EXPERT OPINION

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Current regulatory approaches of bioequivalence testing

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Introduction: Nowadays, reducing medication costs is vital for health care agencies. Prescription of generic drug products can help lower these expenses. A generally accepted assumption is that therapeutic equivalence, between a generic and a brand-name medication, can be claimed if bioequivalence is demonstrated.
Areas covered: This article reviews the current regulatory procedures on bioequivalence testing. Special focus is placed on the guidelines recommended by the European Medicines Agency and the US Food and Drug administration. The authors also describe the evolution of these issues and the alternatives proposed in the literature.
Expert opinion: Defining bioequivalence, as the condition of no significant differences in the *extent* and *rate* of absorption between the generic and the brand-name medication, sounds simple. However, the scientific and regulatory basis of bioequivalence appears rather complicated in practice. Even though the regulatory authorities have elucidated many issues, several aspects of bioequivalence assessment are still unresolved. Examples, of these open questions, in bioequivalence, include the assessment of complex drugs, such as biologics and iron–carbohydrates, the assessment of immunosuppressive agents as well as the role that pharmacogenomics plays in bioequivalence.

Keywords: bioequivalence, biowaivers, complex drugs, European Medicines Agency, Food and Drug Administration, regulatory guidelines, scaled bioequivalence approach

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1. Introduction

During the last years, a continuous rise is observed for the expenses of health care systems and the medication costs particularly [1]. Among the strategic measures to lower the cost of medication, predominant role exerts the prescription of generic drugs. Generics are considered to be equivalent to the innovator's (or brand-name) drugs and can exert a key role in therapeutics and pharmacoeconomics. However, before these products enter the market, it is essential to confirm their quality, efficacy and safety in comparison with the brand-name products. The scientific and regulatory framework for the approval of generic products constitutes the basis of bioequivalence (BE) testing [2].

Assessment of BE relies on the fundamental assumption that a product's clinical effect is a function of the concentration of the active substance in the general circulation. Thus, two drug products are considered bioequivalent if their concentration (C)–time (t) profiles are similar enough to ensure comparable clinical performance [3].

Even though, BE testing is generally applied to the approval process of generics, there are also other situations to which BE concepts are applied. Such cases occur when significant changes are made to a drug product compared with the approved formulation or when the marketed dosage form is different from that tested in the clinical studies [3].

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Article highlights.

- Generic drug products can help reduce medication costs.
- Bioequivalence sets the scientific and regulatory framework to assess the equivalence between a generic and a brand-name product.
- European Medicines Agency and US Food and Drug Administration have issued guidelines, which cover many aspects of bioequivalence assessment and standardize the conditions of the comparison process.
- Important issues in the guidelines include the bioequivalence study endpoints, the clinical design, the required statistical approaches, the appropriate sample size, the moiety (parent drug or metabolite) and the strength to be tested.
- A situation of special interest in bioequivalence is the assessment of highly variable drugs.
- Several aspects of bioequivalence testing, such as biosimilars, immunosuppressive drugs and the role of pharmacogenomics are still not fully resolved.

This box summarizes key points contained in the article.

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During the last decades, BE testing has gained a global attraction, and many regulatory authorities worldwide issued guidelines defining the specific criteria required to demonstrate equivalence between two products. In BE assessment, comparison is made between a new product under study (test, T) and the brand name (reference, R) of the same active substance. Among the regulatory authorities with the greatest advancements in issuing guidelines are the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA). Other regulatory authorities, such as Health Canada, the Japanese Pharmaceuticals and Medical Devices Evaluation Agency, and the World Health Organization for the developing countries, also release scientific guidelines for BE assessment. Finally, efforts are made toward a global harmonization of the regulatory requirements [4,5].

EMA and FDA have released many scientific guidelines on BE, which cover many aspects of the entire evaluation procedure. However, due to the wide field of BE assessment, this article focus on the main regulatory requirements proposed by EMA and FDA in case of the immediate release (IR) oral formulations.

2. Current approaches

Classically, two drug products are considered bioequivalent if they contain the same active moiety, are at the same molar dose and present no significant differences in their *rate* and *extent* of absorption when administered under similar conditions [6-8]. It should be mentioned that this definition of BE does not necessarily imply that two bioequivalent products are also pharmaceutically equivalent. In other words, pharmaceutically alternative products, which might differ in their dosage (e.g., tablet vs capsule) or chemical (e.g., salts, esters, isomers) form or their strength, could be considered bioequivalent [8]. To obtain accurate and unbiased outcomes from the BE assessment, one has to rely on specific guidelines for the comparison between the T and R products.

For this reason, aim of the regulatory guidelines is to standardize the conditions of the BE comparison process. Thus, guidelines include recommendations on many aspects of BE assessment such as clinical design, statistical approaches, sample size of the study, chemical moiety to be measured, strength to be investigated. In addition, BE guidelines refer to situations of special interest as the case of highly variable drugs (HVD), waivers of the need to perform *in vivo* studies and other types of formulations such as modified release products and parenterals.

2.1 Study endpoints

2.1.1 *Type of evidence*

Several types of endpoints can be used to demonstrate BE [8,9]. Based on the selected endpoints, the evidence required to prove BE can be classified, in descending order of importance, into i) pharmacokinetic (PK) studies that focus on drug levels in biological fluids usually blood or plasma, ii) pharmacodynamic (PD) data that rely on appropriate PD models and indices, iii) clinical trials with efficacy and safety endpoints, and finally iv) *in vitro* data.

Nevertheless, pharmacokinetic endpoints (the so-called PK metrics) are usually used in BE assessment and will primarily be discussed in this article.

2.1.2 *Pharmacokinetic metrics*

Classically, BE testing is based on the comparison of the *rate* and *extent* of absorption of two drug products. *Extent* of absorption is usually expressed by the area under the *C–t* curve (AUC) and the area under the *C–t* curve extrapolated to infinity (AUC_{inf}) [10,11]. For the assessment of the *rate* of absorption, indirect metrics are applied: the maximum observed plasma concentration (C_{max}) of the drug and the time (T_{max}) at which C_{max} occurs [10-12].

In the past, several PK metrics have also been proposed for the comparison of two drug products. These included the direct curve comparison metrics for measuring the *C–t* profile dissimilarity between T and R products [13,14], the relative difference metric [15] and metrics intended to compare the entire concentration–time profiles [16,17]. Additionally, the use of the ratios of C_{max}/AUC and C_{max}/T_{max} [18] and the *y*-intercept of $ln(C/t)$ *versus t* plot [19] have been proposed as *rate* metrics for BE assessment. The rationale of these metrics relies on the fact that C_{max} does not exclusively describe the *rate* of absorption, but it is also influenced by the *extent* of absorption.

The concept of *exposure* has been introduced several years ago as a replacement for the *extent* and *rate* of absorption [12,20]. According to the *exposure* idea, the parameters C_{max}, AUC and partial area under the curve (AUC_p) remain fundamental and still used in BE assessment, not in the vein of *rate* and *extent* of absorption, but as measures of *peak*, *total* and *early exposure*, respectively [12,20-27]. *Early exposure* can be estimated by truncating AUC at the T_{max} estimate of the reference product or at a time point alternatively defined, for example, from PK/PD relationships or safety/efficacy data [8,26]. The use of *early exposure*

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metrics is essential in case of IR products where the drug input rate is important [8]. Nowadays, AUC_p is adopted by FDA and EMA as a measure of *early exposure* [7,8].

The abovementioned PK measures of BE are applicable when plasma data are used (in this analysis, no distinction will be made between the terms plasma and blood and both will be used interchangeably). In case of urinary data, the metrics required by the authorities are the cumulative urinary excretion of the unchanged drug from administration until a specific time and the maximal rate of urinary excretion [7]. It is noteworthy that for other circumstances such as steady-state conditions or different formulations (e.g., prolonged release products), additional PK metrics may be required, such as peak-trough fluctuation (%), swing and minimum or pre-dose plasma concentration.

It should be highlighted that for BE purposes, all calculations are made in the *log*-domain of the PK data. The reason for this *log*-transformation is twofold: i) a clinical rationale upon which the comparison focuses on the ratio rather than the difference between the means of the PK parameters [28] and ii) a PK–statistical rationale since a multiplicative model is used [29,30]. In all cases, PK metrics should be calculated using non-compartmental methods [7,8].

2.2 Sampling scheme

An accurate estimation of the PK metrics requires an appropriate sampling scheme, which should adequately describe the entire *C–t* profile of both drugs under comparison. Firstly, the sampling design should be carefully planned to avoid C_{max} being the first point of the *C–t* curve, since these situations can raise questions about the validity of C_{max} estimates [7,8]. Depending on drug's *rate* of absorption, two to five samples in the first hour after administration are usually necessary.

To provide reliable estimates of *total exposure*, the sampling schedule should adequately describe the entire *C–t* profile of the drugs. Hence, it is suggested that *AUC* should be at least equal to 80% of the AUC_{inf} estimate. Particular attention should also be paid to the terminal elimination phase, where at least three to four samples are usually required for an accurate estimation of the terminal slope of the *C–t* curve [8]. Thus, as a rule of thumb, a number of 12 – 18 samples are usually collected for each subject and drug administration. It is noteworthy that special sampling requirements should be applied in case of endogenous substances (e.g., hormones) or entities with complex kinetics [7].

Currently, EMA suggests that in case of IR drugs, sampling schedule can be truncated at 72 h post-dose since at this time the absorption phase has been completed [7]. The same recommendation is also proposed by FDA as soon as the drug exhibits low within-subject variability [8].Presumably, in such cases, the rule for the percentage of AUC/AUC_{inf} ratio being greater than 80% does not apply.

2.3 Statistical approaches

2.3.1 *Average Bioequivalence*

Classically, BE assessment relies on the concept of average bioequivalence (ABE) [6-8,28]. Two drug products are considered bioequivalent if the calculated 90% confidence interval (CI) for the difference of the log-transformed mean measures of BE lies between specific limits imposed by the regulatory authorities. By general consensus and without a clear scientific rationale, the BE limits were arbitrarily set to ± 20%. Due to log-transformation of the PK data, the latter leads to limits (δ) of 80 – 125% (or equivalently 0.80 – 1.25) for the ratio of geometric means (GMR) for the T and R parameters of bioavailability (i.e., C_{max}, *AUC*). Nevertheless, it should be mentioned that in some cases, as for narrow therapeutic index and HVD, different limits are recommended [7,8]. The mathematical expression of ABE is listed in **Table 1**. For reasons of simplicity, no distinction will be made in this article between the *population* and the *sample* terms of the PK metrics and their variabilities.

The PK parameters for all subjects participating in the study are analyzed using a general linear model (i.e., ANOVA). Factors incorporated in the model are *Sequence*, *Period*, *Treatment* and the nested term *Subject-within-Sequence*. According to the EMA 2010 guideline, all these factors should be treated as fixed effects [7]. After applying the linear statistical model to the data, the residual error is estimated, which is then used to make inferences about BE. The within-subject variability (S^2_w) is a component of and is reflected in the calculated residual variability. Finally, the criterion for BE assessment relies on the construction of the 90% CI around the T/R ratio of the PK metrics based on the within-subject variability derived from the ANOVA. This criterion is equivalent to the two one-sided hypothesis testing assuming significance level of 5% [31].

It should be highlighted that application of ABE can assure safety and efficacy between the two products under comparison. However, ABE does not address issues such as *prescribability* and *switchability*. The first term, namely *prescribability*, refers to the physician's choice of the initial medication for a patient who is treated for the first time. Besides, *switchability* reflects the situation of interchangeability between two multisource formulations. The latter is important in case of patients already receiving a specific medication, which should be changed to a different brand of the same drug. In order to deal with these queries, the concepts of population and individual BE were introduced [6,32-40].

2.3.2 *Population bioequivalence*

Population bioequivalence (PBE) was proposed to address the issue of *prescribability* [32-34]. In order to accomplish this task, the total (i.e., sum of within- and between-subject) variability should be known. Two options have been proposed for PBE: a reference or constant-scaled approach (**Table 1**) [6].

2.3.3 *Individual bioequivalence*

Individual BE (IBE) was proposed in order to address the need of *switchability*, namely, to ensure interchangeability of drug products [28,32-40]. IBE requires the conduct of a replicate study, which will allow the estimation of the within-subject variability of both T and R products. Estimation of IBE also requires the inclusion of the additional variance component called as *subject-by-formulation* interaction (**Table 1**). Even though the

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Table 1. Basic aspects of the statistical approaches of bioequivalence proposed by European Medicines Agency (EMA) and Food and Drug Administration (FDA).

Bioequivalence approach	Criterion of bioequivalence*	Regulatory authority
Average bioequivalence[‡]	$-\ln(\delta) \le m_T - m_R \le \ln(\delta)$	EMA, FDA
Population bioequivalence[§]	Reference-scaled: $\frac{(m_T - m_R)^2 + (S^2_{TT} - S^2_{TR})}{S^2_{TR}} \le \theta_p$ Constant-scaled: $\frac{(m_T - m_R)^2 + (S^2_{TT} - S^2_{TR})}{S^2_{P0}} \le \theta_p$	FDA
Individual bioequivalence[¶]	Reference-scaled: $\frac{(m_T - m_R)^2 + (S^2_{WT} - S^2_{WR}) + S^2_D}{S_{wR}^2} \le \theta_I$ Constant-scaled: $\frac{(m_T - m_R)^2 + (S^2_{WT} - S^2_{WR}) + S^2_D}{S^2_{I0}} \le \theta_I$	FDA
EMAs[#]	*Lower/Upper BE limits*: $\begin{cases} CV_{wR} \le 30\% & \Rightarrow 0.80, 1.25 \\ 30\% < CV_{wR} < 50\% & \Rightarrow exp(\pm k \times S_{wR}) \\ CV_{wR} \ge 50\% & \Rightarrow 0.6984, 1.4319 \end{cases}$ *Constraint for point GMR*: 0.80-1.25	EMA
FDAs**	*Lower/Upper BE limits*: $\begin{cases} CV_{wR} < 30\% & 0.80, 1.25 \\ CV_{wR} \ge 30\% & exp(\pm \ln(1.25) \times S_{wR} / S_{w0}) \end{cases}$ *Constraint for point GMR*: 0.80-1.25	FDA

BE refers to bioequivalence; m_T is the mean (in log scale) of the pharmacokinetic metric for T; m_R is the mean (in log scale) of the pharmacokinetic metric for R; δ is the pre-defined limit of average bioequivalence, usually set as $\delta = 1.25$; S^2_{TT} is the total (i.e., within- and between-subject) variability of the T formulation; S^2_{TR} is the total (i.e., within- and between-subject) variability of the R formulation; S^2_{P0} is a constant variance term; ϑ_p is the limit of acceptance for population bioequivalence; S^2_{WT} is the within-subject variability of the T formulation; S^2_{WR} is the within-subject variability of the R formulation; S^2_{I0} is a constant within-subject variance term used in individual bioequivalence; S^2_D is the variance component of subject-by-formulation interaction; ϑ_I is the limit of acceptance for individual bioequivalence; CV_{wR} is the within-subject coefficient of variation for R formulation; k is a scaling factor ($k = 0.760$), set by EMA, for the scaled approach; S_{w0} is a constant referring to the regulatory standardized variation of FDAs approach.

*Mean and variability terms refer to sample data.

[‡]Refers to the classic average bioequivalence approach with the 0.80 – 1.25 limits.

[§]The reference-scaled method is used if $S_{TR} > S_{P0}$, whereas the constant-scaled criterion should be applied when $S_{TR} \le S_{P0}$ [28].

[¶]The reference-scaled method is used if $S_{WR} > S_{I0}$, whereas the constant-scaled criterion should be applied when $S_{WR} \le S_{I0}$ [28].

[#]EMAs refers to the scaled approach proposed by EMA [7].

**FDAs refers to the scaled approach proposed by FDA scientists [41,42].

concept of IBE is rather attractive, it gained only limited application due to practical problems [40].

2.4 Study design

Aim of the planned BE study is to distinguish the *formulation* effect from other factors that can also affect the outcome of the study. For this reason, the trial design, as well as the number of studies required to prove BE primarily, depends on the physicochemical and PK properties of the active substance.

2.4.1 *Standard design*

Conventionally, regulatory authorities recommend the application of a randomized, two-treatment, two-sequence, two-period (2 × 2) design [6-8]. Each subject, participating in the study, receives consecutively the two drug products (i.e., T and R) in two periods, which are separated by an adequate washout period. The duration of the washout period should be sufficiently large (at least five times the elimination half-life) to ensure that no drug can be detected at the initiation of the second period of the study. Due to the nature of this design,

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the within-subject variability of T (S^2_{wT}) and R (S^2_{wR}) cannot be estimated. After applying the ANOVA model, the calculated residual error reflects the estimate of the pooled variability arising from the two drug products. It is assumed that this residual error corresponds to the within-subject variability of the active substance.

2.4.2 Alternative designs

However, under certain circumstances, other designs can be more appropriate for the evaluation of BE. This type of studies may include replicate, two-stage and parallel designs.

2.4.2.1 Replicate designs

In replicate designs, each subject receives the same formulation more than once. The replicate administration may refer to both formulations or to only one of them. In the first case, both T and R preparations are administered at least twice to each subject; this leads to designs with four (or more) periods of drug administration. Plausibly, this type of design allows the estimation of the within-subject variabilities of both T and R products.

Nevertheless, replication may solely apply to one (usually R) formulation. Recently, this semi-replicate design was recommended by EMA and FDA in case of HVD [7,41-43]. According to this design, the R product is administered twice in each subject, while the T product only once. For a semi-replicate design, the possible sequences of drug administration are RTR, RRT and TRR.

In general, replicate designs can be advantageous since they require reduced numbers of subjects and permit comparison of the within-subject variabilities of T and R formulations [6,8,44,45]. For example, a four-period design requires almost 50% of the subjects of a typical 2 × 2 study. Obviously, replicate designs are necessary if the IBE approach is applied. However, they are not obligatory if the average or the population BE approach is used [28].

2.4.2.2 Two-stage design

Group sequential designs have widely been used in clinical trials. However, in the latest EMA guideline on BE, a two-stage design is also proposed as an option for BE studies [7]. If BE is demonstrated after stage I, there is no need to proceed to stage II of the study. However, if no BE is shown in the first step, the applicant is allowed to recruit more subjects and move to stage II.

As in the case of sequential clinical studies, the analyst should take all appropriate measures to preserve the overall significance level, of both stages, to the required maximum value of 5%. In addition, the statistical model used to analyze the data should be modified accordingly to include an effect term of 'stage.' Obviously, the design applied to BE studies appears to be less complicated than the situation encountered in clinical studies, since up to two stages can only be accepted in BE studies.

2.4.2.3 Parallel design

A parallel study design can be very useful in BE studies as in the case of drugs with very long half-lives [7,8]. A potential application of crossover design would require a long washout period, otherwise carryover effects might occur. Nevertheless, parallel designs require larger sample sizes than crossover studies.

2.5 Single and multiple dose studies

For BE purposes of IR drug products, single-dose studies are usually recommended by EMA and FDA [7,8]. This choice can be attributed to the fact that single-dose studies are more sensitive to detect the release of the active moiety from its formulation into the systemic circulation [8]. In such cases, the T and R products are administered only once to each subject at each treatment period. Statistical comparisons and analyses are based on the so-derived plasma *C*–*t* profile.

However, there are a number of situations where a multiple-dose (actually, steady-state) study can be advantageous. This may be due to the fact that the variability in the PK parameter values is often lower after multiple dosing [45-48]; thus, these designs can be useful in the case of HVD. Nevertheless, in actual practice, the use of multiple dosing in IR formulations is very much restricted to the cases where bioanalytical sensitivity is insufficient after single dose. In any case, if multiple dosing is required, then all appropriate measures should be undertaken to ensure that steady-state conditions are reached.

2.6 Sample size

Estimation of the appropriate sample size is very crucial for BE studies. A BE study should include an adequate number of subjects in order to attain the ability to prove equivalence whenever it exists. At the same time, BE studies should also avoid unnecessary human exposure to drugs [49]. Usually, a value of at least 80% of power is required for BE studies.

Besides power, the required number of subjects in a BE study is a function of several other parameters, which include the maximum allowable limits of BE (e.g., 0.80 – 1.25), the significance level (usually set equal to 5%), the type of design (e.g., parallel, crossover, replicate), the expected deviation of T from R formulation and the estimated residual (or within-subject) variability of the data after applying the statistical model. The sample size should also be sufficient enough to allow for any possible dropouts. It should be mentioned that both EMA and FDA set 12 subjects as the minimum value of sample size [7,8].

2.7 Highly variable drugs

A critical issue for sample size estimation is the within-subject variability of the active substance. As this variability increases, it becomes more difficult to prove BE, unless a large number of subjects are recruited. Thus, the issue of sample size estimation is of crucial importance in the case of HVD, namely, drugs where the within-subject coefficient of variation (CV_w) is ≥ 30% [50,51]. It should be mentioned that in this article the terms *highly variable drug* and *highly variable drug product* will be used interchangeably even though they are not actually the same. The underlying causes of variability can be ascribed to physiological (e.g., gastrointestinal motility

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and secretions), physicochemical (e.g., products' batch-to-batch similarity), pathological conditions of the subjects and any drug-related issues such as pre-systemic metabolism, which can contribute significantly to variability [52].

In order to face the problem of recruiting a large number of volunteers for HVD, several methods have been proposed. These approaches include the widening of BE limits to pre-fixed constant values [6,53,54], the application of simple [55], mixed [56], GMR dependent [57] and leveling-off [58,59] scaled approaches. Recently, scientists from EMA and FDA proposed new scaled approaches in order to resolve the problem of high variability encountered in BE studies [7,41,42].

According to the latest EMA guideline on BE assessment, a mixed-scaled approach (EMAs) could be applied for drugs with CV_w for the R formulation (CV_{wR}) greater than 30% [7]. The proposed procedure suggests the use of replicate (three- or four-period) studies where, at least, the R product should be administered twice. The latter allows the estimation of S^2_{wR}, which is then used to construct scaled limits (**Figure 1**). The mathematical expression of the EMAs approach is quoted in **Table 1**. It should be underlined that EMA recommends the application of the mixed-scaled approach only in cases where CV_{wR} lies between 30 and 50%. For CV_{wR} values lower than 30% and greater than 50%, constant BE limits equal to 0.80 – 1.25 and 0.6984 – 1.4319, respectively, should be used. In other words, the limits are not allowed to scale freely, but until a maximum leveling-off value [60]. In addition, EMA recommends this scaled approach only for C_{max}, whereas for AUC the constant 0.80 – 1.25 limits should always be applied irrespectively of the level of variability. Finally, the approach proposed by EMA includes a secondary criterion on the point estimate of GMR constraining it in the range 0.80 – 1.25.

Scientists from FDA have also proposed a reference-scaled approach (FDAs) in case of HVD [41,42,61]. It is suggested that for HVD, a semi- or full-replicate design can be applied, that is, similar to what is quoted in the latest EMA guideline. Again, these designs allow the estimation of S^2_{wR}, which is further used to construct scaled BE limits (**Table 1**). It is worth mentioning that these FDAs limits expand unendingly with variability after CV_{wR} 30% (**Figure 1**) [62]. Similarly, a complementary constraint criterion on point GMR estimate is also applied.

The FDAs approach has three major differences from the EMAs method; the former approach i) is applied to both C_{max} and AUC, ii) utilizes a scaling factor with greater value (0.893 vs 0.760) and iii) does not involve an upper limit for scaling, that is, no leveling-off behavior (**Figure 1**) [61]. These differences lead to different performances between the EMAs and FDAs scaled approaches, which are more prominent when CV_{wR} exceeds 50% [61,63]. Basically, the FDAs method is more permissive than EMAs, an attribute that becomes evident for high CV_{wR} values (e.g., 50%) and sample sizes lower than 50. In addition, the complementary GMR constraint is quite essential for FDAs; otherwise drugs that may differ significantly in their GMR values could have been accepted. For EMAs, the GMR-constraint criterion becomes prominent when a large sample size is used and CV_{wR} is approximately 50%. As it is expected, the increase in sample size affects the EMAs more than the FDAs performance [61,63].

Obviously, an increased exposure of humans to drug is anticipated for EMAs and FDAs approaches, since both of them comprise more than two periods of drug administration [64]. However, these methods are clearly advantageous, in terms of human exposure, at higher variability values.

2.8 Measured analyte

Another important issue in BE testing is the choice of the analyte that will be measured and included in the statistical assessment. This dilemma applies mainly to the following cases: i) parent drug (P) or metabolite(s) (M) and ii) drugs that exist in enantiomers.

2.8.1 *Parent drug–metabolites*

In most of the cases, BE studies are focusing only on the parent drug. The major advantage of using P relies on the fact that the *C–t* profile of the parent drug reflects better differences in formulation performance [65,66]. In other words, C_{max} estimate of P describes better the *rate* of drug absorption. However, measurement of P should not be considered as a rule since in some instances determination of M or both M and P could be advantageous [65-69].

In this vein, FDA proposes that M data are preferable when the P levels in the biological fluids are relatively low to allow a reliable analytical measurement [8]. It is also recommended that both M and P should be measured for a metabolite that contributes significantly to safety and/or efficacy.

In the case of EMA, BE assessment is primarily based on the measurement of P [7]. The same opinion is also extended to inactive prodrugs, where again P data are preferable. The use of metabolite data is suggested when i) administration of parent drug results in low plasma concentrations and ii) a sensitive analytical method cannot be reliably applied to P even after administrating the drug at the highest dose strength. Nevertheless, the use of M data should be justified on the basis that these data are capable of reflecting parent drug and that the formation process of metabolite is not saturated [7].

2.8.2 *Enantiomers – racemates*

Another controversial issue in BE assessment is encountered when the active drug is a stereoisomer. In such cases, the question posed is whether to measure the individual enantiomers or the racemate. EMA and FDA generally propose measurement of the racemate mixture. Determination of a specific enantiomer is recommended in cases when the enantiomers exhibit the following properties: different PK and PD properties, as well as different absorption kinetics, and there is no interaction or interconversion between the isomers. Also if the previous characteristics are not known, measurement of the individual enantiomers is necessary. A sole enantiomer can be measured if this enantiomer is mainly responsible for

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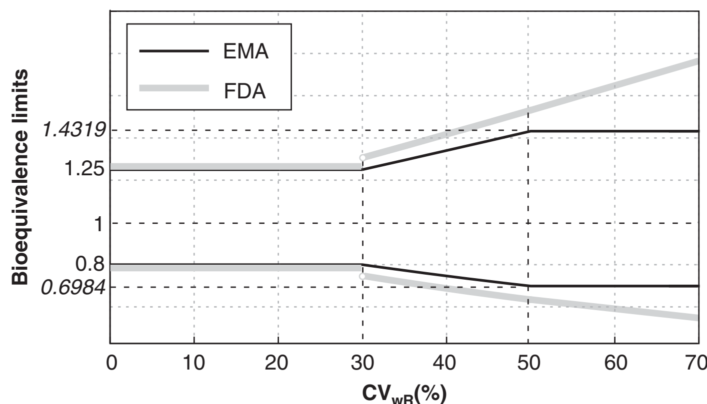

Figure 1. Bioequivalence limits for the scaled approaches of European Medicines Agency (EMA) and Food and Drug Administration (FDA) as a function of within-subject coefficient of variation of the reference product (CV_{wR}). At CV_{wR} = 30%, a discontinuity becomes apparent for FDA limits. After CV_{wR} = 30%, FDA limits expand endlessly, whereas EMA limits expand only up to the extreme range of 0.6984 – 1.4319. FDA limits include a greater scaling factor than EMA and become more liberal as CV_{wR} increases.

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the clinical effect (e.g., when the other enantiomer is inactive or has negligible effect) [7,8].

2.9 Long half-life drugs

For drugs with long half-lives, the sampling schedule should be adequate to allow a complete description of the entire *C–t* profile. The recent EMA guideline suggests that estimation of *AUC* can be truncated at 72 h, irrespectively of the drug's elimination half-life, since at that time it is anticipated that the absorption phase is completed [7]. A similar statement can be found in the FDA guideline, where truncation at 72 h is also proposed for IR drugs with low within-subject variability in distribution and clearance [8].

If a crossover design is used, then an adequate washout period should be used for long half-life drugs; alternatively, a study with parallel design could be considered.

2.10 Narrow therapeutic index drugs

For some active substances (such as phenytoin, theophylline, lithium), the difference between the therapeutic and toxicity levels is very small. FDA does not suggest specific criteria for the BE assessment of narrow therapeutic range drugs, but provides only general directions toward the need that applicants should encompass all possible testing to ensure the quality of their drug product.

In case of EMA, more specific recommendations appear for the treatment of narrow therapeutic index drugs [7]. Thus, a stricter acceptance interval (0.90 – 1.11) is suggested for *AUC*. For C_{max}, two options are available: i) if C_{max} is important for efficacy and safety reasons, the 0.90 – 1.11 limits should be preferred, and ii) if the drug is highly variable, wider acceptance limits can be claimed.

2.11 Fasting–fed studies

The presence of food in the gastrointestinal tract can modify the *rate* and *extent* of absorption of a drug through various mechanisms (e.g., delay of gastric emptying, stimulation of secretions) [70-73]. In order to minimize this variability, BE studies should also be standardized in respect to food.

In case of EMA, BE studies for IR products are routinely performed under fasting conditions that are more sensitive to distinguish the absorption profiles of two drug products. Similarly, fasting conditions should also be used when the labeling of the R product indicates that the drug should be administered in an empty stomach or when no statement is made about the food intake [7]. Besides, EMA recommends the conduct of a BE study in the fed state if the labeling of the R formulation quotes concomitant food intake. Studies in both the fasting and fed state are proposed for certain categories of formulations such as microemulsions and solid dispersions. In all cases, specific recommendations are provided for the type of meal and the time of its administration [7].

FDA generally recommends that for BE reasons, studies should be conducted under both fasting and fed conditions [73]. For an IR product, a fed study can be omitted if both T and R are highly soluble, rapidly dissolving and highly permeable drugs (see also Section 2.14.4). Similarly, studies only under the fasting state are accepted if according to the labeling, the product is intended to be administered in an empty stomach or no labeling information is provided regarding the effect of food [73].

It should be highlighted that in case of modified release (MR) products, specific requirements are in effect for BE studies. In such cases, the rule is that studies should be conducted under both fasting and fed states [73,74].

2.12 Strength and dose

When the applicant claims for a market authorization for multiple strengths, it is possible to select only one, the most appropriate, strength for BE testing. If dose-independent kinetics have been established, a BE study at only one strength may be

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sufficient [7,8,75]. BE assessment at only one strength prerequisites that other factors, such as the proportionality in composition between the different strengths, are fulfilled [7,8]. Generally, the administered strength of choice is the one that provides the higher sensitivity of detection of any possible differences between the two products. For example, if linear kinetics is proved, then usually BE studies are usually held at the highest strength [75]. In case of additional strengths of the product, the applicant may grant for a waiver of conducting BE studies at all strengths (refer to Section 2.14.1 below).

2.12.1 *Bracketing approach*

A special situation occurs when BE studies are performed at more than two strengths, for example, due to deviation from the rule of proportionality composition. In such cases, the EMA guideline offers the opportunity to conduct totally two BE studies; namely, utilizing the minimum and the maximum strengths [7].

Furthermore, if studies are required under both fasting and fed states for two strengths, then it is possible to ignore the need of conducting both of them (i.e., fasting or fed) at each strength. Alternatively, if the applicant provides convincing justification, then both studies (fasting and fed) can be conducted at only one strength. At the other product's strength, a single fasting or fed study can be applied.

2.13 Data other than plasma concentrations

BE studies are based on drug measurements in biological fluids. In the vast majority of cases, BE assessment is based on the concentration–time data from plasma samples. In certain cases, other types of evidence can be used. These include PD endpoints, therapeutic equivalence data as well as urinary or *in vitro* data.

2.13.1 *Urinary data*

For BE purposes, urinary excretion data are used when it is difficult to obtain reliable measurements of *C*–*t* data [7,8]. However, urinary data suffer from sensitivity to characterize *peak drug exposure*. For this reason, reliable C_{max} estimates should be used in conjunction with urinary data. In accordance with plasma sampling, urinary data can be collected for up to 72 h.

2.13.2 *In vitro* data

In vitro information of a drug product is always necessary during the development process as a quality control tool [7,8,76]. In addition, comparative dissolution tests between the T and R products should be provided, as complementary evidence to the BE study. Finally, *in vitro* data can be used as substitutes for *in vivo* evidence as in the case of *in vitro*–*in vivo* correlations (IVIVC). In IVIVC, a relationship is established between an *in vitro* property of a drug product (e.g., dissolution rate) and a relevant *in vivo* parameter (e.g., *extent* of absorption).

The dissolution profiles of the two medicinal products are usually compared at three pH values: 1.2, 4.5 and 6.8, unless otherwise specified [7,8]. The similarity factor (f_2) is used for the comparison of the dissolution profiles; an f_2 value greater than or equal to 50% implies similarity. However, similarity of the two dissolution profiles is proved if the drug is rapidly dissolving, namely, more than 85% dissolution is achieved within 15 min [7,8,15,77].

2.14 Waivers of *in vivo* studies

In vitro data not only are included as a complementary material to *in vivo* evidence, but can also be used in regulatory biowaiver applications, namely, to replace *in vivo studies*. The conditions, where *in vitro* data can replace BE studies, comprise the case when the sponsor applies for additional strengths of a product, post-approval changes and certain types of formulations [7,8]. Besides, aspects of Biopharmaceutics Classification System (BCS) and Biopharmaceutics Drug Disposition Classification System (BDDCS) have been incorporated into the guidelines [78,79].

2.14.1 *Additional strengths*

Under certain circumstances, the need of conducting BE studies at all dose strengths can be waived (see also Section 2.12) [7,8]. Dissolution data can be used in regulatory biowaiver applications for additional dose strengths. Overall, the conditions that allow the applicant to ask for a biowaiver of additional strengths are linear drug kinetics, same manufacturing process, same qualitative composition of the different strengths and proportionality in formulation composition.

2.14.2 *Post-approval changes*

In vitro evidence can also be used as a biowaiver in cases of post-approval changes for a drug product already in the market [8]. In this case, comparative dissolution tests should be performed between the pre- and post-approval product.

2.14.3 *Biowaivers for special formulations*

This section refers to waivers of *in vivo* data for particular types of drug products. In this vein, biowaivers can be claimed when both the T and R products are aqueous oral solutions of the same active moiety and at the same concentration [7]. A basic prerequisite for granting biowaivers is that excipients should not affect gastrointestinal motility, drug solubility and uptake through the intestinal membranes.

In addition, no BE studies are necessary for intravenous aqueous solutions as long as they contain the same active substance and excipients do not alter its disposition. Intramuscular and subcutaneous preparations can also waive the need of conducting BE studies when the T and R products contain the same active moiety, are at the same concentration, the solution is of the same type and consist of the same excipients in similar amounts [7].

2.14.4 *BCS- and BDDCS-based biowaivers*

2.14.4.1 *BCS*

BCS was proposed in 1995 as a scientific tool to optimize drug development [78]. According to BCS, drug substances

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are classified into four categories based on their solubility and permeability properties. Drugs with high aqueous solubility and high permeability are considered as class I drugs, while those with high permeability and low solubility are characterized as class II. Besides, drugs exhibiting low permeability belong to either class III if they are high soluble or class IV if they exert low aqueous solubility. Based on this classification, BCS can predict *in vivo* absorption of drugs and can be used as a surrogate of BE in place of *in vivo* studies [80,81]. Demonstration of high solubility is a physicochemical issue, which is proved experimentally. For the determination of high permeability, several approaches have been proposed such as mass balance studies, urinary recovery of unchanged drug and studies on *in vivo* perfusion and *in situ* permeation [80].

Currently, both EMA and FDA offer the opportunity to grant for biowaivers based on BCS [7,82]. The latest EMA guideline allows biowaivers for BCS I and III drugs. In particular, these biowaivers apply to oral IR formulations with no narrow therapeutic index drugs. However, a biowaiver cannot be granted i) in case of sublingual, buccal and modified release formulations, ii) when the active substance in the T product is a different ester, ether, complex and isomer, and iii) excipients that may affect absorption are not in the same qualitative and quantitative composition. In case of class I drugs, a BCS-based biowaiver can be claimed if the drug dissolves either very rapidly or rapidly in media with pH values 1.2, 4.5 and 6.8 [7,8]. The rationale for biowaivers of class III drugs relies on the postulation that if a drug is highly soluble, then no differences in absorption can be anticipated, unless the excipients affect the absorption process [7]. For class III drugs, the requirements for dissolution testing are stricter, since very rapid dissolution of the products should be demonstrated [7].

It should be mentioned that a BCS-based biowaiver can be granted by the FDA only for class I drugs that are formulated as IR formulations proven to exert rapid dissolution [82].

2.14.4.2 *BDDCS*

Ten years after the development of BCS, the Biopharmaceutics Drug Disposition Classification System (BDDCS) was proposed as a framework to handle issues of drug disposition, drug interactions and transporter–enzyme interplay in the intestine and liver [79,80,83-85]. According to BDDCS, drugs are classified into four categories based on metabolism and solubility. Class I and II comprise compounds that are eliminated primarily via metabolism, while the major route of elimination for class III and IV drugs is the kidneys or the bile. Therefore, for drugs already in the market, BDDCS utilizes knowledge of metabolism to predict drug permeability, namely, no need of conducting human permeability studies [86]. Nevertheless, BDDCS can also be used during early drug discovery for new molecular entities [87]. In addition, the EMA 2010 guideline allows in essence granting for a biowaiver in case of a highly metabolized drug.

Through the last years, it was observed that the classification of compounds based on BDDCS was not always identical to that derived from BCS [86,88]. For example, highly permeable drugs according to BCS do not always exhibit high metabolism and they are not in accordance with BDDCS findings [89]. This discrepancy arises from the fact that BCS considers primarily permeability in terms of *extent* of absorption, whereas BDDCS views permeability as a kinetic factor that is related to the metabolism in the liver and the intestine [80].

3. Conclusions

This article focuses on the main aspects of BE assessment, which are in effect today in the case of IR solid oral forms. The definition of BE, in terms of no significant differences in the *extent* and *rate* of absorption between two drug products, sounds simple. Nevertheless, the scientific and the regulatory basis of BE is complicated and unclarified issues may occur in practice [90].

Due to space limitations, not all aspects of BE can be discussed in the current analysis. Other topics of special interest include fixed combination dosage forms, the role of outliers in BE assessment, modified release drug products, locally applied and locally or systemically acting formulations as well as orally administered products for local action.

4. Expert opinion

Aim of BE testing is to compare two pharmaceutical products of the same active moiety in a way that will ensure their safety and efficacy similarity upon the administration. Currently, regulatory agencies, such as EMA and FDA, have issued and continue to publish guidelines that intend to cover as many as possible aspects of BE testing. Even though the authorities worldwide have elucidated many issues, it seems that some aspects of BE assessment require further clarification. The latter should be attributed not only to the fact that BE is a wide scientific field, but also to the continuous rise of new issues. Known problems that are neglected in the current guidelines include the detailed management of studies with endogenous substances and the multiple peak phenomenon [91]. Nevertheless, open questions in BE are not pending only for low molecular weight drugs, but also for the so-called complex drugs. The term "complex drugs" is used to define a wide variety of drugs such as liposomes, biologics, iron–carbohydrate complexes and glatiramoids [92]. Among them, only liposomes are considered to be well characterized and allow the application of the general BE procedures [92].

A difficult and controversial issue in BE assessment is that of biosimilars, namely to set the regulatory framework for demonstrating equivalence between biological or biopharmaceutical products. This need is of crucial importance, since biologicals represent a large proportion of the market, the patent of many original products has expired and biosimilars

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provide a mean to decrease health expenditures. However, the classic generic approach is not appropriate for biologicals due to their complexity and the fact that they are subtle to even minor manufacturing differences [92]. Thus, any biological product, before given to the patients, should be clearly identified in terms of quality, safety and efficacy [93,94]. More specifically, these issues require a complete characterization of the manufacturing and disposal process, the long-term toxicity and the potential immunogenicity. In order to face these problems, EMA has pioneered by adopting general and product-specific guidelines as in the case of human insulin, low-molecular-weight heparins, erythropoietins, interferon alpha, granulocyte-colony stimulating factor and somatropin [95]. For other biologicals such as interferon beta, follicle stimulation hormone, monoclonal antibodies, regulatory guidelines are still in progress [96]. Conclusively, biosimilars' substitution is a topic under extensive discussion without clear consensus, which is reflected on the fact that a case-by-case assessment is made for biologics and only a centralized procedure is currently allowed by EMA.

In the same vein, glatiramoids comprise a heterogeneous mixture of polypeptides with immunomodulatory activity [97]. The reason for this complexity is multiple-fold and can be attributed to the fact that these products do not appear in the systemic circulation, exhibit a complex mechanism of action, are immunogenic and can lead to serious toxic effects. The latter might imply a regulatory treatment similar to that of biologics. Nevertheless, equivalence assessment of glatiramoids becomes more complicated since their *in vivo* behavior is even more complex than biologics.

Equivalence of iron–carbohydrate (i.e., iron–sucrose) complexes represents another unresolved issue. Iron–sucrose preparations differ from the classic intravenous products since they are considered as nanoparticle medicinal products with several characteristics such as variation in size, different physicochemical properties, stability issues and different rates of degradation. Ultimately, it is anticipated that all these factors may have an impact on the safety (e.g., iron toxicity, oxidative stress) and efficacy profile. However, a precise regulatory framework is still missing for the assessment of 'generic' iron–sucrose products [98].

Another open question in BE assessment refers to immunosuppressive agents used in transplantations for the prevention of graft rejection. This is an emerging problem since the patents of many immunosuppressive compounds have recently expired, and generic formulations have already appeared in the market. Even though no specific guidelines are recommended for these products, there is a controversy whether the typical BE criteria can also be applied to generic immunosuppressives. For this reason, stricter criteria have been proposed for the evaluation of this kind of drugs [99].

Finally, pharmacogenomics is a promising field of application in BE testing. This term refers to the study of the expression variability of individual genes to drug response. Safety and efficacy of a drug treatment are influenced by the within-subject variability in genes, which affects encoding of drug transporters, metabolizing enzymes and drug targets. It is anticipated that determination of genetic polymorphism, such as identification of single nucleotide polymorphisms, can reduce between-subject variability. For this reason, it is quoted in the latest EMA guideline on BE that phenotyping and/or genotyping of subjects may be considered for safety or PK reasons [7]. In this vein, a new EMA guideline, on the use of pharmacogenomics methodologies in the PK assessment of drugs, will be in effect from August 2012 [100].

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Declaration of interest

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