REVIEW

Generic drugs: myths, facts, and limitations

Miti, realtà e limiti riguardanti i farmaci generici

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Summary Bioequivalence (BE) has always been an important pharmaceutical area, particularly (but not solely) in Mediterranean region, where the use of generic drugs is a relatively recent development. The lack of new therapeutic molecules has concentrated primary research in the hands of a few large pharmaceutical companies. For smaller companies, this has created opportunities for the development of new formulations of existing drugs (orodispersible tablets that dissolve in the mouth, extended-release tablets, transdermal delivery systems, generic drugs). These applications take advantage of the Abridged New Drug Application (ANDA) procedure, which exempts them from a series of expensive investigations and limits the requirement for clinical testing to bioequivalence trials. Since 1991, bioequivalence trials have been regulated by US Food and Drug Administration (FDA) and European Medicines Agency (EMA) guidelines that provide precise indications on the most specific procedures to be adopted. In spite of these guidelines, however, some aspects of the process have not been fully defined, the most important of which regards the management of endogenous substances. Additional problems are how to manage bioequivalence protocols with drugs that have long half-lives and those whose clearance is characterized by high intrinsic variability. The view that bioequivalence data would be more reliable if they were based on studies in target populations is a myth to be discredited. The present paper reviews issues relative to pharmacokinetics (PK), bioavailability (BA), and bioequivalence, also from an historical viewpoint, and includes a stimulating “questions and answers” section on some key aspects of the bioequivalence of generic drugs.

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Introduction: from pharmacokinetics to bioavailability and bioequivalence

The term “pharmacokinetics” was introduced the first time into life sciences by Dorst on 1953 [1]. At that time, the only way to follow plasma concentrations of drugs was to administer drugs labelled with radioisotopes, mainly \(^1^{14}C\) and \(^1^{3}H\), which allowed total scintigraphy to be measured. A pioneer approach was taken in the 1950s by Okita, who grew Digitalis purpurea L. in an air-tight chamber in the presence of \(^1^{3}CO_{2}\).
and then extracted and purified the resulting labelled digitoxin, a positive inotropic agent, which was administered to dogs and human beings, thus producing the first kinetic data on this drug [2—4]. This method is very sensitive, but is not specific, since also metabolites are assayed together with the parent compound. The total scintigraphy was extensively used in that period. A catalyzed chromatographic H/D exchange procedure allowed various molecules to be easily radio labeled with $^3$H, and then to be studied for their pharmacokinetics. Digitoxin, a positive inotropic drug cleared via urine as such, was extensively investigated with this procedure by Doherty [5—9]. Further studies using a more specific biassay confirmed the pharmacokinetic data published by Doherty on digitoxin [6], as this molecule is not metabolized and thus the radioisotopic tracing followed the digitoxin molecule. In contrast, data obtained by Okita on digitoxin [2—4] were not confirmed by further specific bioassay as digitoxin is a molecule that is cleared by biotransformation.

The term “bioavailability” and its widespread use started after the fully unexpected data from Lindebaum and colleagues on 1971 [10], who were able for the first time to bioassay digoxin in plasma with the radioimmunodissociation (RIA) method, just set up by Smith and Butler on 1969 [11]. Lindebaum et al. observed very relevant differences in plasma concentration of four different digitoxin formulations [10]. Their paper coined the term “biological activity”, further abbreviated in “bioavailability”.

U.S. Food and Drug Administration (FDA), as well as other regulatory authorities, proposed a dissolution method and related cut-off levels to accept or refuse digoxin preparations. Within a few years, the absorption of digoxin was regularized and the term of “bioavailability” widespread everywhere in scientific audience not only as a new name, but also as a new concept.

The radioisotopic approach was prevalent until 1960; thereafter, gas chromatography (GC) and, then, high performance liquid chromatography (HPLC) with various detectors allowed less sensible but more specific assays of drugs to be carried out [12]. These methods increased the knowledge of most drugs, mainly those possessing a low distribution volume, thus being present in plasma at large concentrations (in the $\mu$g/ml range). Further analytical developments led to the tandem mass spectrometry (LC-MS-MS) that, together with the last realizations, allows to assay drugs and drugs metabolites with a very high specificity (in the pg/ml concentrations range), even allowing run times of the analysis lasting about 3 min [13].

Now, pharmacokinetic data of existing drugs are well known. The different absorption and clearance of drugs are available also in special populations, such as infants, elderly, patients with liver, renal and heart diseases, dialysis and thyroid dysfunctions. Data on drug-drug, drug-food, drug-antacids interactions are now exhaustively described.

The definitions of four terms—namely bioavailability, bioequivalence, pharmacological equivalence and pharmaceutical alternatives—are reminded from European Medicines Agency (EMA) guideline, and are reported in Box 1 [14,15].

The bioavailability is, thus, related and restricted to the performance of a pharmaceutical formulation, e.g. disgregation, dissolution rates, and to the permeation of the active ingredient through the biological epithelia of gut [16].

### Box 1. Definitions of bioavailability, bioequivalence, pharmaceutical equivalence and pharmaceutical alternatives, as reminded from EMA guideline [14,15]

#### Bioavailability:
“Bioavailability is understood to be the extent and the rate at which a substance or its active moiety is delivered from a pharmaceutical form and becomes available in the general circulation and, thus, at the site of action”.

#### Bioequivalence (BE):
“Two medicinal products are bioequivalent if they are pharmaceutically equivalent or pharmaceutical alternatives and if their bioavailabilities after administration in the same molar dose are similar to such degree that their effects, with respect to both efficacy and safety, will be essentially the same”.

#### Pharmaceutical equivalence:
“Medicinal products are pharmaceutically equivalent if they contain the same amount of the same active substance(s) in the same dosage forms that meet the same or comparable standards. Pharmaceutical equivalence does not necessarily imply bioequivalence as differences in the excipients and/or the manufacturing process can lead to faster or slower dissolution and/or absorption”.

#### Pharmaceutical alternatives:
“Medicinal products are pharmaceutical alternatives if they contain the same active moiety but differ in chemical form (salt, ester, etc.) of that moiety or in the dosage form or strength”.

The disposition of the active ingredient when entered the body, e.g. metabolism, elimination, distribution, protein interaction, is not covered by bioavailability nor by bioequivalence studies, but resides in the domain of pharmacokinetics.

### Questions and answers

#### Are studies concerning healthy subjects and patient populations significant for their external validity and generalizability?

The clinical pharmacokinetic development of a given active ingredient includes the following steps:

- **descriptive pharmacokinetics (PKs) in healthy volunteers:** finger print PK parameters, linearity, absorption-distribution-metabolism-excretion (ADME), metabolic pathway, interactions (with food, drug/drug, or antacids), studies in steady state conditions;
- **PKs in special populations:** target population, elderly, liver disease, kidney failure, heart failure, thyroid dysfunctions, dialysis, polymorphic metabolism.

Thus, in the case of a new active compound, the regulatory authorities require for approval, in addition to non-clinical development, the whole PK profile that should include trials in healthy volunteers for PK and tolerability, and in target population for activity, PK and tolerability.

A different situation occurs when the application deals with a well known molecule, like the case of bioequivalence to approve a generic drug. This procedure is known as an Abridged New Drug Application (ANDA). In this case, all
pre-clinical procedures and clinical trials to test activity and safety and the complete PK profile are exempted, as these data are already well known and described in the registration dossier of the Reference [17], the brand name already on the market.

Regulatory authorities have reported on operating guidelines what is required for the registration of a generic drug, that is:
- only one bioequivalence (BE) trial on healthy volunteers in single dose, Test (candidate generic agent) vs Reference (a brand name already on the market), in fasting situation for immediate-release formulations;
- two BE trials—one after a high fat content breakfast and another in fasting status in healthy volunteers, in the case of enteric coated tablets both in single dose;
- the above two trials and another trial in steady state in healthy volunteers, in the case of an extended release formulation.

Bioequivalence focuses on the rate and extent of absorption of a given drug in the comparison Test vs Reference that must result close each other. This would mean that possible differences in PK parameters of the absorption process must be contained within a pre-defined statistical range, normally 0.80-1.25 range of 90% confidence intervals (C.I.s). This is checked in healthy volunteers.

Checking BE in target patient population is not required and would need a very higher pool size of subjects for the higher variability intrinsic in target population, producing less reliable results.

The high variability is due to concurrent pathologies, concurrent drugs and physical conditions.

Is the use of volunteers correct in bioequivalence studies? Should not we apply quite the contrary, because Test and Reference must be equivalent in sick, not in healthy subjects?

Bioequivalence is the demonstration that the rate and extent of systemic absorption of a given Test (candidate generic) is equivalent when compared to the Reference, the first drug registered with a complete dossier. This demonstration is carried out in healthy volunteers using the crossover design, namely the same subjects are treated with Test and Reference after a wash-out period.

The pool size of volunteers is selected from the intrinsic variability of the active compound, with volunteers being in very strict and documented healthy situations, with haematocellular parameters within the normal ranges, in absence of current or previous pathologies and concomitant medications, non smoking. If we could carry out the same BE trial on target population, a relevant increase of variability should be expected, that would mean that almost twice or even more volunteers would be enrolled.

BE means that the enteral absorption of the active ingredient from the pharmaceutical formulation into the systemic circulation of the Test is equivalent with that of the Reference. This is easier checked in healthy volunteers than in target populations.

Bioequivalence and bioavailability deal with drug concentrations and related PK parameters and not with therapeutic activity. In conclusion, the BE design on healthy volunteers is the most appropriate strategy and is predictive of the Test/Reference behaviour also in the target population.

When a generic drug product is approved, are the required rigorous standards established, with respect to identity, strength, quality, purity and potency, the same all over the world?

The conclusion of BE means that the new Test drug is equivalent to the Reference drug selected for the comparative trial. This conclusion is valid in each country where that specific Reference is marketed. This usually occurs in European member states. The FDA asks the demonstration of BE between the new Test drug compared with the Reference drug marketed in the USA.

Operating guidelines require the following requisites for both Test and Reference:
- the titre of the active compoud to be less different than 5%;
- the dissolution test must give similar profiles of Test and Reference at three pH, namely 1.2, 4.5, 6.8;
- BE must be demonstrated statistically with 90% C.I. within 0.80-1.25, or 80-125%.

May current bioequivalence requirements not be sufficient for drugs that exhibit a high degree of variability in PK variables?

BE is achieved when 90% C.I.s of pharmacokinetic parameters are comprised within the 0.80-1.25 range, with a statistical power ≥80%. This is achieved with the needed pool size, that is strictly correlated with the intrinsic variability of the active ingredient. The variability is expressed by the intra-subject coefficient of variation (CVintra) obtained from residual error of ANOVA as follows

\[ CV_{\text{intra}} = \sqrt{\text{residual error}} - 1. \]

Thus, the variability does not affect the BE conclusion, in the sense that it affects the pool size to achieve the statistical demonstration of bioequivalence.

How does variability impact on bioequivalence?

High variability of drug plasma concentrations and related PK parameters is an intrinsic characteristic of a given molecule. One cause of PK variability is a relevant first pass effect.

The variability is directly related to bioequivalence in terms of pool size of volunteers to be enrolled in a specific trial. Variability, in fact, can be managed by increasing the pool size.

BE operators usually consider an intrasubject CV of 25% as the cut-off; higher values of CV are expression of high or very high variability, whereas lower values are expression of medium or low variability. The pool size (=n) is calculated as intrasubject CVintra × 392 [18].

Now, in Table 1 we consider four cases of variability that would need different pool sizes.

In case of very high variability (CV = 60%), the pool size is very high, namely 141 subjects. In these situations, the replicate design can be adopted, which requires volunteers to be treated twice with the Test and twice with the Reference. In this case, the CV intrasubject obtained is lower than
the above set up to 60%. We can hypothesize the new CV for the replicate design to be 65% of the above CV, namely CV = 0.65 X 60% = 0.39%. The pool size in this case is: n = 0.39^2 X 392 = 60. That is less than half of that evaluated with the simple crossover design.

**Is it acceptable to enlarge the 90% C.I. with Cmax?**

The first EU guideline on bioavailability and bioequivalence allowed an enlargement of 90% C.I. with Cmax, but did not give how much to enlarge it [19]. From various publications, the enlargement was set up to 0.70-1.43 [20]. The further EU guideline set up this enlargement to 0.75-1.33 [14]. The last EMA guideline does not allow the enlargement of 90% C.I. of Cmax unless the intrasubject variability be ≥30% and the replicate design is adopted [15]. The enlargement required in this case is reported in Table 2.

**There is an ongoing debate about generic drug use for a multitude of conditions including epilepsy, psychosis, hypertension, post-organ transplantation, and several infectious diseases. Most concerns involve drugs with narrow therapeutic indices. What about their safe use in these conditions?**

As already punctualized on above question/answer sections, the BE conclusion is achieved on healthy volunteers and is strictly related to the performance of the pharmaceutical formulation and to the intrinsic variability of the active compound in crossing epithelium strata of the gut.

In the case of drugs with narrow therapeutic index, guideline operating in EU asks BE to be demonstrated with more restricted 90% C.I., e.g. 0.90-1.10 (90-110%).

**How does ethics impact on bioequivalence?**

Any clinical trial, and thus all BE trials, must be preventively approved by Ethics Committee and regulatory authorities.

| Variability | Pool size (No. of subjects) |
|-------------|-----------------------------|
| Low variability, CV% = 16% | 0.16^2 X 392 = 10 |
| Medium variability, CV% = 24% | 0.24^2 X 392 = 23 |
| High variability, CV = 40% | 0.40^2 X 392 = 63 |
| Very high variability, CV = 60% | 0.60^2 X 392 = 141 |

The study design will comply with ethics rules, namely Helsinki Declaration, and operating guidelines, and Good Clinical Practice as well.

Apart from cytotoxic agents that cannot be administered to healthy subjects, some existing drugs must be administered to healthy volunteers with specific precautions reported in their Summary of Product Characteristics (SPCs).

However, some drugs that can be administered in single dose to healthy volunteers could produce relevant safety problems when administered in a repeated-dose regimen to steady state. Steady-state trials are in fact required by EU operating guidelines for modified-release formulations of drugs. Some examples are as follows. Carbamazepine requires about 30 days to achieve the steady state [21]. Morphine, marketed as 10, 30, 60, 100 and 200 mg modified-release formulations, can induce vomiting and cardiorespiratory adverse events at relatively high doses and tolerance with repeated-dose regimens [22]. Warfarin, with an elimination half-life (t1/2) of 2 days, is associated with relevant adverse events, if given as a repeated-dose regimen [23]. In addition, the abrupt interruption of warfarin treatment can cause a rebound effect on the activation of the haemostatic system, which does not occur with single-dose administration. Cyclosporine can affect renal clearance [24] and flutamide causes gynecomastia in males [25]. In these cases, the repeated-dose regimen to achieve steady state must be avoided.

That above described is considered one of the open questions on bioequivalence, that has not yet been focused on by operating guidelines [26].

**Are both males and females suitable to be enrolled in bioequivalence trials?**

In most cases, subjects of both sexes should be enrolled. Some exceptions however must be considered. For instance, drugs that are designed for only one sex must be tested on this sex only.

This could be inter alia the case of drugs affecting prostate, penis erection, indicated for men only, and drugs for female infertility, for females only.

**What drugs with long half-lives?**

The crossover design, the most adopted for BE trials, needs a blood sampling covering a period of at least 3 times the drug half life and a washout between the consecutive study periods lasting about 7 times the drug half-life.

This would mean that with long or very long t1/2, the crossover design is problematic. In a specific publication, the case of tamoxifen was considered the cut-off, namely drug with t1/2 >15 days could be studied with the parallel group design [27].

Operating guideline allows to trunk blood sampling period at 72 hours after dosing. However, adopting the crossover design, the wash-out cannot be reduced, this to assure the lack of carryover concentrations from the first to the consecutive study period.

The parallel group design, however, requires more volunteers than the crossover design, in order to achieve the same statistical performance.

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**Table 1** How variability affects the pool sizes.

| Variability               | Pool size (No. of subjects) |
|---------------------------|-----------------------------|
| Low variability, CV% = 16%| 0.16^2 X 392 = 10            |
| Medium variability, CV% = 24%| 0.24^2 X 392 = 23           |
| High variability, CV = 40%| 0.40^2 X 392 = 63            |
| Very high variability, CV = 60%| 0.60^2 X 392 = 141         |

**Table 2** Enlargement of 90% C.I. of Cmax in the case of replicate design, according to the last EMA guideline [15].

| Intrasubject CV | 90% Confidence Interval (C.I.) |
|-----------------|--------------------------------|
| 30              | 80.00-125.00                   |
| 35              | 77.23-129.48                   |
| 40              | 74.62-134.02                   |
| 45              | 72.15-138.59                   |
| ≥ 50            | 69.84-143.19                   |
What does it happen with endogenous substances?

That of endogenous substances is the most relevant open question on bioequivalence [28]. This issue was described in various publications for PK, bioavailability and BE applications.

The differences between endogenous substances and general drugs are as follows [29]:
- the body preserves endogenous substances at the concentrations that are considered adequate;
- the enteral absorption of endogenous substances is controlled by the body with various mechanisms preserving homeostatic equilibrium;
- the dilution of the newly absorbed amount with that already present in the body is a physical mechanism to buffer the concentration of endogenous substances;
- the renal threshold of endogenous substances cleared via urine assures a controlling mechanism of homeostatic equilibria;
- the reversible multimetabolic interconversion and specific body storages are additional homeostatic mechanisms;
- the rhythms that increase/decrease some of these substances during the day (melatonin) or the month (female sexual hormones).

The above mechanisms make the PK, bioavailability, and thus the BE of endogenous substances the most difficult problem in this area.

Usually, the plasma concentrations of most of these substances do not produce a well defined shape of the absorption profile, but more likely they fluctuate around baseline, that does not allow any baseline subtraction for the very relevant increase of coefficient of variation, as follows:

- baseline concentration 100 ± SD 10, CV=10%
- after the dose concentration 110 ± SD 10, CV=9%
- net value 110 - 100 = 10 ± SD 10, CV=100%.

This situation was considered by US FDA in the case of potassium bioequivalence, suggesting to not consider plasma concentration, but only cumulative urinary excretion (CUE) of potassium as the parameter to assess Test vs Reference bioequivalence [30–32].

This approach is applicable to all endogenous substances cleared via urinary excretion, like sodium, potassium, L-carnitine, some aminoacids.

But, what to do with endogenous substances cleared through biotransformation? The case of levothyroxine can give us a useful example, here again from US FDA requirements. Thyroxine offers an approach for BE, useful for other endogenous substances cleared via biotransformation. An appropriate BE design with this substance requires to work with a long-lasting repeated-dose regimen, and working on whole post-dose concentrations without baseline subtraction, assuming that baseline values are a result of the repeated dose regimen [33,34].

Are brand name drugs safer than generic drugs?

To achieve the approval, a generic drug needs, in comparison to the Reference (brand name):
- to be pharmaceutical equivalent or pharmaceutical alternative;
- to possess similar in vitro dissolution data at three pH and titre being different no more than ± 5%;
- to be bioequivalent.

The above items lead to conclude that Test and Reference of a given active compound must not differ in activity and safety.

May physiologic changes associated with age, polytherapy and comorbidities affect drug absorption, distribution, metabolism, excretion and a safe use of the generic drug in the geriatric population?

The above situations, if able to affect PK of a given compound, must act in the same extent with Test and Reference.

Generic drugs are chemically equivalent to their brand-name counterparts in terms of active ingredients but may differ in peripheral features, such as pill colour or shape, inner binders and fillers, and the specific manufacturing process. Can these factors negatively influence compliance and adherence to therapy, mostly in elderly?

The rules to check bioequivalence do not consider some pharmaceutical aspects as colour and shape. Any role of the binder in the absorption process must be ruled out as it would have led to difference in bioequivalence.

Active ingredients contained in the generic formulation may be the same as the branded, but may be present in a different form, as pharmaceutical alternatives. These alternatives may have different salts, esters, or complexes of the active moiety or may be different in dosage forms or strengths. The form may have different absorption characteristics and solubility properties and these differences may explain differences in clinical efficacy and safety. What about this issue?

According to operating guidelines, pharmaceutical alternatives can be registered as generic drugs if they demonstrate to be bioequivalent with the Reference.

Although evidence does not support the notion that brand-name drugs used in cardiovascular disease are superior to generic drugs, a substantial number of editorials counsel against the interchangeability of generic drugs. What about this issue?

If the whole procedure to compare Test and Reference is correctly done, no activity differences between Test and Reference are expected.
However, problems of switchability are described in literature not depending from the performance of the generic drug [35, 36]. These considerations led FDA to edit a guideline on individual bioequivalence to be studied with the replicate design that is as follows: Test — Reference — Test — Reference [37]. This design assesses the within-subject variability as well as the subject-by-formulation interaction that should reduce the switchability problems met shifting from Reference to a generic drug, as well as from a given generic drug to another generic drug.

FDA prefers the individual approach in assessing bioequivalence. This design is very rarely adopted in EU.

Conclusive remarks

Guidelines operating in EU and in the USA require that a new generic drug must prove to possess the same rate and extent of absorption into the systemic circulation when compared to the Reference, a brand name registered with the complete dossier.

The above issues are related to the pharmaceutical technology adopted and to the absorption process including the intrinsic variability of the molecule. Any consideration about the efficacy must be regarded as a further event.

Thus generic drug can be registered with a complete chemistry and pharmacy dossier that must describe in details the pharmaceutical procedures, and with the bioequivalence file, that includes one, two or three clinical trials, according to the kind of pharmaceutical formulation, e.g. immediate release, enteric coated, extended-release, or without bioequivalence trial in case of exemption.

When Test vs Reference similar plasma concentration-time behaviour and then the bioequivalence has been demonstrated in healthy volunteers, the similarity of activity and safety is a consequence of the similar bioavailability. The high variability, typical of some molecules, is an issue statistically managed in the pool size evaluation to achieve the bioequivalence conclusion.

In conclusion, when the bioequivalence of Test and Reference is correctly demonstrated, the consequence is that also their activity and safety are expected to be equivalent.

Conflict of interest

The authors have no conflict of interest to declare.

References

[1] Gladtke E. History of pharmacokinetics. In: Pecile A, Rescigno A (Eds.), Pharmacokinetics. New York. 1988:1–10.
[2] Okita GT, Kelsey FE, Walaszek EJ, Geiling EM. Biosynthesis and isolation of carbon-14 labelled digoxin. J Pharmacol Exp Ther 1954;110:244–50.
[3] Okita GT, Talso PJ, Curry Jr JH, Smith Jr FD, Geiling EM. Metabolic fate of radioactive digoxin in human subjects. J Pharmacol Exp Ther 1955;115:371–9.
[4] Okita GT, Talso PJ, Curry Jr JH, Smith Jr FD, Geiling EM. Blood level studies of C14-digoxin in human subjects with cardiac failure. J Pharmacol Exp Ther 1955;113:376–82.
[5] Doherty JE, Perkins WH. Studies with tritiated digoxin in human subjects after intravenous administration. Am Heart J 1962;63:528–36.
[6] Doherty JE, Perkins WH. Tissue concentration and turnover of tritiated digoxin in dogs. Am J Cardiol 1966;17:47–52.
[7] Doherty JE. The clinical pharmacology of digitalis glycosides: a review. Am J Med Sci 1968;255:382–414.
[8] Doherty JE, Kane JJ. Clinical pharmacology and therapeutics use of digitalis glycosides. Drugs 1973;6:182–221.
[9] Doherty JE, De Soya N, Kane JJ, Bissett JK, Murphy ML. Clinical pharmacokinetics of digitalis glycosides. Prog Cardiovasc Dis 1978;21:141–58.
[10] Lindebaum J, Mellow MH, Blackstone MO, Butler Jr VP. Variation in biological availability of digoxin from four preparations. N Engl J Med 1971;285:1344–7.
[11] Smith TW, Butler Jr VP, Haber D. Determination of therapeutic and toxic serum digitoxin concentrations by radioimmunoassay. N Engl J Med 1969;281:1212–6.
[12] Marzo A. Chromatographic methods and selective detectors in pharmacokinetics. Bolt Chim Farm 1989;128:45–53.
[13] Marzo A, Dal Bo L. Tandem mass spectrometry (LC-MS-MS): a predominant role in bioassays for pharmacokinetic studies. Arzneimittelforschung 2007;57:122–8.
[14] EMA 2001. Note for guidance on the investigation of bioavailability and bioequivalence 1401/98, London 26 July 2001.
[15] EMA 2010. Guideline on the investigation of bioequivalence, 1401/98 Rev 1/Corr, London 20 January 2010.
[16] Amidon GL, Lennernäs H, Shah VP, Crison JR. A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. Pharm Res 1995;12:413–20.
[17] Marzo A. Clinical pharmacokinetic registration file for NDA and ANDA procedures. PharmacoRes 1997;36:425–50.
[18] Marzo A, Balant LP. Bioequivalence: an updated reappraisal addressed to applications of interchangeable multi-source pharmaceutical products. Arzneimittelforschung 1995;45:109–15.
[19] EMA 1991. Investigation of bioavailability and bioequivalence. III/54/89, December 1991.
[20] Diletti E, Hauschke D, Steinijans VW. Sample size determination: extended tables for the multiplicative model and bioequivalence ranges of 0.9 to 1.11 and 0.7 to 1.43. Int J Clin Pharmacol Ther Toxicol 1992;30(Suppl 1):559–62.
[21] Bertilsson L, Tomson T. Clinical pharmacokinetics and pharmacological effect of carbamazepine and carbamazepine 10,11-epoxide. Clin Pharmacokinet 1986;11:117–98.
[22] Osborne R, Joel S, Treed D, Slevin M. Morphine and metabolite behaviour after different routes of morphine administration: demonstration of the importance of the active metabolite morphone-6-glucuronide. Clin Pharmacol Ther 1990;47:12–9.
[23] Wingard Jr LB, O’Reilly RA, Levy G. Pharmacokinetics of warfarin enantiomers: a search for intrasubject correlations. Clin Pharmacol Ther 1978;23:212–7.
[24] Bennett WM, Pulliam JP. Cyclosporine nephrotoxicity. Ann Intern Med 1983;99:851–4.
[25] Jacobo E, Schmidt JD, Weinstein SH, Flocks RH. Comparison of flutamide (SCH-13521) and diethylstilbestrol in untreated advanced prostatic cancer. Urology 1976;8:231–3.
[26] Marzo A. Bioequivalence behind the scenes. Pharm Dev Regul 2003;1:179–89.
[27] Marzo A. Crossover design in tamoxifen bioequivalence: a borderline situation. J Pharm Pharmacol 1998;50:1433–4.
[28] Marzo A. Open questions on bioequivalence: an updated reappraisal. Curr Clin Pharmacol 2007;2:179–89.
[29] Marzo A, Rescigno A. Pharmacokinetics of endogenous substances: some problems and some solutions. Eur J Drug Metab Pharmacokinet 1993;18:77–88.
[30] Marzo A, Vuksic D, Crivelli F. Bioequivalence of endogenous substances facing homeostatic equilibria: an example with potassium. Pharmacol Res 2000;42:523–5.

[31] Cerny I, Dighe SV. Guidance for in vivo bioequivalence study for slow-release potassium chloride tablets/capsules. Rochville (MD): FDA Centre for drug evaluation and research; 1987 May 15.

[32] US FDA Guidance for Industry. Potassium chloride modified-release tablets and capsules: in vivo bioequivalence and in vitro dissolution testing. US Dept of Health and Human Services, FDA Centre for Drug Evaluation and Research. October 2005.

[33] Dong BJ, Hauck WW, Gambertoglio JG, Gee L, White JR, Bubp JL, et al. Bioequivalence of generic and brand-name levothyroxine products in the treatment of hypothyroidism. JAMA 1997;277:1205–13.

[34] Cerutti R, Rivolta G, Cavalieri L, Di Giulio C, Grossi E, Vago T, et al. Bioequivalence of levothyroxine table administered to a target population to a steady state. Pharmacol Res 1999;39:193–201.

[35] Anderson S. Individual bioequivalence: a problem of switchability. Biopharm Reports 1993;2:1–11.

[36] Chen ML. Individual bioequivalence. A regulatory update. J Biopharm Stat 1997;7:5–11.

[37] US FDA Guidance for Industry. In vivo bioequivalence studies based on population and individual bioequivalence approaches. US Dept of Health and Human Services, FDA Centre for Drug Evaluation and Research. October 1997.