



Bioequivalence

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Abstract

Exposure in the central circulation is an essential prerequisite for efficacy and safety of systemic drugs. After oral administration the rate and extent of absorption from the gastrointestinal

tract may be significantly determined by the biopharmaceutical properties of both, the drug substance as well as the drug formulation. This is the reason why rate and extent of bioavailability of oral dosage forms needs to be characterized thoroughly during drug product development for regulatory submission. In case of generic medicinal products assessment of bioequivalence in comparison to an appropriate reference product is the basis for marketing authorization applications and the approval process. The

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requirements for this procedure are therefore clearly defined in the bioequivalence guidelines published by the competent regulatory authorities, e.g. EMA in Europe or FDA in the USA.

Most relevant parameters to be considered in this context are discussed in this chapter. Since there are still certain differences between the main guidelines activities have been started to discuss the basis for science-driven regulations and to harmonize the existing requirements. In particular the Global Bioequivalence Harmonization Initiative (GBHI) of the European Federation for Pharmaceutical Sciences (EUFEPS) has achieved significant contributions to this process.

Introduction and Definitions

Bioavailability (BA) and bioequivalence (BE) are essential elements in clinical research and drug product development. The characterization of systemic exposure is equally relevant for innovative as well as generic preparations considering that systemic drugs develop their efficacy only if the active ingredient is absorbed into the bloodstream and becomes available at its site of action. It is, therefore, mandatory for all newly developed medicinal products to demonstrate their appropriate bioavailability in a dossier submitted along with the Marketing Authorisation Application. In case of generic products, the assessment of bioequivalence is the most essential prerequisite in order to confirm their therapeutic equivalence with the innovator's reference product in terms of efficacy as well as safety.

The definitions of bioavailability and bioequivalence are very similar in the global regulatory landscape. As an example the following definitions were taken from the CHMP Note for Guidance 2001, which was the last European guideline describing standards for BA and BE:

- **BA:** "Bioavailability means the rate and extent to which an active substance or active moiety is absorbed from a pharmaceutical form and becomes available at the site of action." However, considering that in the majority

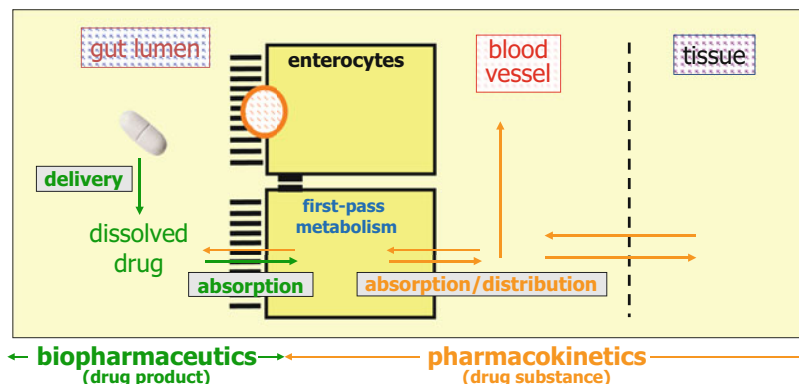
of cases measurement at the site of action is not possible, a more practical definition was suggested taking into account that the substance in the general circulation must be in exchange with its presence at the site of action: "Bioavailability is understood to be the extent and the rate at which a substance or its active moiety is delivered from a pharmaceutical form and becomes available in the general circulation."

- **BE:** "Two medicinal products are bioequivalent if they are pharmaceutically equivalent or pharmaceutical alternatives and if their bioavailabilities 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."

It is important to consider that the assessment of bioavailability should reflect the *in vivo* performance of a drug product after its administration in line with the recommendations given by the labeling. In case of oral products, this includes several important biopharmaceutical processes, starting with disintegration of a solid dosage form in the stomach or proximal intestine, followed by drug release from the preparation and dissolution of the compound. All these processes may have an essential impact on drug absorption into the body. Moreover, also a pre-systemic "first-pass" effect is of importance, which may occur during absorption in the intestinal enterocytes (gut wall first-pass metabolism) or after absorption in the liver (hepatic first-pass metabolism). Major steps in this context are elucidated in Fig. 1:

But there are also other aspects which can significantly affect the bioavailability of oral drug products. In this context the administration conditions – fasted or fed – play a relevant role as they may impact the transit of non-disintegrating solid oral dosage forms through the gastrointestinal (GI) tract. In particular gastric emptying will be significantly delayed in fed state. However, GI transit can also be modified by certain excipients, e.g., accelerated by sugar alcohols such as mannitol, or vary depending on tablet size and shape.

Fig. 1 Disposition of systemic drugs: pharmacokinetic processes



As a consequence, for product comparisons – e.g., with the intention of BE assessment in case of generic drug development – constant administration conditions are essential, while various study settings, fasted and fed state, should be investigated in order to properly characterize and understand product performance in changing situations of treatment.

Concept of BA/BE Assessment

Even though the general study design for BA and BE assessments is very similar (except the statistical sample size estimation and confirmative evaluation of study objectives in case of BE), all regulatory guidelines clearly differentiate between both scenarios. This seems useful considering that only more general suggestions are given for BA studies, while very restrictive requirements have been defined for BE projects. The latter includes also procedures how the pharmacokinetic parameters should be calculated and which to be used for evaluation.

Characterization of Bioavailability

As bioavailability is an essential prerequisite for the efficacy and safety of systemic products, proper characterization of their systemic total and peak exposure as well as pharmacokinetic properties is requested for regulatory purposes of all medicinal products – with only very few

exceptions of preparations with self-explanatory exposure, e.g., gases for inhalation.

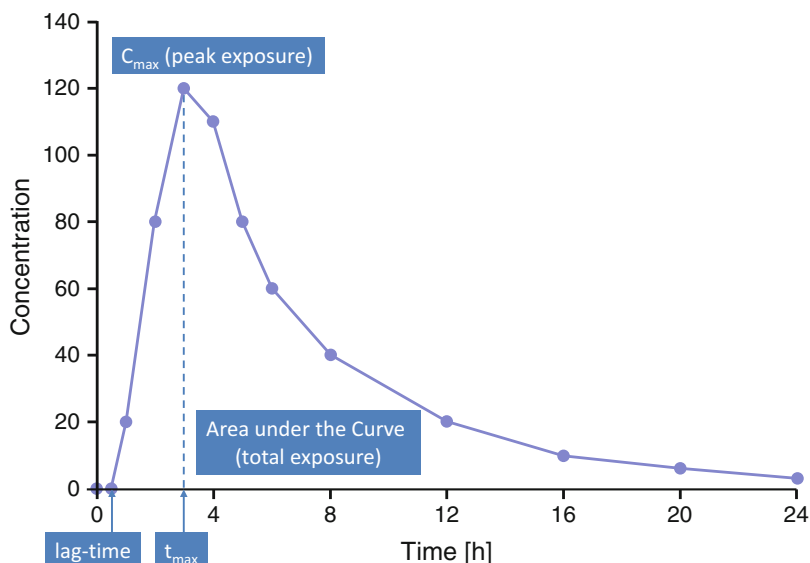
In this context it should be carefully differentiated between properties of the drug substance and characteristics of the drug product. Absorption rate constant, volume of distribution, drug biotransformation, and elimination rate constant as well as total, hepatic, and renal clearances are primarily drug substance-related properties. They should be properly characterized for all active ingredients. However, some of those parameters can also considerably be affected by the properties of the pharmaceutical form, in particular drug absorption. As consequence, systemic total and peak exposure need to be determined as well.

Pharmacokinetic Properties

In order to determine the pharmacokinetic characteristics of an active compound, the drug substance should be administered in a way which allows investigating its *in vivo* properties (absorption, distribution, metabolism, and elimination/excretion) widely unmodified by the biopharmaceutical properties of a pharmaceutical formulation, which can in particular affect drug absorption, which can in particular affect drug absorption but also first-past metabolism to certain extent.

This goal is, however, not easily achievable as any administration of a drug substance needs certain application form which, at the same time, can modify GI transit and drug absorption. The best option in this context is to investigate an aqueous solution either for intravenous (i.v.) or oral administration. Under such conditions uptake of the

Fig. 2 Typical plasma profile after fasted oral administration of a conventional IR tablet



active ingredient should occur widely unaffected by excipients or pharmaceutical technology measures, e.g., tableting.

- **i.v. application:** Pharmacokinetic parameters determined after i.v. application of an aqueous solution are the best measures to characterize drug substance properties as they will not be affected by any modification of drug absorption.
- **Oral versus i.v. administration:** Comparison of orally and intravenously administered aqueous solutions opens the opportunity to determine the rate of drug absorption widely unaffected by a pharmaceutical formulation and its potential impact on other pharmacokinetic parameters like first-pass metabolism which may be affected to certain extent also by the rate of absorption.

Bioavailability

Bioavailability studies generally need to be designed as comparative investigations. Reliable conclusions will not be possible from clinical trials which include only one study arm as the pharmacokinetic (PK) parameters determined in such a case may be modified significantly by the properties of the study population as well as other study conditions. Only comparisons with known

(e.g., approved reference product) or well-established (e.g., oral solution) *in vivo* performance will allow proper conclusion on the bioavailability of a newly developed product, which should reflect the rate and extent of drug absorption.

The main BA characteristics derived from plasma profiles (Fig. 2) are:

- **Total exposure:** The degree of intestinal drug absorption (extent of bioavailability) can be determined as the area under the drug concentration versus time curve (AUC) which has been shown to be proportional to the amount of the active ingredient reaching the systemic circulation.
- **Peak exposure:** This parameter represents the highest value (C_{\max}) in a plasma concentration-time profile. It is dependent on both, the rate of absorption (the more rapid absorption occurs, the higher concentration peaks will be) and the extent of absorption (C_{\max} normally increases with rising AUC). As consequence, this is a “hybrid” PK parameter and, thus, not a perfect characteristic for the rate of drug absorption. On the other hand, C_{\max} is clinically important as it will impact both, efficacy and safety of a drug.

- **Time-point of peak exposure (t_{\max}):** This parameter represents the time-point when the maximum concentration (C_{\max}) of a plasma concentration-time profile is achieved. More rapid absorption will result in shorter t_{\max} values.
- **Lag time:** In cases of a delayed onset of drug absorption, the period between the time-point of drug administration and the occurrence of the first measurable plasma concentration is defined as lag time (t_{lag}). This parameter is primarily affected by drug formulation properties, e.g., in case of gastro-resistant products.

Determination of the parameters C_{\max} , t_{\max} , and t_{lag} deviates between BA and BE studies. While certain degrees of freedom are accepted in BA studies with respect to the calculation of these parameters (including an assessment by use of pharmacokinetic models), restrictive requirements have been defined for BE assessment as clearly stated in the guidelines. C_{\max} and t_{\max} should be directly derived from the measured plasma concentration-time profile as the highest observed concentration and the time-point of its occurrence.

With regard to the study concepts and the preparations administered, the following scenarios should be differentiated:

- **Assessment of absolute bioavailability:** In this case preparations administered on a non-parenteral route will be compared with an intravenous application form. Values determined as absolute bioavailability allow conclusions on certain loss in exposure due to limited absorption or first-pass metabolism. Such conclusions will be best if the compound administered via the non-parenteral route is given as (aqueous) solution.
- **Assessment of relative bioavailability:** In this case preparations are administered via the same route in order to allow comparison of their biopharmaceutical properties. However, also systemic exposure of products applied on different ways – e.g., orally versus topically – may be compared. In the latter case an

appropriate interpretation of the results needs to consider also information on divergent conditions for drug absorption (and first-pass metabolism) at the different sites of application.

While the absolute bioavailability is more a drug substance-related characteristics, the relative bioavailability should allow relevant conclusions on the biopharmaceutical properties of drug products and, thus, the quality of their formulation.

Assessment of Bioequivalence

In contrast to more general suggestions for BA studies, very strict and binding requirements have been defined in the guidelines for the assessment of bioequivalence as basis for an “abridged application” in Europe resp. an “Abbreviated New Drug Application” in the USA. This includes, among others, the following aspects in particular:

- **Prospective study planning:** All conditions for study conduct and evaluation of the results should be pre-specified in the protocol. Later changes or post hoc extensions of the project will not be acceptable in the majority of cases.
- **Statistical sample size estimation:** This essential element of a prospective study planning should assure that the number of subjects included into the clinical part will be sufficient for confirmation of BE (in case the products are comparable enough for such conclusion).
- **Administration conditions:** In general fasted state is considered most discriminative and, thus, most sensitive to detect differences between products. However, BE may deviate between fasted and fed administration. The US-FDA therefore often requests BE assessments under both conditions. In the EU those conditions should be applied for BE assessments which are suggested for the reference product in its labeling.
- **Reference product:** The reference product should be a medicinal product which has been approved based on full clinical documentation.

In the USA these products are defined by the FDA in the *Orange Book* as “Reference Listed Drugs.” In Europe the identification of the appropriate reference product is often not as easy, especially in cases of older products (“grandfather’s drugs”), and in such cases it may be advisable to contact the agencies for confirmation of the right selection.

- **Characterization of systemic exposure:** As mentioned above clear rules have been defined for the determination of plasma profiles (e.g., use of a validated bioanalytical procedure, sufficient number and proper distribution of sampling time-points over the entire curve, etc.). Compliance with these requirements is normally strictly enforced by the reviewers in BE studies conducted for regulatory purposes, e.g., for generic drug applications.
- **Statistical evaluation and bioequivalence assessment:** The statistical methods to be used for BE assessment and the general acceptance criteria for a conclusion on bioequivalence are also clearly defined in the guidelines and need to be considered in studies for regulatory submission.

Even though guidelines only present suggestions concerning the appropriate study design and clinical conduct of BE investigations as well as the statistical evaluation of study results, applicants are well advised to principally follow those suggestions. However, one should be aware that BE requirements can never apply to a “one-size-fits-all” principle, and thus, it is essential to adequately “adjust” those suggestions given by the guidelines to the individual question(s) to be investigated.

A proper clinical rationale should be the guiding principle to define all details in study design, clinical conduct, and statistical evaluation. All these aspects should be laid down a priori in the study protocol, and all measures need to follow these definitions. This includes also all relevant aspects of the “Good Clinical Practice” requirements described in the international guidelines as well as the ethical principles laid down in the Declaration of Helsinki.

Design and Conduct of In Vivo Bioequivalence Studies

The general concept and basic principles of bioequivalence studies will be described in the following. More practical details may be found in regulatory guidelines and product-specific guidances relevant for each jurisdiction.

Concept and Essential Elements of BE Studies

Assessment of bioequivalence as basis for generic drug approval should be established on a comparison of medicinal products containing same active ingredient(s) in same molar dose in “comparable” (US-FDA: “same”) dosage forms, e.g., tablets with tablets or also tablets with capsules.

Normally these studies are conducted in healthy volunteers following a crossover design as this is considered most appropriate in order to reduce the within-subject variability and, thus, should allow a more discriminative comparison between the products. Only in exceptional cases BE studies will be conducted in a patient populations, in particular if investigations in healthy subjects do not seem adequate and acceptable due to safety or tolerability concerns, e.g., in oncology. Thus, medical and ethical aspects need to be taken into account as well.

Single-Dose Studies

Assessment of systemic peak and total exposure after single-dose administration is the most conventional approach in the clinical development of generic drug products. Such design is generally considered most sensitive in detecting potential differences between formulations.

Administration occurs in fasted or fed state together with 240 mL of non-carbonated water. In this context different requirements are relevant in Europe (similar in Canada and Australia) or in the USA. While the EU guidelines generally suggest an administration according to the labeling of the reference product, the US-FDA more often requires BE assessments after both fasted and fed administration, also in cases of immediate-

release dosage forms. Detailed recommendations are given by product-specific guidances.

Plasma concentration versus time profiles will be determined in order to properly characterize the absorption, distribution, and elimination phases of the active ingredient. All relevant parts of a profile should be adequately described by measured values. In order to determine the bioavailability of the medicinal products sufficiently, minimum of 80% of the area under the curve (representing the extent of absorption) should be covered by measuring points.

For BE assessment total and peak exposure need to be considered and compared between the products on an individual basis (test and reference comparison for each subject). Total exposure – representing the extent of absorption – should be appropriately described by the AUC (area under the plasma concentration-time curve) which is calculated from all measured values. Peak exposure (C_{\max}) will be taken directly from the plasma profiles as the highest determined concentration.

Other pharmacokinetic parameters of interest are the time of peak concentration (t_{\max}), the lag time (t_{lag}), or the elimination half-life ($t_{1/2}$). They are, however, only considered descriptively (and not for confirmative analysis) as they are normally highly variable and, moreover, are predefined by the time-points for plasma sampling (t_{\max} , t_{lag}). Thus, they are not considered suitable – and not mandatory – for assessment of bioequivalence.

Multiple-Dose Studies

Even though multiple dosing can be considered most relevant for the treatment of chronic diseases and, thus, should reflect therapeutic practice more realistically, such setting is not recommended for BE assessment. Rationale for this regulatory decision is that the within-subject variability will be dampened by multiple dosing, and consequently, studies at steady state may be less discriminative and, thus, also less sensitive to detect differences between products.

Exceptions from this general rule are only (1) studies in patient populations who need chronic treatment of their diseases and (2) investigations of modified-release products which release their active ingredient(s) so slowly that significant

accumulation may occur after multiple dosing. The latter may be expected in cases of preparations developing plateau-like plasma profiles with less than 90% of total AUC covered within the intended dosing interval ($AUC_{0-\tau}$). In such cases the European authorities request for additional multiple-dose studies. This is a Europe-specific requirement which is not supported by the US-FDA which does generally not recommend multiple-dose studies for BE assessment except in patient populations.

Administration conditions (fed or fasted) for multiple-dose studies should be defined in line with the recommendations given by the Summary of Product Characteristics of the reference product. This includes normo-caloric instead of high-fat, high-calorie meals and also fasting periods adapted to realistic treatment situations (e.g., if fasted state is suggested by the SmPC, the post-dose fasting period must not be maintained for 4 h).

It is essential to confirm that steady state is achieved at the profiling day. Plasma profiles need to be characterized for at least one dosing interval; however, blood sampling over 24 h may be considered in order to take also diurnal variations in pharmacokinetics into account.

Similar to the assessment of BE in single-dose settings, total and peak exposure should be considered for comparison between the products also at steady state. Total exposure will be appropriately described by the AUC calculated from the measured values during the dosing interval (it has been demonstrated that this area [$AUC_{0-\tau}$] equals the $AUC_{0-\infty}$ in case of single-dose studies). Peak exposure (C_{\max}) will be taken directly from the plasma profiles as the highest determined concentration. An additional important parameter for BE assessment at steady state is the trough value determined at the time-point of next dosing (end of dosing interval). This concentration is an important efficacy-related characteristic.

Another pharmacokinetic parameter of interest is PTF, the peak-trough fluctuation, which describes the range of fluctuation between maximum and minimum concentrations of a profile. PTF is, however, only considered descriptively as its variability is normally high and, thus, is

normally not suitable for confirmative BE analysis. Relevant steady-state parameters are shown in Fig. 3.

Drug-Food Interactions (Food Effect Studies)

The intake of food (or caloric beverages, including alcoholic) will immediately initiate a significant change in gastrointestinal physiology. Relevant alterations include an increase in gastric pH, bile secretion, and blood supply to the intestine. These modifications may impact the solubility of compounds and, consequently, their dissolution from medicinal products and often also the rate and extent of drug absorption. First-

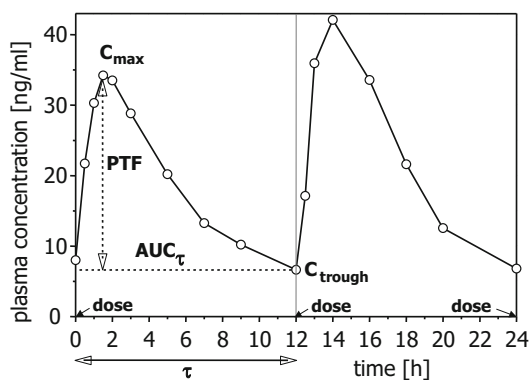


Fig. 3 Typical plasma profile and PK parameters after multiple dosing/at steady state

pass effect may be affected as well – especially in the gut wall – and decrease in certain cases with the consequence of rising systemic exposure of the drug substance. On the other hand, drug absorption might also be reduced due to binding or complexation of compounds by food ingredients.

These are reasons why the US-FDA and some other authorities request BE studies in both, fed and fasted state, even in case of immediate-release drug products. So far such investigations are less frequently required in Europe.

There is, however, another essential change in gastrointestinal physiology induced by food intake: the GI transit of chyme – and non-disintegrating solid oral dosage forms as well – will occur retardedly after administration in the fed state. In this context a delay in gastric emptying is of particular relevance. Prolongation of gastric residence time can significantly impact the systemic drug exposure, especially in case of modified-release dosage forms.

Such meal-induced delay in gastric emptying is elucidated in Fig. 4: This example shows a generic alternative of the innovator's nifedipine OROS (*oral osmotic pump*) preparation for once daily administration (Schug et al. 2002). The generic matrix tablet had been developed as enteric-coated form. The rationale for this surprising measure (there is no obvious reason for such coating in case of nifedipine tablets) was the

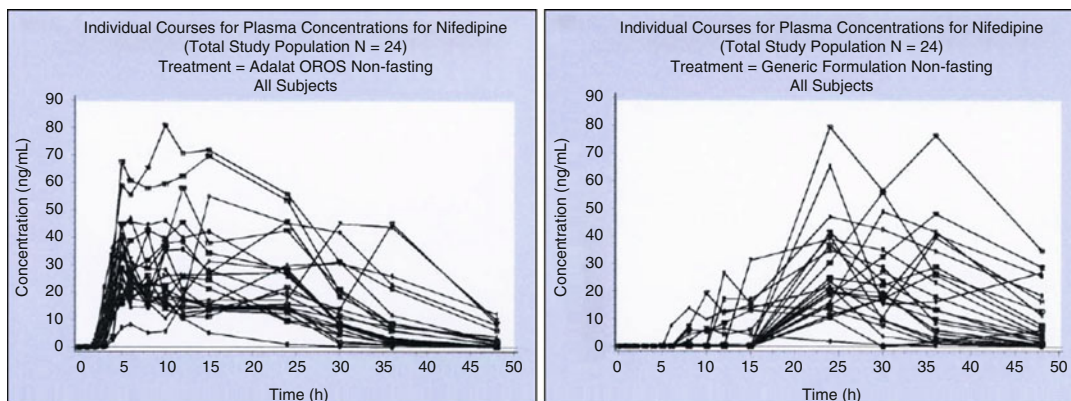


Fig. 4 Nifedipine plasma profiles after fed administration of enteric-coated tablets (left) or the OROS preparation (right) (Schug et al. 2000)

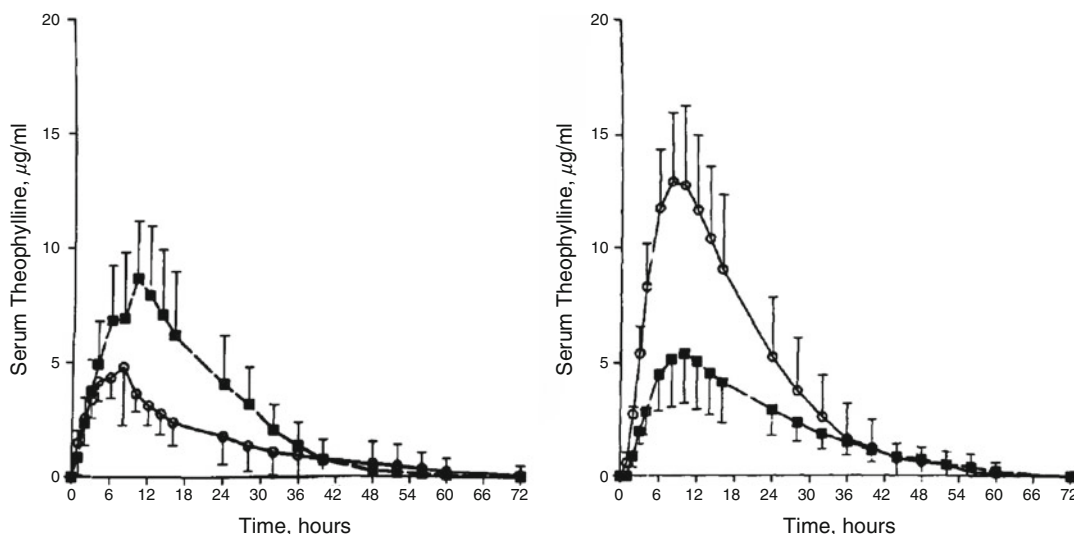


Fig. 5 Food-induced changes in exposure of nifedipine prolonged-release tablets: increase in AUC in one case (left graph) and reduction in another (right graph) (Karim et al. 1986)

attempt to mimic the delayed-release character of the innovator product (typical for an OROS). The coating avoids drug release during gastric residence. The consequences are, however, drastic – and certainly unintended: in fed state onset of drug absorption was delayed by 16 h or even longer in the majority of subjects while only by 2–4 h after intake of the osmotic system.

Numerous examples can be found in the literature demonstrating food-induced changes in exposure which can, moreover, vary between modified-release products containing the same active ingredient. Systematic investigations in the 1980s with several theophylline prolonged-release preparations marketed in the USA discovered considerably different and sometimes even opposite food effects between the products. Two very distinctive examples are shown in Fig. 5 (Karim et al. 1986).

These examples indicate that the changes initiated by food ingestion are not drug substance related but caused by the biopharmaceutical properties of the formulation. Based on these findings – which elucidate the general importance of food-induced changes in the exposure of modified-release preparations – such food interaction studies are meanwhile requested for all MR products by the international guidelines.

Food effect studies should be designed in order to investigate “worst-case” scenarios. Thus, the products should be administered immediately after a high-fat, high-calorie meal (50% fat, 800–1000 kilocalories) in comparison with intake after an overnight fast. All other conditions for BE assessment are identical as in studies after fasted administration.

Thus, in case of modified-release generic products, BE needs to be demonstrated after fasted as well as fed administration in comparison to the approved reference preparation.

Bioanalysis

Optimum conditions for the analytical determination are necessary in order to obtain reliable information from bioequivalence studies. Moreover, results reported from the studies need to be transparent and traceable from raw data to the finally listed values. All measures should assure reproducibility of the data. Parameters like selectivity, accuracy, and precision of the method as well as stability of the samples in all phases of the determination are essential elements in this context.

Bioanalytical method validation has, therefore, become one of the major issues in developing science-based regulations. Detailed requirements for pre-study and within-study validation

procedures have been defined in CHMP and US-FDA guidelines, and compliance with these is critically reviewed during the regulatory assessment of reports submitted along with the Marketing Authorisation Application. It is, thus, recommendable in BE studies for regulatory submission to carefully consider the suggestions given by these guidelines.

Analytes to Be Measured

Generally BE assessments should be established on the plasma concentration-time profiles determined for the parent compound. This requirement is supported by the assumption that these data reflect best the in vivo performance of the drug product after administration.

In certain cases measurement of metabolites may be recommended, especially if the parent drug concentrations are very low – e.g., in case of compounds undergoing pronounced first-pass metabolism – and, thus, their reliable analytical determination is difficult even with high-sophisticated analytical procedures. However, also in such situations, other attempts should be taken into consideration with the intention to achieve measurable plasma concentrations of the parent drug, e.g., administration of higher (event supra-therapeutic) single doses.

These rules should also be applied to inactive prodrugs as even in these cases the parent compound should reflect most appropriately the performance of the products.

Statistical Analysis and Conclusion on Therapeutic Equivalence

The statistical procedures to be used for BE assessment are also clearly defined in the major international guidelines. After intensive discussions in the 1980s and 1990s, broad consensus has been achieved, and, accordingly, 90% confidence intervals for the ratio of the population geometric means should be calculated for the parameters used for confirmative BE analysis (e.g., AUC and C_{\max}). This approach is equivalent to a two one-sided test procedure. Basis for such statistical calculations is an analysis of variance (ANOVA) applied to the parameters under consideration. The use of non-parametric analyses is generally not recommended.

Details of the procedure used for statistical analysis should be pre-specified in the protocol.

Results obtained from parametric statistical analysis need to be evaluated and interpreted for BE assessment by use of the preset bioequivalence acceptance criteria. There is meanwhile international consensus to apply an 80.00–125.00% acceptance range in this context. As long as the 90% confidence interval lies completely inside this range, conclusion on BE is accepted. Otherwise bioequivalence cannot be confirmed by the study results. In case of narrow therapeutic index drugs, even more restrictive requirements may need to be applied, e.g., 90.00–111.00% acceptance limits. In specific cases this will be indicated in the product-specific guidances published by EMA or US-FDA.

It is common understanding to conclude from bioequivalence on therapeutic equivalence of generic medicinal products. This is in line with the concept of an abridged application in the EU (resp. the Abbreviated New Drug Application procedure in the USA) considering that all information of the clinical documentation submitted for the reference product may also apply for a generic alternative with confirmed BE.

Immediate-Release Versus Modified-Release Oral Products

Conditions and requirements for BE assessment deviate considerably between immediate-release and modified-release medicinal products. Consequently, separate guidelines have been edited for both types of products in the majority of jurisdictions.

Immediate-Release Products

This group of products includes solid oral dosage forms like tablets or capsules but also some other preparations, e.g., effervescent tablets, granules, as well as oral dispersible forms.

In these cases, BE assessment based on the PK characteristics AUC (for total exposure) and C_{\max} (for peak exposure) is generally considered sufficient to describe the products' BA and to conclude on BE. In most cases studies under one condition

Fig. 6 Calculation of partial AUCs for better description of product's profiles according to the CHMP guideline for MR products

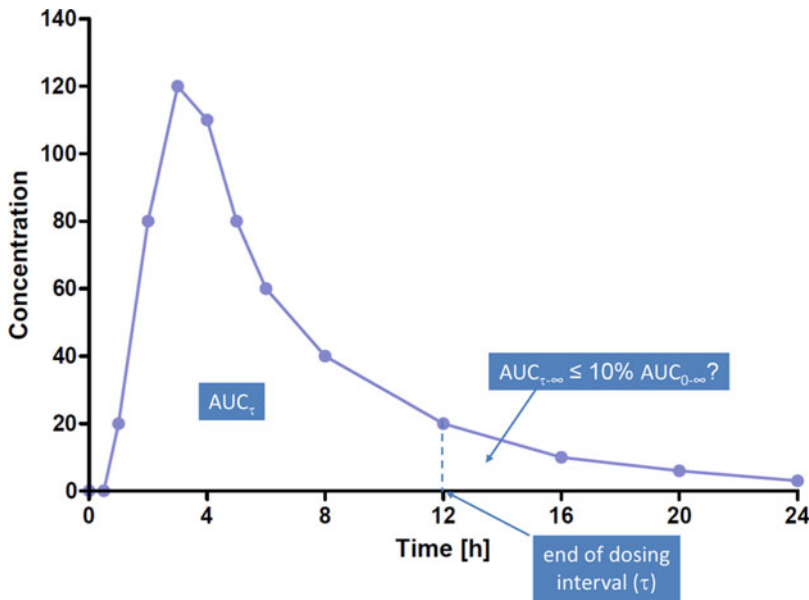
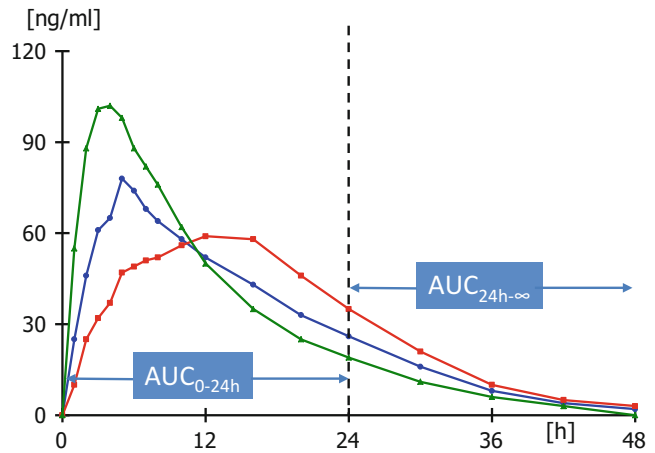


Fig. 7 MR products: evaluation of likelihood of accumulation ($AUC_{0-\tau}$ covers less than 90% of $AUC_{0-\infty}$)

– in the more discriminative fasted state (FDA) or considering the suggested conditions for the reference product (EU) – may suffice; however, fasted and fed administration are requested with increasing frequency.

In certain cases, *in vivo* studies may be replaced by *in vitro* bioequivalence assessments as described more in detail in section “BCS-Based Biowaiver Concept.”

Modified-Release Products

This group of medicinal products is more complex, and thus, the biopharmaceutical properties of the products require a more comprehensive characterization. Thus, consideration of AUC and C_{max} as exposure parameters is not sufficient – despite the regulatory practice for decades.

Prolonged- or sustained-release preparations, delayed-release (e.g., enteric-coated) forms, and – more recently – also products with “pulsatile” or “multiphasic” release are allocated to this group,

and often the terminology used in this context is not precise, and thus, deviating names may be used for the same type of products.

Considering the more complex nature of these preparations and their biopharmaceutical properties, additional pharmacokinetic parameters need to be included in order to describe, e.g., a “plateau-like” or “biphasic” profile. The most appropriate procedure should be defined case by case. A way suggested by the revised CHMP guideline (2010) is the calculation of partial AUCs in order to describe the course of the profile more properly and allow adequate BE comparison. Figure 6 describes such an example:

Moreover, European authorities also request for conduct of multiple-dose studies in case of such MR products where an accumulation is “likely” during maintenance treatment (thus after multiple dosing). This may be assumed if less than 90% of the total AUC after single-dose administration is achieved during the intended dosing interval (Fig. 7).

The BE requirements of MR products deviate, thus, considerably between EMA and FDA. While FDA principally applies same criteria as for IR preparations, EMA suggests additional parameters for single-dose studies (i.e., partial AUC in order to compare the shape of profiles more appropriately) and requests for investigations at steady state in case of products with certain likelihood for accumulation. The requirement of conducting fed state studies in case of all MR forms is identical in both regions.

Special Application Forms

The principles developed for BE assessment of orally administered systemic products can generally also be applied to non-oral formulations and under certain conditions even to non-systemic products.

Non-oral Systemic Drug Products

Considering that the active ingredient needs to be absorbed into the central circulation in case of all systemic drugs, conclusions on bioequivalence should also be established in case of non-oral

forms based on PK parameters derived from plasma concentration-time profiles. Thus, in this context there is no relevant difference between oral and non-oral preparations. However, the specificities of site and way of administration via the non-oral route need to be carefully taken into account.

Topical (Non-systemic) Drug Products

The situation is more complicated in case of non-systemic (“topical”) drugs. In certain cases concentrations in plasma remain on such low level that reliable measurement and profiling is not possible, even with advanced analytical techniques. On the other hand, conclusions from the exposure in plasma to the concentration-time courses at the site of action should also be possible in certain situations. These, however, need to be defined and justified case by case.

Generally, the following paradigms have been developed for non-systemic drugs:

- **Plasma concentrations as safety measure:** Comparison of plasma concentrations measurable after administration of the generic alternative and the innovator product is considered an appropriate option to conclude on potential safety differences between both forms.
- **Assessment of therapeutic equivalence:** As long as the bioequivalence approach is not applicable (with convincing justification), clinical studies in patient populations will be needed in order to confirm therapeutic equivalence of both products.

Finally it will be a case-by-case decision how therapeutic equivalence can be appropriately confirmed in case of non-systemic drugs.

Special Challenges and Specificities in Bioequivalence Assessment

In some cases BE assessments require special approaches, e.g., if certain particularities of a reference product hamper the confirmation of bioequivalence of generic medicinal products.

Highly Variable Drugs

High variability of pharmacokinetic parameters makes BE assessment difficult and requires special measures in most cases. Sources of variability can be drug substance related but may also be caused by unfavorable properties of the drug product. Often the application of carefully controlled and consistent conditions in the clinical conduct and the bioanalytical determinations is not sufficient, and inclusion of a considerably higher number of subjects can be unavoidable.

Such increase in the number of volunteers can, however, approach or even exceed limits where reliable clinical conduct cannot be guaranteed any more. Special features have been developed for such exceptional situations, in particular scaling procedures considering the high within-subject variability determined for the reference product in the same study. This approach is only applicable to those drugs with a within-subject variability above 30%. Moreover, scaling is limited to AUC in Europe, while it is also applicable to C_{\max} in the USA.

Details for study design and evaluation are described in the relevant guidelines.

Narrow Therapeutic Index (NTI) Drugs

There is a long and controversial international discussion on the question whether more restrictive BE acceptance criteria (e.g., 90.00–111.00%) need to be applied for NTI drugs.

On the other hand, there are strong arguments suggesting that especially the NTI drugs will tend to exhibit relatively low variabilities as otherwise safe treatment would not be possible with such drugs. Improvement of constant dosing from tablet to tablet (content uniformity) and stability of the given dose confirmed in smaller acceptance ranges until end of shelf life is often considered a more relevant criterion than tightening the BE acceptance range. This was, for example, the outcome of the discussion on bioequivalence in case of levothyroxine.

Hierarchy in BE Assessment Procedures

In general there are various alternatives for the investigation of bioequivalence or therapeutic equivalence, i.e., in vitro testing, PK comparison,

and pharmacodynamic or clinical studies. There is, however, a clear hierarchy of these options with absolute priority for BE assessment by means of PK measurements or the BCS-based biowaiver approach, if applicable.

On the other hand, pharmacodynamic or clinical investigations are only acceptable in such rare cases where PK measurements are not reliably possible, and thus, generally preferred approaches cannot be realized.

BCS-Based Biowaiver Concept

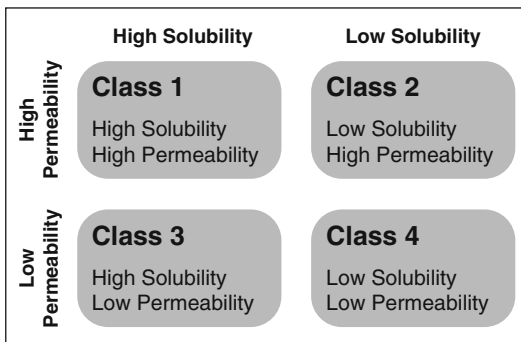
In certain cases the conclusion on bioequivalence can be established on comparative in vitro dissolution testing, and thus, in vivo clinical studies may be waived.

History and General Concept

For this purpose a Biopharmaceutics Classification System (BCS) has been developed in the 1990s (Amidon et al. 1995) and subsequently introduced into regulatory guidelines, first in the USA and later also in Europe.

The development of the BCS concept was initiated by a general discussion on the dissolution properties of solid oral dosage forms and their predictive value for the in vivo performance at the BIO-international conference 1994 in Munich, Germany. It was hypothesized that the systemic exposure should approach that of an oral solution and thus be self-evident in cases of immediate-release forms which dissolve their active ingredient(s) rapidly and completely during gastric residence. In such cases the active ingredient is emptied in solution from the stomach to the intestine where absorption will occur. Consequently, in vivo BE assessments might be waived for such products.

This concept was considered applicable for highly soluble and highly permeable compounds in rapidly dissolving drug products. Consequently drug substances were classified according to their biopharmaceutical properties as follows:



The conditions for allocation of compounds to these BCS classes as well as the dissolution requirements were defined considering the physiological environment in the GI tract:

- **Solubility:** “High solubility” is concluded if the highest dose strength (EU: highest dose) is completely soluble in 250 mL (considering that tablets should be taken with a full glass of water) of aqueous media of pH 1.2 (representing the fasted stomach), 4.5 (representing the fed stomach), and 6.8 (representing the small intestine).
- **Permeability:** Considering that highly permeable drugs should be completely absorbed, compounds with measured extent of absorption exceeding 85% are classified as “high permeability” drugs.
- **Dissolution:** Dissolution is considered as “complete” if minimum 85% of labeled content are in solution and as “rapid” if this limit is achieved within 30 min, which is assumed as mean residence time of tablets in the stomach. Experiments should be performed in buffered solutions of pH 1.2, 4.5, and 6.8 (see above).

For a BCS-based biowaiver application, the dissolution properties of the test formulation (e.g., a generic development product) and the reference product (e.g., the innovator product) need to be compared and their “similarity” confirmed. This is the case if dissolution is complete (>85%) within 15 min, while in cases of slower drug release, dissolution profiles should be compared statistically, e.g., by use of the f_2 equation (details defined in the guidelines).

Extension of BCS-Based Biowaiver Applications

Already during the development of the BCS concept, it was argued that class III drugs (high solubility, low permeability) should be even better candidates (than class I) for a BCS-based biowaiver (Blume and Schug 1999). Consequently, an extension to class III compounds was suggested. However, it took more than 10 years to implement this proposal into the current guidelines, first in the EU (2010) and later in the USA (2015).

Nowadays BCS-based biowaivers are applicable to BCS class I and class III compounds in rapidly dissolving products. In this context the guidelines define more restrictive dissolution requirements for class III (>85%/15 min) than for class I drugs (>85%/30 min) in order to be on the safe side with this extension. There are, however, findings from simulation experiments indicating that the risk of a false-positive conclusion on bioequivalence based on the BCS concept is considerably lower in case of class III than in class I compounds.

On the other hand, compounds with incomplete – and often site-dependent – absorption may be more sensitive to interactions with excipients which might impact GI transit, intestinal absorption, and metabolism in the gut wall. Thus, the potential influence of differences in excipients used in both investigational products (test and reference) needs to be carefully considered and justified.

Regulatory Requirements and Need for Harmonization

The scientific basis for regulatory requirements in bioequivalence has continuously been developed and further optimized during the last four decades. On the other hand, deviations between the regions – e.g., Europe, North America, and Japan – still exist in several details, and this makes global development of medicinal products, especially generics, difficult. As consequence more

than one BE study may be necessary to fulfill the regional requirements.

With the intention to fill this gap and to harmonize the divergent regulations, several initiatives have been started, in particular the series of BIO-international conferences in the 1990s and currently the EUFEPS Global Bioequivalence Harmonization Initiative (GBHI).

The BIO-International Conferences: Toward Science-Driven Regulations

Primary intention of the BIO-international conferences was originally to discuss open issues in bioequivalence and to support the development of science-driven regulations. Scientist from academia and industry contributed to this process and important progress was achieved in exchange with experts from regulatory authorities. Examples for essential advancements were, among many other detailed achievements, the development of the Biopharmaceutics Classification System or the scaling procedure for the investigation of highly variable drugs.

Results and conclusions of the discussions have been summarized in conference reports (McGilveray et al. 1990; Blume and Midha 1993; Blume et al. 1995; Midha et al. 1996, 2005) and, even more important, were incorporated in new or revised guidelines. Considering that this implementation was not identical in all cases, discussions on harmonization were started more recently in smaller BIO-international conferences held in the 2000s in London.

The EUFEPS Global Bioequivalence Harmonization Initiative

A more systematic approach in harmonization of BE requirements was started by EUFEPS, the European Federation for Pharmaceutical Sciences, with its network on biopharmaceutics and bioavailability. Essential for the success of this Global Bioequivalence Harmonization Initiative is that key regulatory scientists from EMA as

well as the US-FDA were prepared to support this process significantly from the very beginning.

This harmonization initiative is structured by means of specific conferences which take place every 18 months alternatively in Amsterdam, the Netherlands, or Rockville, USA. Meanwhile three conferences have been held in March 2015, September 2016, and April 2018. Results and conclusions from the discussions are summarized in conference reports (Chen et al. 2018, 2019; The global bioequivalence harmonization initiative: report of EUFEPS/AAPS third conference [Manuscript in preparation](#)) in order to give the scientific community the chance to further contribute to those issues still open even after the intensive debate during the meetings.

It is desirable that these activities will continue further on and will achieve essential success in harmonization. For this purpose significant contribution by all relevant jurisdictions globally is aspired. This initiative might also be supportive for the ICH process which obviously plans taking up BE issues as well, e.g., as first topic the BCS-based biowaiver concept.

Conclusions and Future Perspective

Concepts and requirements for BE assessment have been developed and further optimized during the previous decades. Relevant open issues have been identified and systematically resolved. Meanwhile, most of the international guidelines include appropriate requirements and suggestions for the majority of relevant BE issues. Nonetheless, the scientific community should continue their effort in contributing to further improvement of the current regulations.

Therapeutic Equivalence and Interchangeability

For the clinical use of medicinal products approved based on BE assessments, in particular generic alternatives, the question of their proven therapeutic equivalence in comparison to the innovator product is of essential relevance.

Considering that assessment of BE is generally understood as confirmation of therapeutic equivalence, all generic products may be declared as therapeutic alternatives of the approved reference products. On the other hand, comparisons between the generic products are normally missing, and thus, therapeutic equivalence between all of them may be questionable.

In this context a discussion on “individual bioequivalence” in the 1990s in the USA may be reflected which concluded that BE assessment confirmed the “prescribability” of the generic products, while their “interchangeability” has not been reliably demonstrated. The therapeutic experience with generic substitution in all major jurisdictions did, however, not discover major clinical problems. Nonetheless, every exchange of products during maintenance treatment should be handled with care.

Approval Policy and Reimbursement Decision

In most jurisdictions different committees are responsible for the decisions on marketing authorization and reimbursement. Therefore, marketing authorization for medicinal products does not automatically include reimbursement. In increasing number of countries, the latter depends on the outcome of price negotiations between pharmaceutical industry and health insurance companies. In other countries, e.g., Germany, specific fixed-price limits have been defined for the reimbursement of generic medicinal products. These fixed-price limits are set case by case on a compound- and dose-strength-specific basis by a national reimbursement committee. This system, at the same time, supports the general understanding in the public that all medicinal products listed in such a fixed-price group are interchangeable and, thus, may be used for generic substitution.

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