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Review Article

Theme: Facilitating Oral Product Development and Reducing Regulatory Burden through Novel Approaches to Assess Bioavailability/Bioequivalence
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Bioequivalence Requirements in the European Union: Critical Discussion

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Abstract. The aim of the present paper is to summarize the revised European Union (EU) Guideline on the Investigation of Bioequivalence and to discuss critically with respect to previous European requirements and present US Food and Drug Administration guidelines its more relevant novelties such as the following: in order to facilitate the development of generic medicinal products, the EU guideline includes the eligibility for Biopharmaceutics Classification System (BCS)-based biowaivers not only for BCS class I drugs but also for class III drugs with tighter requirements for dissolution and excipient composition. The permeability criterion of BCS classification has been substituted with human absorbability, as per the Biopharmaceutical Drug Disposition Classification System. The widening of the acceptance range for C_{max} is possible only for highly variable reference products with an additional clinical justification. This scaled widening is carried out with a proportionality constant of 0.760 which is more conservative than the FDA approach and maintains the consumer risk at a 5% level when the intra-subject CV is close to 30%, due to the smooth transition between the scaled and the constant criteria. The guideline allows for the possibility of two-stage designs to obtain the necessary information on formulation differences and variability from interim analyses as a part of the pivotal bioequivalence study, instead of undertaking pilot studies. The guideline also specifies that the statistical analyses should be performed considering all factors as fixed, which has implications in the case of replicate designs.

KEY WORDS: bioequivalence; generic medicinal products; regulatory requirements.

INTRODUCTION

Although bioequivalence (BE) principles have been clearly defined since the early 1990s (*i.e.*, 20% acceptance range (80–125%) for the 90% confidence interval of the ratio between test and reference least square means after log-transformation of the pharmacokinetic parameters of interest, C_{max} and area under curve (AUC)), there is no international consensus on many of the details regarding the requirements for the design, conduct, and evaluation of bioequivalence studies because it has never been a subject of the International Conference of Harmonization. Consequently, each regulatory region, *e.g.*, USA (1–3), Japan (4), European Union (EU) (5,6), Canada (7–10), and South Africa (11), has issued its own corresponding guidelines.

The first BE guideline of the EU "Investigation of Bioavailability and Bioequivalence" was published in June 1992 as part of the Rules Governing Medicinal Products in the European Communities. This guideline was revised as a "Note for Guidance on the Investigation of Bioavailability and Bioequivalence," released in July 2001 (12). Subsequently, clarification on specific topics has been given through Questions and Answers documents (13). Beginning in May 2007, a global update of this guideline was undertaken by the Pharmacokinetic Subgroup of the Efficacy Working Party, now Pharmacokinetic Working Party, and was adopted by the Committee for Human Medicinal Products (CHMP) in January 2010 as "Guideline on the Investigation of Bioequivalence" (effective 1st August 2010) (5). This update was necessary, on the one hand, to clarify the requirements in order to increase the homogeneity within the different member States of the EU so as to reduce disagreements and arbitrations to the Coordination Group for Mutual Recognition and Decentralised procedures (human) (CMD(h)) and CHMP, and on the other hand, to take into account the scientific advances in the field of BE, *e.g.,* requirements for highly variable drugs and biowaivers based on the Biopharmaceutical Classification System.

This manuscript represents the personal opinion of the authors and does not necessarily represent the views or policy of the Spanish Agency for Medicines and Health Care Products or Health Canada.

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In this paper, the revised EU Guideline on the Investigation of Bioequivalence, whose content is said to be limited to immediate release dosage forms with systemic action although it contains general principles applicable to BE studies for any dosage form and defines requirements for several other dosage forms in Appendix II, is summarized and discussed critically. The specific BE requirements for modified release products are defined in a different guideline (5) that is presently under review.

LEGAL ISSUES: GENERIC MEDICINAL PRODUCTS *VERSUS* OTHER TYPES OF APPLICATIONS

For those outside of the EU, it is important to first understand the concept of a generic medicinal product as defined in Directive 2001/83 (14), which is different than the US Food and Drug Administration (US-FDA) concept of generic. In the EU, those products that show equivalence by means of pharmacodynamic or therapeutic equivalence trials with clinical endpoints, *i.e.,* locally acting and locally applied products like inhalation, nasal, cutaneous, gastrointestinal, ophthalmic products, *etc.* are not considered to be generics, but hybrids. Generics are only those whose BE is demonstrated by means of bioavailability studies, *i.e.*, pharmacokinetic studies. In addition, different dosage forms are acceptable in the case of immediate release oral dosage forms, *i.e.*, oral solution and tablet. Furthermore, pharmaceutical alternatives such as different salts, ester, ethers, isomers, mixtures of isomers, complexes, or derivatives of an active substance are considered to be the same active substance, unless they differ significantly with regard to safety and/or efficacy. Some, if not all, of these differences are somewhat difficult to understand when generics are considered to exist to be interchangeable with the reference product. The reason for such criteria is that, in the EU, the pharmaceutical legislation only deals with the approvability or prescribability of medicinal products. A product is approved if the benefit–risk relationship is positive, but this does not mean that it is interchangeable with the reference product. The substitution policy is a national issue that is not regulated by the EU.

Another issue that may be difficult to understand for those outside the EU is that a product that fails to show BE in comparative bioavailability (pharmacokinetic) studies can be approved based on pharmacodynamic/clinical studies showing equivalence, even though these studies are less sensitive to detect differences between products. Again, this is because, in the EU, the objective is not to interchange these hybrid products but to approve them based on a positive benefit–risk relationship.

HOW MANY STUDIES ARE REQUIRED?

The number of BE studies required in the EU has to be deduced based on the physico-chemical characteristics of the substance (*e.g.*, solubility and chirality), its pharmacokinetic properties (*e.g.*, linearity or dose proportionality, food effect/food intake recommendations in the Summary of Products Characteristics (SPC)), and proportionality in composition (to waive studies for proportional strengths). Presently, the European Medicines Agency (EMA) does not publish BE recommendations like the 'Bioequivalence Recommendations for Specific Products' presented on the FDA web page(15), which simplifies notably the development of generic products for pharmaceutical industry.

WHAT STUDIES SHOULD BE SUBMITTED?

In contrast to past practice, the revised Guideline requires the submission of all studies performed with the formulation proposed in the application (*i.e.*, same composition and manufacturing process) with the reference medicinal product marketed in the EU (synopsis only for pilot studies). In addition, synopses of studies conducted during the formulation development should be submitted.

STUDY DESIGN

The only study design change in the revised version relative to the previous guideline is that an additional multiple dose study is not required for immediate release products with non-linear pharmacokinetics (PK; dose- or time-dependent PK). In comparison with the US-FDA guideline no major differences seem to exist with regard to study design since the standard single-dose 2×2 design is recommended in both regions. Obviously, the parallel design is acceptable for drugs with very long half-lives if demographic characteristics (*e.g.,* age, body weight, sex, ethnic origin, smoking status, and metabolic status) that may affect the PK of the drug in both treatment groups are comparable. Therefore, phenotyping and/or genotyping is necessary in parallel designs. Furthermore, replicate designs are recommended for highly variable drugs in order to estimate the within-subject variability of the reference product with the aim of widening the acceptance limits for C_{max} (16).

Multiple dose studies are only acceptable when single dose studies are not feasible due to the following: (1) tolerability/safety concerns that require that the study be performed in patients that cannot have a passive wash-out period or (2) in exceptional cases of low analytical sensitivity that precludes the estimation of the plasma concentration-time profile after a single dose, but that is able to detect the higher plasma levels that occur after accumulation in steady state. As C_{max} after multiple doses is less sensitive to detect formulation differences than C_{max} after a single dose (17,18), the use of a single supra-therapeutic dose is preferred if there are neither solubility nor tolerability limitations.

SELECTION OF THE REFERENCE PRODUCT

Although the definition of reference medicinal products in Directive 2001/83 (14) states clearly that "reference medicinal product" shall mean a medicinal product authorised under Art. 6, in accordance with the provisions of Art. 8, the Notice to Applicants (19) and the Guideline on the Investigation of Bioequivalence (5) have widened the legal basis that a reference product can have. Now, not only products applied based on Art. 8(3), but also those based on article 10a, 10b, or 10c of Directive 2001/83/EC can be considered as an appropriate reference product. As the reference product has to be based on a complete dossier (Art. 8(3)), it is understandable that a licence (Art. 10c) of the innovator could be used as reference when the innovator

is not on the market or that a fixed dose combination (Art. 10b) is a complete dossier for the combination. However, an application based mostly on literature data plus one or only a few clinical studies, which is considered a mixed dossier, a type of complete dossier (Art. 8(3)), is a more controversial reference product since generics of the innovator will be confounded with generics of the mixed dossier. From a scientific point of view, it is evident that those bibliographical applications (Art. 10a) of drugs that are considered to be of well-established use simply because they have been marketed in the EU should not be considered appropriate reference products since these products are approved based on the literature data obtained with other products and in most cases no experiments were carried out with the Art. 10 a formulation. Therefore, usually no BE or comparative bioavailability study has been performed on these products before reaching the market of the EU to link the bioavailability of the proposed product to the product described in the literature, the one with a well-established use. In the USA, it must be challenging to understand how a marketing authorisation can be granted to a product that lacks pre-clinical and clinical data. In fact, those EU legislators that assume that the bioavailability of the new product will not change significantly and the benefit–risk relationship will be similarly positive may be wrong is some cases, *e.g.,* the use of a small amount of sodium laurylsulphate (SLS) in a product approved based on a bibliographical application, will increase the bioavailability of alendronate five- to sixfold (unpublished data). If it is questionable that a bibliographic product should be marketed, it is easy to understand that generics of such a product should not be acceptable.

Finally, although liposomes are not considered to fulfil the EU definition of generic since clinical and/or preclinical studies may be necessary in addition to bioequivalence pharmacokinetic studies, the EMA has validated as a generic/hybrid medicinal product (Doxorubicin Sun) (20) an application making reference to a product (Caelyx® 2 mg/ml concentrate for solution for infusion) approved as a hybrid application (formerly Art. 4.8.(a) (iii) of the EEC Directive 65/65) (21). The reference product containing liposomal doxorubicine was considered a hybrid application that referred to the reference product of conventional doxorubicine (22). Although an abbreviated application cannot refer to another abbreviated application, in this case the generic application refers simultaneously to the liposomal product (hybrid) and the conventional intravenous solution (complete dossier).

Apart from that, the EU guideline is sound in asking for comparisons against the same dosage form of the reference product when available. When the innovator company develops a line extension, it is recommended that comparison of the new dosage form be made with the one nearest to the formulation used in phase III trials.

Finally, as per the revised guideline, the applicant should justify that the batch of the reference product investigated is representative of the reference product in the market comparing at least two batches from the EU market.

NUMBER AND SELECTION OF SUBJECTS

As seen in other similar guidelines, a minimum number of 12 subjects has been defined as a requirement to ensure reliable estimates. Interestingly, the guideline stresses that the model of healthy volunteers is adequate in most instances to extrapolate the results to other populations, but the rare instances where the extrapolation is not adequate are not identified. Therefore, unless these rare instances are identified in the literature, it will have to be assumed that the model of healthy volunteers is always applicable.

STUDY STANDARDIZATION

Standardization of study conditions is in the interest of the sponsor in order to reduce variability and increase the likelihood of demonstrating BE. In the revised guidance, the over-night fasting time has been reduced to at least 8 h and the volume of fluid to be taken with the treatments is identified as at least of 150 ml.

When a study is to be conducted in the fed state, the revised guideline indicates that the timing of food administration must follow the SPC of the reference product. If this information is not provided in detail in the SPC, the administration of the treatments should follow 30 min after the start of the meal, which should be eaten within 30 min.

The guideline states that although concomitant medications should be avoided, contraceptives are permitted as are any other medications considered necessary to treat emergent issues, however, the use of these medications must be reported and it must be demonstrated that they neither interfere analytically nor interact pharmacodynamically.

For those drugs that are taken always in combination with another drug (*e.g.*, drugs to be boosted with ritonavir), the study may be performed in combination or isolation, because BE in one of these scenarios indicates BE in the other since the extent of the interaction will be the same for both products.

FASTING OR FED CONDITIONS

With respect to the administration of food during BE studies, the revised EU guideline still differs from the US-FDA regulations. The approach of the US-FDA (1) is that in order to demonstrate BE a study conducted under fed conditions is required in addition to a fasted study except for in the following situations: (1) class I drugs when both test product and Reference Listed Drug (RLD) are rapidly dissolving and have similar dissolution profiles, (2) when the SPC of the RLD states that the product should be taken only on an empty stomach, or (3) when the RLD label does not make any statement about the effect of food on absorption or administration. In contrast, in general in the EU (5), only a single study conducted in the fasting state is required assuming that it is the most sensitive condition to detect formulation differences. Therefore, the food effect may exist but it is not believed that products with conventional pharmaceutical technology will be equivalent in fasting state and bioinequivalent in fed state as the fasting state is considered more discriminative. Consequently, it is not considered necessary to increase the regulatory burden for such products.

Based on this principle, for drugs that are taken only in the fasted state or irrespective of food, a BE study with that drug must be conducted in fasted state. However, in situations where it is recommended in its labelling that a reference

product be taken only in the fed state, a BE study conducted with that product should generally be conducted in fed state. This "generally" means that if the fed state is recommended in the SPC in order to avoid tolerability problems associated with chronic use in patients, a fasted state study is acceptable as a single dose in healthy volunteers but, if the fed state is required for pharmacokinetic reasons resulting in a systemic exposure that is notably different, the study should be performed in fed state. There is an exception to this approach for products (test or reference) employing special (not conventional) technology (*e.g.*, microemulsions and solid dispersions) that can be taken irrespective of food in that for these products BE has to be shown in both fasted and fed state (*e.g.*, cyclosporine microemulsion).

The advantage of testing the performance of products in the fasted state and the fed state with a high-fat, high-calorie meal, such as is required for many conventional products in the USA, is that the extremes of the food effect are tested and BE with intermediate meals can be assumed. In the EU, if the SPC of the reference product indicates administration with food but does not make specific recommendations with respect to the composition of the meal, studies should employ a high-fat, high-calorie meal and hence, bioequivalence when products are taken with meals with a different more moderate composition, which might be more realistic, is not investigated. The demonstration of bioequivalence in the fasting state and after a high-fat high-calorie meal would represent a bracketing approach where all intermediate meal compositions could be assumed. In contrast, demonstration of bioequivalence in the worst-case scenario of a high-fat high-calorie meal could be considered as not representative of all possible meal compositions. The high-fat, high-calorie meal might be representative of a dinner or a lunch of some European countries but, would not normally be considered a typical breakfast.

Another issue of debate is the composition of the high-fat, high-calorie meal. In the US-FDA, the ingredients of the high-fat, high-calorie meal are defined, *i.e.*, an example test meal would be two eggs fried in butter, two strips of bacon, two slices of toast with butter, four ounces of hash brown potatoes, and eight ounces of whole milk. Substitutions in this test meal can be made as long as the meal provides a similar amount of calories from protein, carbohydrate, and fat and has comparable meal volume and viscosity. In the EU, however, only the caloric content of each component of the meal is defined, which leaves room to employ different types of food according to the dietary habits of the study site. Consequently, the volume, texture, and viscosity of the meal may vary markedly, which could affect the extent of the food effect.

PHARMACOKINETIC PARAMETERS

Non-compartmental methods should be used to estimate conventional PK parameters, *e.g.*, $AUC_{(0–t)}$, $AUC_{(0–\infty)}$, residual area, C_{max}, t_{max}, λ_z, and $t_{1/2}$ in single-dose studies. The parameters to be analysed statistically in a single dose study are C_{max} and $AUC_{(0–t)}$, instead of $AUC_{(0–\infty)}$. For the first time, AUC truncated at 72 h ($AUC_{(0–72)}$) is accepted in BE studies as a substitute of $AUC_{(0–t)}$ (23). For studies conducted at steady state, the parameters for statistical analysis for immediate release dosage forms would be $C_{max,ss}$ and $AUC_{(0–\tau)}$. If urinary data are employed, $Ae_{(0–t)}$ and R_{max}, if C_{max} in plasma is not detectable, should be measured.

Urinary data are only acceptable if the parent cannot be measured in plasma, and it can be justified that urinary excretion reflects plasma exposure. However, the guideline does not indicate a preference between the possible approaches: a study after steady state for the parent drug in plasma, a single dose study with a metabolite in plasma, or a single dose study for the parent drug in urine. This is a case by case decision. Interestingly, in case of a multiple dose study, C_{max} of the parent in plasma does not need to be measured, even if measurable, after the first administration, whereas in case of a single-dose study for the parent drug in urine the C_{max} in plasma, if measurable, should be used instead of R_{max}.

ANALYTE TO BE MEASURED: PARENT OR METABOLITE?

In principle the parent drug has to be measured, even if inactive, due to the higher sensitivity of its C_{max} to detect formulation differences in release rate (18,24,25). However, in the case of pro-drugs or drugs with very low contribution to activity, where BE is very difficult to show due to high variability associated with low plasma levels that disappear very quickly, it is acceptable to measure only the main active metabolite for practical reasons (*e.g.*, mycophenolate mofetil *vs.* losartan) (26). The use of a metabolite as a surrogate of an active parent drug is discouraged. Such a situation would only be considered if the state-of-the-art analytical technology is not able to measure the low concentrations of the parent drug after a single dose. In this case, it would be necessary to justify that a supra-therapeutic dose is not feasible due to tolerability/safety reasons or solubility limitations, and that the metabolite formation is not saturated at therapeutic doses so that the metabolite exposure reflects the parent exposure.

Active metabolites do not need to be measured if the parent drug is measured, even if the PK system is non-linear, although there is experience with some statins showing discordant results between parent and metabolite. However, as these are very exceptional cases, the risk is considered to be minor.

In contrast, in the case of active metabolites formed as a result of gut wall or other pre-systemic metabolism, the US-FDA recommends that the metabolite and the parent drug be measured, but only the parent drug has to be analyzed using a confidence interval approach. The metabolite data are used as supportive evidence of comparable therapeutic outcome and could highlight the existence of marked differences in metabolite exposure. This approach seems to be more adequate for the assessment of statins but unnecessary for most drugs. In addition, the absence of formal statistical analysis makes the data difficult to interpret.

ENANTIOMERS

The revised guideline introduces new recommendations on the need of chiral bioanalytical methods for enantiomer drugs. Chiral methods are necessary when three conditions are met (or unknown): (1) the enantiomers exhibit different pharmacokinetics, (2) the enantiomers exhibit pronounced

difference in pharmacodynamics, and (3) the exposure (AUC) ratio between enantiomers is modified by a difference in the rate of absorption.

In contrast to the US-FDA requirements, it is not necessary that the primary efficacy and safety activity resides with the minor enantiomer, because even if both eutomer and distomer have similar exposure the bias of the achiral method remains (27). In addition, when the AUC ratio between enantiomers is modified by a difference in rate of absorption, at a given rate both enantiomers may exhibit similar PK, but at another rate of absorption the PK will differ (28). Therefore, the first requirement is fulfilled if the third is fulfilled. Consequently, non-chiral methods are acceptable only if it is possible to show that enantiomers have similar pharmacodynamic activity (*e.g.*, omeprazole) or that nonlinear absorption is not present (as expressed by a change in the enantiomer concentration ratio with change in the input rate of the drug) for both enantiomers. Interestingly, the US-FDA compares enantiomer concentration ratio, which changes with time, whereas the EU compares AUC for simplicity, but seems to be less accurate. Unfortunately, according to the revised EMA guideline, a chiral bioanalytical method would not be necessary for etodolac although it was the example that illustrated the non-linear absorption since it only affects C_{max} (29).

Although it is not indicated in the guideline, it can be deduced that for drugs that are pure enantiomers where enantiomer inter-conversion exists and inter-conversion depends on rate of absorption, chiral bioanalytical methods would be necessary.

ENDOGENOUS SUBSTANCES

In BE studies of endogenous substances, factors like dietary intake that may affect the baseline levels should be standardized and baseline correction should be used to estimate pharmacokinetic parameters. Supra-therapeutic doses, if well tolerated and without solubility limitations, facilitate the measurement of the concentrations over baseline provided by the treatment. The type of baseline correction must be pre-defined case by case depending on the characteristics of the substance. In some cases the approach will involve the subtraction of a constant baseline level, which can be the mean of several pre-dose concentrations of each subject, or subtraction of the pre-dose AUC of each subject, when the endogenous levels are not constant. However, these two scenarios do not address the possible feedback mechanisms that may occur after the exogenous administration of the endogenous substance. Therefore, the sponsor is expected to justify the adequacy of a proposed baseline correction strategy. In rare cases where the endogenous levels are negligible with respect to the exogenous ones, baseline correction is not necessary (*e.g.*, supra-therapeutic doses or patients without or with very low endogenous values) (30).

Interestingly, the guideline clarifies that it is essential to ensure the sensitivity of the study by demonstrating separation in exposure following administration of different doses, either in a pilot study or as part of the BE study using different doses of the reference formulation, if this has not been established previously.

STRENGTH TO BE INVESTIGATED

If an application includes multiple strengths and these strengths fulfil certain criteria, it may be sufficient to demonstrate BE at only one or two strengths. The criteria to waive BE studies for some strengths are as follows:

(a) The pharmaceutical products are obtained by the same manufacturing process. It should be noted that it is **now** possible to manufacture them in different manufacturing plants.
(b) The qualitative composition of the different strengths is the identical, although certain excipients like colorants can differ.
(c) The composition of the different strengths are quantitatively proportional, *i.e.*, the ratio between the amount of each excipient to the amount of active substance(s) is the same for all strengths. For immediate release products, coating components, capsule shell, colour agents, and flavours are not required to follow this rule.
In addition, some deviation from exactly proportional compositions are acceptable when the amount of the active substance(s) is less than 5% of the tablet core weight, or the weight of the capsule content, in the strength used in the BE study and the strength to be waived, and one of the following conditions applies:

1. The amounts of the different excipients are the same and only the amount of active substance is changed, or
2. The amount of a filler is changed to account for the change in amount of active substance and the amounts of other excipients is kept constant.

(d) Appropriate *in vitro* dissolution data should confirm the similarity of the dissolution profiles, and
(e) BE has been investigated at the strength(s) that are most sensitive to detect a potential difference between products. The strengths to be tested depend on the pharmacokinetic linearity, more specifically on AUC dose proportionality. It is important to note that C_{max} is not taken into account due to its higher variability, which could make the conclusion of PK linearity/dose proportionality more difficult, although C_{max} is generally more sensitive than AUC to detect solubility-limited absorption (*e.g.*, glimepiride). A simple criterion to conclude AUC dose-proportionality has been included in this guideline for this purpose only: the difference in dose-adjusted mean AUCs should be no more than 25% between the investigated strength and the to be waived strength (*i.e.*, a ratio within 0.75 and 1.33)

In the case of drugs with linear PK, it is sufficient to establish BE with only one strength, usually the highest but, if the drug is highly soluble, any lower strength is acceptable. In any case, for reasons of tolerability or safety, studies with a lower strength will be accepted. On the contrary, a supra-therapeutic dose using preferably multiple units of the highest strength may be acceptable for analytical reasons, if there is neither tolerability problems nor absorption/solubility limitations at that dose.

In the case of non-linear PK with greater than proportional increases in AUC with increasing dose, the BE study should be conducted at the strength in the curve part of the AUC *vs.* dose curve, which is generally the highest strength.

In the case of non-linear PK with less than proportional increase in AUC with increasing the dose, it is essential to identify the cause of the non-linearity. In the case of saturation of transporters (*e.g.,* gabapentin), the lowest strength or any strength in the linear part of the AUC *vs.* dose curve should be tested since at the highest strength the curve is flat and insensitive. In contrast, in the case of non-linearity caused by solubility/dissolution limitations, the lowest and the highest strengths should be studied. The lowest strength (or any strength in the linear part of the AUC *vs.* dose curve) would be the most sensitive if both formulations exhibit a similar non-linearity but, if the new formulation were able to avoid the solubility/dissolution limitations or, in the extreme case exhibit dose-proportionality, the highest strength would be the most sensitive.

For reasons of safety/tolerability or low bioanalytical sensitivity, the dose can be modified as described above for drugs with linear PK.

In addition, the guideline now includes the bracketing approach to investigate only two strengths (extreme cases) when the formulations do not fulfil the criteria to waive BE studies at some strengths (*e.g.,* formulations are not quantitatively proportional in composition and dissolution profiles are not similar). Interestingly, when two strengths have to be investigated and fed and fasting studies are required, it may be sufficient to assess only one strength in both fasting and fed state. Waiver of either the fasting or the fed study at the other strength has to be based on previous knowledge or the information obtained with the strength tested in both fasted and fed state to select the most sensitive condition (fasted or fed). This is controversial since the Applicant should justify which study can be waived and it may be difficult to agree during assessment (*e.g.*, sirolimus immediate release products since the different strengths of the reference product are not bioequivalent when they are tested at the same dose level).

In the case of fixed combinations the proportional composition requirement should be fulfilled for all active substances taking into account that when considering the amount of each active substance, the other active substance (s) can be considered as excipients. In the case of bilayer tablets, each layer may be considered independently.

It is noteworthy that the guideline refers only to strength and does not address the possible need of testing the administered single dose when the single dose is higher than the maximum strength, *e.g.,* in case of low solubility drugs the differences might be detected only at the highest administered dose since the low solubility might not be critical at the maximum strength.

EVALUATION

Potency correction is only acceptable when the difference in potency between the tested products is larger than 5%. Deviations of greater than 5% are only acceptable when it is not possible to find in the European market a batch of the reference product with a potency difference lower than 5% with respect to the batch of the test product. This strategy should be clearly pre-defined in the study protocol according to the certificate of analysis of both products.

The guideline stipulates that subjects that do not provide data for both test and reference product in a cross-over trial (or one period in a parallel study) should not be included in the statistical analysis. Therefore, the use of statistical methods that impute the missing observations based on the observations of the other subjects are not acceptable.

Data from treatments that are not relevant for the comparison of interest should be excluded, *e.g.*, data from references outside of the EU or fed/fasted arms in a 4 period study when investigating alternatively BE in the fasted and the fed state. Otherwise, all subjects receiving treatment should be included in the statistical analysis. In fact, "spare subjects," who are treated but whose samples are analysed only if other subjects withdraw, are not acceptable and all treated subjects should be analysed even if there are no drop-outs.

The guideline stresses that the decision to withdraw a subject must be made before the analysis of his/her samples. Reasons for withdrawal are acceptable if pre-defined in the protocol (*e.g.*, vomiting, diarrohea, need to administer concomitant medication) but, removal on the basis of the statistical analysis (*i.e.*, outliers) or for pharmacokinetic reasons (*e.g.*, implausible values, extrapolation of AUC larger than 20%) is not accepted. However, as described also in the US-FDA guideline, those subjects with significant pre-dose levels (>5% of C_{max}) should be excluded since such a carry-over effect might be unequal between sequences and bias the BE point estimate. Interestingly, an additional reason to exclude "a" subject has been included in the guideline, but it is not clear if it refers to only one exceptional case or if more cases (*e.g.*, two or three) are acceptable, and how many cases are necessary to conclude that the study validity is questionable. According to the guideline, if a subject exhibits no levels or insignificant levels (<5% of the geometric mean of the other subjects) and this erratic behaviour is observed with the reference product, the test product should not be penalised and, consequently, that subject could be removed from the statistical analysis. However, this might question the reliability of the study, similar to cases when AUC extrapolation is more than 20% in more than 20% of the subjects.

The statistical analysis recommended in the guideline is based on the conventional 90% confidence interval for the ratio of the population least square means test/reference of the pharmacokinetic parameters of interest after log-transformation (geometric means). Interestingly, the revised guideline does not require a non-parametric 90% confidence interval for t_{max} but, simply a visual inspection of medians and variability if the onset of action is relevant for efficacy or safety.

The statistical model should be pre-defined in the protocol. Traditionally in the EU, the factors of the ANOVA in a 2×2 cross-over design are sequence, period, subject nested in the sequence and formulation. It is not common to consider the phase within the period when all subjects cannot be dosed on the same day. Importantly, the model has to be analysed as if all factors were fixed. Therefore, subjects should not be considered as random. This has no implication in 2×2 designs since subjects with missing data are excluded

as imputation like the one performed by SAS® Proc Mixed is not acceptable. Therefore, SAS® General Lineal Model (GLM) and SAS® Proc Mixed give the same results when there are not missing data, however, the results will be slightly different in case of replicate designs (26).

The guideline also clarifies that the observation of a significant sequence effect (or period effect) is inconsequential since the existence of a (unequal) carry over effect can be addressed directly with pre-dose samples. However, this is not applicable to endogenous substances.

For the first time, this guideline acknowledges the possibility of a two-stage design to show BE. In this instance, the following should be noted:

(a) The first stage is an interim analysis and the second stage is the analysis of the full data set. The second data set cannot be analysed separately.
(b) In order to preserve the overall type I error, the significance level needs to be adjusted to obtain a coverage probability higher than 90%. Therefore, it is not acceptable to perform a 90% CI at the interim analysis and a 95% confidence interval in the final analysis with the full data set.
(c) The plan to spend alpha must be pre-defined in the protocol. The same or a different amount of alpha can be spent in each analysis. If the same alpha is spent in both stages, the Bonferroni rule (95% confidence interval in both analyses) is too conservative and 94.12% confidence interval can be used. It is also possible to distribute the alpha differently, and as an extreme case, it is acceptable to plan no alpha expenditure in the interim analysis when it is designed to obtain information on formulation differences and intra-subject variability and 90% CI are not estimated at the interim stage.
(d) A term for the stage should be included in the ANOVA model. However, the guideline does not clarify what the consequence should be if it is statistically significant. In principle, the data sets of both stages could not be combined.

Although the guideline is not explicit, even if the final sample size is going to be decided based on the intra-subject variability estimated in the interim analysis, a proposal for a final sample size must be included in the protocol so that a significant number of subjects (*e.g.*, 12) is added to the interim sample size to avoid looking twice at almost identical samples. This proposed final sample size should be recruited even if the estimation obtained from the interim analysis is lower than the one pre-defined in the protocol in order to maintain the consumer risk.

In the revised guideline, the acceptance range has now been defined with two decimal units (80.00–125.00%, except for narrow therapeutic index drugs), like in the US-FDA.

When several studies have been performed the complete body of evidence must be considered. It is not acceptable to ignore failed studies simply because another one has passed. The reasons for the failure should be discussed (*e.g.*, lack of statistical power). A combined analysis (meta-analysis) of all studies can be provided if relevant, however, it is not acceptable to combine failed studies to show BE.

NARROW THERAPEUTIC INDEX DRUGS

In contrast to US-FDA, NTI drugs have a tighter acceptance range in the EU. This revised guideline has defined a 90.00–111.11% acceptance range for AUC of all NTI drugs. However, the classification of drugs as NTI drugs depends on the CHMP and they are not listed in the guideline. C_{max} acceptance range has to be tightened to 90.00–111.11 if it is of particular importance for efficacy or safety of drug monitoring, which is again a decision of the CHMP. For example, requirements for AUC and C_{max} of immediate release cyclosporine formulations have to be tightened both in fasted and fed state studies while only the AUC requirement for immediate release tacrolimus formulations needs to be tightened (26).

HIGHLY VARIABLE DRUG PRODUCTS

In order to confirm that a product is highly variable (CV, >30%) for a given pharmacokinetic parameter, it is necessary to perform a replicate design to estimate its intra-subject variability.

In contrast to the US-FDA, the EU guideline only accepts widening of the acceptance range of C_{max}, not for AUC, and it is necessary to demonstrate that a larger difference in C_{max} is clinically irrelevant. Previously, such justification was required to widen the acceptance range to 75–133%. Now, this decision depends on the intra-subject variability of the reference product, the one in the market whose large variability generally has no clinical relevance, and it can vary from 80.00 to 125.00 when variability is 30% to 69.84–143.19 when it is 50%, the maximum that is accepted. Intra-subject variabilities larger than 50% are not frequent. Although the proper statistical methodology is to scale the average BE, in the guideline, the limits have been scaled for simplicity.

The guideline gives a table as example with the acceptance range that corresponds to different intra-subject variabilities but, the values for other intra-subject variabilities can be obtained with the following formula: $(U, L)=\exp(\pm k \cdot s_{WR})$, where U and L are the widened limits, s_{WR} is the intra-subject variability of the reference product and k is the regulatory constant that has been defined as 0.760 to be consistent with the variability where scaling starts (CV=30%). This has been done in order to have a smooth transition between scaling and no scaling, and to avoid an excessive consumer risk at intra-subject variability slightly higher than 30%, which are very frequent (31). In contrast, the US-FDA employs a proportionality constant that is more permissive (wider limits) and there is a lack of consistency between the CV that corresponds to that constant and the CV where scaling starts to be acceptable (CV= 30%), which increases the consumer risk.

It is worth noting that the guideline clarifies that the estimation of the intra-subject variability has to be reliable and not the result of outliers, the point estimate has to be constrained within 80.00–125.00, and any replicate design is acceptable.

IN VITRO DISSOLUTION AND VARIATIONS

In vitro dissolution tests of the test and reference biobatches at three different buffers (usually 1.2, 4.5 and 6.8) and

the Quality Control media have to be reported for quality purposes and to define specifications but, *in vivo* studies prevail if *in vitro* data differ. However, the discrepancy should be addressed and justified. Similarly, if *in vitro* data do not reflect the *in vivo* data or are unable to discriminate between batches with acceptable and non-acceptable *in vivo* performance, all attempts should be made to develop an alternative method.

The same dissolution test should be carried out to waive proportional formulations. However, where sink conditions are not achievable at certain pH values, the profiles might differ between strengths. To show that this difference is simply due to the different dose, the sponsor should perform studies at the same dose per vessel (*e.g.*, two tablets of 5 mg *vs.* one tablet of 10 mg) or, alternatively, demonstrate the same trend in the reference product by comparing each strength of the test with the corresponding strength of the reference.

The BE guideline is the only guideline in the EU that addresses specific technical requirements for variations since there is no specific guideline similar to Scale-Up and Post Approval Changes guidelines in the US-FDA, but only a Regulation (32) and a Directive (33) about classification. This revised guideline stresses that after reformulation or a change in the manufacturing method that may affect bioavailability, an *in vivo* study is required unless *in vitro* data are considered a valid surrogate. This would only be true in instances of an existing level A *in vitro in vivo* correlation (IVIVC) defined taking into account such a change, or in the case of a Biopharmaceutics Classification System (BCS) biowaiver approach. Therefore, for products containing a low solubility drug where an IVIVC has not been established, a new BE study is always required for changes that may impact bioavailability. The guideline does not specify what may affect bioavailability and it must be decided according to current knowledge.

For BE studies required for a variation, the reference product should again be the innovator product in case of generics or hybrids, and the previous formulations in the case of applications that did not make reference to another product (*i.e.,* complete dossiers, mixed dossiers, fixed dose combinations, and licences). It seems somewhat illogical to require a BE study for a change in bibliographical product when such a comparative BE study *vs.* the product described in the literature was not required for its authorisation.

In those cases where dissolution studies are considered sufficient to ensure equivalent *in vivo* performance after a change, the guideline refers to other guidelines of the Quality section, but it can be assumed that the new product has to be compared with the existing one.

The comparison of dissolution profiles should be performed with the f_2-similarity factor, taking into account not more than one mean value with more than 85% dissolved for any of the formulations and other prerequisites.

BIOEQUIVALENCE REQUIREMENTS FOR SPECIFIC DOSAGE FORMS

Although the guideline deals only with immediate release formulations, its Appendix II provides some guidance not only for immediate release dosage forms, but also for other types of formulations.

According to the guideline, a BCS biowaiver might be considered for orodispersible tablets if it is demonstrated that the active substance is not absorbed in the mouth. However, as the BCS biowaiver is based on the intake of the tablet with a glass of water (*i.e.,* solubility in 250 ml) and the orodispersible tablets are usually taken without water, it would seem appropriate that the solubility criterion be amended accordingly and dissolution should be compared both in the conventional vessels and in vessels, for example, resembling the dissolution in the mouth (*e.g.*, 5 ml of volume) that have not yet been developed.

It is noteworthy that the demonstration of BE without water is considered the worst case scenario and it is assumed that the formulation will be also equivalent with concomitant intake of water. However, such an assumption is questionable when either the test or the reference orodispersible tablet contains mannitol since the presence of water might increase the differences in absorption due to the osmotic effect of mannitol.

For studies conducted without water, the guideline specifies a method of administration to standardize the administration conditions and to ensure the availability of enough saliva (to wet the mouth with 20 ml of water directly before the administration and not to take water within 1 h of administration). The same rules apply for similar dosage forms: orodispersible films, buccal tablets, sublingual tablets, and chewable tablets.

The guideline stresses the importance of excipients in oral solutions since in the past, low solubility drugs, in solution thanks to the addition of co-solvents in the formulation, were not required to show BE. However, different co-solvents might have a different solubilisation capacity and precipitation might differ between different formulations, which in turn might affect bioavailability. Similarly, excipients affecting gastrointestinal transport, absorption, or *in vivo* stability have to be assessed more carefully since a low amount of sorbitol can affect certain drugs like risperidone (34) or small amounts of surfactants are able to increase the bioavailability of low permeability drugs like alendronate, which can be increased up to five- to sixfold (35).

For intravenous aqueous solutions, a waiver of BE studies is not possible if there are differences in composition with respect to excipients that interact with the drug (*e.g.,* complex formation). For intravenous aqueous solutions with a different concentration compared with the concentration of the reference product in a hybrid application, a waiver can be granted since the drugs are diluted in the plasma, as long as there are no safety/tolerability issues related to a higher concentration.

For other parenteral routes, the importance of similarity in viscosity has been highlighted in the revised guideline when different excipients, but comparable ones, are used. This is ensured if the same qualitative and (similar) quantitative composition is employed in the test product.

The guideline also clarifies that demonstration of BE is not required for lipids for intravenous parenteral nutrition.

BE requirements for comparison of intravenous emulsions can be waived if the composition is qualitatively and quantitatively the same and the physicochemical characteristics (*e.g.,* size distribution, Zeta potential, and rheology) are similar, although the guideline does not indicate how similar these have to be, and the conventional quality characterisation does not include a proper comparability exercise.

Similarly, BE requirements for intravenous micelle forming formulations can be waived if the micelles disassemble upon dilution in plasma and the composition is qualitatively and quantitatively the same. Furthermore, such a waiver is also extended to cases with minor changes in qualitative or quantitative composition, as long as the surfactant is not altered. However, it is not evident that other excipients (co-solvents) or differences in their amount do not affect bioavailability or the safety/tolerability profile. For example, a change in co-solvents may cause a different stability and more frequent precipitation in storage, which does not preclude marketing but, may facilitate misuse since these products are not interchangeable. Again, the guideline suggests some *in vitro* test (*e.g.,* critical micelle concentration, solubilisation capacity, free, and bound drug and micelle size) but it does not define a complete list of tests and their corresponding acceptance ranges to ensure similarity.

The guideline clarifies that a waiver of clinical studies is only possible for locally acting and locally applied products formulated as solutions with the same qualitative and quantitative composition, or with minor differences in excipients, as long as it is justified that the minor differences do not alter the local availability of the drug and, therefore, therapeutic equivalence. Importantly, the guideline stresses the need of comparative bioavailability studies with only a superiority limit of 125.00% for safety reasons when there is a risk of systemic adverse reactions. This highlights that the clinical point of view prevails in locally acting, locally applied products as a quality approach would require BE within 80.00–125.00% since a safer product can be a different but not an interchangeable product. In the EU, the clinical demonstration of efficacy would prevail over pharmacokinetic differences, even if clinical endpoints are less sensitive than PK, because products are approved to be marketed, not to be interchangeable. As mentioned earlier, interchangeablility is a national policy which can be impaired by the way the medicinal products are assessed and approved.

BCS BIOWAIVERS

The main advancement of the EU guideline in the area of BCS biowaivers is the acceptance of biowaivers not only for class I drugs, which was mentioned in the previous version, but also for class III drugs under strict conditions. Although there are several differences in approach compared with the US-FDA approach, like the US-FDA, narrow therapeutic index drugs are excluded and the biowaiver policy only applies to products with the same immediate release solid oral dosage forms (capsule *vs.* tablets is not acceptable, although this is allowed by the definition of generic medicinal products in Directive 2001/83). Similarly, in spite of the fact that different ester, ethers, isomers, mixtures of isomers, complexes, or derivatives of an active substance are considered to be the same active substance for the EU definition of generic medicinal product, only different salts of class I drugs are acceptable for biowaivers.

Although the guideline states that it only applies to products with systemic action, the same scientific principles could be applicable to gastrointestinal locally acting products (*e.g.,* acarbose). In contrast, it is not applicable to systemically acting products that are not absorbed in the gut (*e.g.,* sublingual and buccal) and orodispersible tablets since satisfactory dissolution methodology is not developed yet and, as explained above, the orodispersible tablets are usually taken without water, therefore, the definition of solubility based on 250 ml does not apply.

In this guideline, the classification as of a drug as highly soluble is based on the maximum single dose and not simply the maximum strength, the pH range of interest varies from 1 to 6.8 instead of 7.5 (36), and the pH characterisation requirements do not include the $pK_a \pm 1$, but only pK_a.

In this document, the concept of permeability has been changed to absorbability and the criterion of highly absorbable is based exclusively on "human" absorption (37,38), determined by means of mass balance studies or absolute bioavailability studies, greater, or equal to 85% of the administered dose. Data from animals or culture cells are only considered to be supportive. The data from the mass balance studies have to be interpreted in the light of the Biopharmaceutical Drug Disposition Classification System (39), taking into account that oxidative and conjugative metabolites are formed only systemically after absorption.

Although the guideline indicates that BE between a solid oral dosage form and an oral solution is supportive, as it is indicative that absorption limitations due to the dosage form are negligible, it does not signal that absorption is complete. In such situation, dissolution similarity is less relevant for class III drugs as BE between solid dosage forms and solutions is generally more easily accomplished for low permeability drugs than for extremely permeable drugs.

As per the guideline, dissolution profiles should be compared at pH 1.2, 4.5, 6.8, and the pH of minimum solubility in more than one batch of test and reference products. The agitation speed for these studies has been defined as usually 50 rpm for the paddle and 100 rpm for the basket apparatus. There is no guidance on when a different speed would be acceptable. A different agitation speed, *e.g.,* 75 rpm with the paddle apparatus as recommended by World Health Organization (40), is questionable since it would facilitate the demonstration of similarity.

Dissolution profiles must be similar and rapid (>85% in 30 min) for class I drugs, and similar and very rapid (>85% in 15 min) for class III drugs (36). Although rapid dissolution is less critical for some products containing class III drugs (perhaps not for those with an absorption window), the requirement of a very rapid release is to ensure that a solution is emptied from the stomach and therefore it can be considered as similar to oral solutions.

In the EU guideline, special attention is paid to excipients as excipients that may affect bioavailability have to be included in identical amounts in test and reference products. In contrast, the US-FDA asserts that large amount of surfactants or mannitol and sorbitol are necessary to alter bioavailability (1). However, experience in the EU has shown that small amounts of surfactants (*e.g.*, SLS) and sorbitol affect the bioavailability of drugs (*e.g.,* 4 mg of SLS increases five- to sixfold the bioavailability of alendronate, and 7 mg of sorbitol decreases the C_{max} of risperiodone with 60 mg also decreasing the AUC).

For class I drugs, excipients that are not known to affect bioavailability can be different but, for class III drugs, even these excipients have to be the same and in very similar amounts.

CONCLUSIONS

By incorporating important advances in the area of BE, including requirements for BCS-based biowaivers for class III drugs and direction on the scaling of C_{max} acceptance limits for highly variable drugs, the EU "Guideline on the Investigation of Bioequivalence" represents the most progressive BE guideline currently available in the ICH region. The principles for the study of BE described in this guideline are consistent with those presented in the earlier EU guidance and in the current FDA documents. However, there are refinements in the EU guideline, such as those mentioned above, that are novel. The value of these novelties and a comparison of the revisions to the EU approach presented in this guideline relative to previous EU guidelines and to current FDA guidance have been presented to encourage a better understanding of current EU requirements for the examination of BE.

Conflict of Interest None.

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