5%-level.

According to present views concentrations and concentration-related characteristics (e.g. AUC, MRT) should preferably be tested after logarithmic transformation.

If the assumption of a lognormal (AUC, C_{\max}) distribution or normal (t_{\max}) distribution in the parametric approach is doubtful, a corresponding non-parametric approach is recommended. This approach may also be chosen as the general statistical approach to evaluate all bioavailability characteristics throughout a given study.

Further cf. Appendix III. Assumptions on the design or statistical analysis should be discussed.

3.7 “In vitro” Dissolution

The results of “in vitro” dissolution tests, obtained with the batches of test and reference products that were used in the bioavailability or bioequivalence study should always be reported.

The specifications for the “in vitro” dissolution of the product should be derived from the dissolution profile of the batch that was found to be bioavailable or bioequivalent.

3.8 Reporting

The report of a bioavailability or bioequivalence study should give the complete documentation of its protocol, conduct and evaluation complying with GCP-rules. This implies that the authenticity of the whole of the report is attested by the signature of the study monitor. The responsible investigators should sign for their respective sections of the report.

Names and affiliations of the responsible investigators, site of the study and period of its execution should be stated. The names and batch numbers of the products used in the study as well as the composition(s) of the test product(s) should be given. In addition the applicant may submit a signed statement, confirming the identity of the test product with the product which is submitted for registration.

All results should be presented in a clear way. The way of calculating the characteristics used (e.g. AUC) from the raw data should be specified. Deletion of data should be justified. If results are calculated using pharmacokinetic models the model and the computing procedure used should be justified. Individual plasma concentration/time curves should be drawn on a linear/linear, and facultatively also on a lin/log scale. All individual data and results should be given, also of eventually dropped-out subjects. Drop-out and withdrawal of subjects should be reported and accounted for. A representative number of chromatograms should be included. The analytical validation report should be reported.

4. APPLICATIONS FOR PRODUCTS CONTAINING NEW ACTIVE SUBSTANCES

4.1 Bioavailability

In the case of new active substances (new chemical entities) intended for systemic action the pharmacokinetic characterisation will have to include the determination of the systemic

availability of a substance in comparison with intravenous administration or – if this is not possible – of the bioavailability relative to a suitable oral solution or standardised suspension. In the case of a prodrug the intravenous reference solution should preferably be the therapeutic moiety.

4.2 Bioequivalence

The dosage recommendations for the market form of a new active substance should be validated by a comparative bioavailability study against the forms used in the clinical trials, especially those used in the dose finding studies, unless its absence can be justified by satisfactory *in vitro* data.

5. APPLICATIONS FOR NEW PRODUCTS CONTAINING APPROVED ACTIVE SUBSTANCES

5.1 Bioequivalence and comparative bioavailability studies

If a new product is intended to be a substitute for an approved medicinal product as a pharmaceutical equivalent or alternative, the equivalence with this product should be shown or justified. Bioequivalence studies should be carried out when bio-inequivalence may have therapeutic significance. Therefore, bioequivalence studies are conducted if there is:

- a) a risk of bio-inequivalence and/or
- b) a risk of pharmacotherapeutic failure or diminished clinical safety.

The following sequence of criteria is useful in assessing the need for *in vivo* studies:

1. Oral immediate release products with systemic action:

- 1.1 indicated for serious conditions requiring assured response;
- 1.2 narrow therapeutic margin;
- 1.3 pharmacokinetics complicated by absorption < 70% or absorption window, nonlinear kinetics, presystemic elimination > 70%;
- 1.4 unfavourable physicochemical properties, e.g. low solubility, metastable modifications, instability, etc.;
- 1.5 documented evidence for bioavailability problems;
- 1.6 no relevant data available, unless justification by applicant that “*in vivo*” study not necessary.

2. Non-oral immediate release products.

3. Modified release products with systemic action (cf. separate guidance).

If none of the above criteria is applicable, comparative dissolution studies *in vitro* will suffice. The justification of the choice may lie with the applicant.

As many old approved products may biopharmaceutically not be up to date in such cases it may be useful to include a suitable solution or suspension as a primary reference preparation. If bioequivalence has to be shown but for any reasons cannot, the dosage recommendations of the new product will have to be supported by controlled clinical studies.

5.2 Exemptions

In the following situations the applicant will not normally be required to submit bioequivalence studies:

- a) the product differs only in strength of the active substance it contains, provided all following conditions hold:
 - pharmacokinetics are linear;
 - the qualitative composition is the same;
 - the ratio between active substance and the excipients is the same, or (in the case of small strengths) the ratio between the excipients is the same;
 - both products are produced by the same manufacturer at the same production site;
 - a bioavailability or bioequivalence study has been performed with the original product;
 - under the same test conditions, the dissolution rate in vitro is the same.
- b) the product has been slightly reformulated or the manufacturing method has been slightly modified by the original manufacturer in ways that can convincingly be argued to be irrelevant for the bioavailability. The bioavailability of original product has been investigated and the dissolution rates in vitro under the same test conditions are equivalent.
- c) the product is to be parenterally administered as a solution and contains the same active substance(s) and excipients in the same concentrations as a medicinal product currently approved.
- d) the product is a liquid oral form in solution (elixir, syrup, etc.) containing the active substance in the same concentration and form as currently approved medicinal product, not containing excipients that may significantly affect gastric passage or absorption of the active substance.
- e) an acceptable correlation between dissolution rate in vivo and in vitro has been shown and the dissolution rate in vitro of the new product is equivalent with that of the already approved medicinal product under the same test conditions as used to establish the correlation.

5.3 Suprabioavailability

If suprabioavailability is found, i.e. if the new product displays a bioavailability appreciably larger than the approved product, reformulation to a lower dosage strength assuring therapeutic equivalence will be necessary. The biopharmaceutical development should be reported and a final comparative bioavailability study of the reformulated new product with the old approved product should be submitted. The name of the new product should preclude confusion with the older approved product(s) e.g. by an appropriate prefix or suffix.

If the plasma concentration effect relation is insufficiently known the dosage recommendations will have to be supported by clinical studies. If marketing authorisation has been obtained the new product will be considered as a potentially new reference product.

6. SITUATIONS IN WHICH BIOAVAILABILITY STUDY IS NOT RELEVANT

Studies of bioavailability are generally not required if one of the following situations applies:

- a) The product is a simple solution intended only for intravenous administration or a gas for inhalation.
- b) Products for local use (after oral, nasal, ocular, dermal, rectal, vaginal, etc. application) intended to act without systemic absorption are outside the scope of this recommendation. This does not however exclude the potential need for safety studies in case of unintended partial absorption.

To prove therapeutic equivalence clinical or pharmacodynamic studies are required.

APPENDIX I

Therapeutic Equivalents

Therapeutic equivalents contain the same active substance or therapeutic moiety and, when administered clinically, show the same efficacy/safety as the medicinal product whose efficacy and safety are established (cf. 2.6).

Accordingly, therapeutically equivalent products refer to products which are identical, similar or related to “innovator” or “pioneer” medicinal products. Related products cover pharmaceutical alternatives, of which differences from pharmaceutical equivalents with respect to the dosage form (i.a), the chemical form (i.b), the strength (ii.) are considered to be medically meaningless.

- i) Generally, therapeutic equivalents consist of Bioequivalent forms:
 - either pharmaceutical equivalents (essentially similar medicinal products),
 - or, sometimes, pharmaceutical alternatives differing in:
 - a) the dosage form (e.g. capsules v. tablets);
 - b) other chemical forms (e.g. the salt or ester) of the same therapeutic moiety, provided that evidence is supplied that such differences do not induce changes in pharmacokinetics, pharmacodynamics and/or in toxicity which could be clinically meaningful (i.e. theophylline and aminophylline).
- ii) Therapeutic equivalence may also include pharmaceutical equivalents or alternatives showing suprabioavailability if a comparative bioavailability study has shown that the product in question after dose adjustment displays the same bioavailability as the corresponding innovator product.

APPENDIX II

Explanation of the symbols in paragraph 3.3

C_{\max} :	maximal plasma concentration;
C_- :	minimal plasma concentration;
C_{av} :	average plasma concentration;
t_{\max} :	time passed since administration at which the plasma concentration maximum occurs;
AUC_t :	area under the plasma concentration curve from administration to time t ;
AUC :	AUC extrapolated to infinite time;
AUC :	AUC during a dosage interval in steady state;
MRT :	mean residence time;
Ae :	cumulative urinary excretion from administration until time t ;
Ae_{∞} :	cumulative urinary excretion extrapolated to infinite time;
$d\text{Ae}/dt$:	urinary excretion rate;
t :	plasma concentration half-life;
F :	$(C_{\max} - C_-)/C_{\text{av}}$.

APPENDIX III

Technical Aspects of Bioequivalence Statistics

The pharmacokinetic characteristics to be tested, the procedure for testing and the norms to be maintained should be stated beforehand in the protocol. A post hoc change of the methods described for the statistical evaluation is only acceptable if protocol adherence would preclude a meaningful evaluation and if such change of procedure has been fully justified.

In testing for equivalence of the main characteristics AUC and C_{\max} , the multiplicative model is used which has as consequence that data should be logarithmically transformed before statistical analysis.

Acceptance ranges for main characteristics:

AUC-ratio: The 90% confidence interval for this measure of relative bioavailability should lie within a bioequivalence range of 0,80-1,25. In case of an especially narrow therapeutic range the acceptance range may need to be tightened. A larger acceptance range may be acceptable if inevitable and clinically acceptable.

C_{\max} -ratio: This measure of relative bioavailability is inherently more variable than e.g. the AUC-ratio, and a wider acceptance range may be necessary. The range used should be justified taking into account safety and efficacy considerations.

t_{\max} -diff: Statistical evaluation of t_{\max} only makes sense if there is a clinically relevant claim for rapid release or action or signs for a relation to adverse effects. The non-parametric 90% confidence interval for this measure of relative bioavailability should lie within a clinically determined range.

others: In the case of other characteristics the 90% confidence intervals have to be calculated for the difference or the ratio of expected medians depending on the assumption of, respectively, an additive or a multiplicative model.