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Computer Simulations as a Tool for Optimizing Bioequivalence Trials

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1. Introduction

The analyte to be measured in a Bioequivalence study when an oral drug undergoes a metabolic step in intestine or liver is still today a controversial issue with different recommendations in European Medicines Agency (EMEA/EMA) and Food and Drug Administration (FDA) guidance documents (EMA, 2010; EMEA, 2001; FDA, 2002).

In the current EMA guidance it is stated that in principle, evaluation of bioequivalence should be based upon measured concentrations of the parent compound (also for inactive pro-drugs) as the Cmax of the parent compound is usually more sensitive to detect differences in absorption rate than the Cmax of the metabolite. Only for some pro-drugs with very low plasma concentrations and quickly eliminated it is acceptable to demonstrate bioequivalence for the main active metabolite without measurement of parent compound. Nevertheless in these exceptional cases the applicant should adequately justify that it is not possible to reliably measure the parent compound after single dose administration (even with supra-therapeutic doses) and moreover the applicant should present any available data supporting the view that the metabolite exposure will reflect parent drug and that the metabolite formation is not saturated at therapeutic doses (EMA, 2010)

FDA guidance recommends metabolite measurement if it is formed as a result of gut-wall or other pre-systemic metabolism and if the metabolite contributes meaningfully to safety and/or efficacy. In this case parent drug data is used to confidence interval approach whereas metabolite data is used as supportive evidence of comparable therapeutic outcome (FDA, 2002).

The extent of pre-systemic metabolism and the non-linearity of the metabolic processes are the controversial aspects that require harmonization with regards to analyte selection. The lack of agreement in FDA and EMEA/EMA recommendations and the changes in the new

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* This article reflects the author's personal opinion and not necessarily the policy or recommendations of the AEMPS.

EMA guideline makes evident that the simulations on which those recommendations were based, if any, were performed in different set of scenarios under a different set of assumptions leading to different answers to the same question.

2. Simulation models of bioequivalence scenarios

The critical issues that have been considered in the literature to create the simulated scenarios, apart from the true differences in extent and/or rate of absorption are

a.   the extent of pre-systemic metabolism, intestinal or hepatic
b.   the non-linearity of the metabolic processes
c.   the intrinsic clearance magnitude (high or low) and
d.   the intra-subject variability (high or low)

For instance Chen and Jackson (Chen & Jackson, 1991,1995) and Jackson (Jackson, 2000) constructed models of two compartments with and without a linear metabolic step. They considered the difference in absorption rate with Cmax as target parameter and the final criterion to select the best analyte was intra-individual variability.

The factor of parent drug or metabolite variability, nevertheless, is an arguable aspect to make a decision about the analyte. Once a study design is selected, the larger the intra-subject (inter-occasion) variability of the analyte, the lower the percentage of successful bioequivalent studies for a given real difference. In another words, the lack of power can be solved by increasing the number of patients in the study but the lack of sensitivity cannot be improved once the insensitive analyte has been selected. The ability to reflect the formulations differences in the estimations (accuracy) should not be confounded with the variability of the estimations (precision). The analyte selection should be based on the accuracy of the estimations. Statistically, the consumer and producer risk offered by each analyte (with the adequate sample size) should be the main determinants for this decision (Fernandez-Teruel et al., 2009b).

The issue of parent drug and metabolite variability has been addressed in other papers based on simulations with controversial conclusions (Blume & Midha, 1993; Jackson, 2000; Rosenbaum, 1998). Many of these simulation works have employed the percentage of failed studies as end-point to select the analyte to be measured. This depends not only on the difference between formulations but also, and in a higher extent, on the variability of the analytes. In spite of the interest of sponsors in decreasing the percentage of failed studies, to select the analyte based on its rate of failures should never be the regulatory criterion. On the contrary, the study design and analyte should be defined according to their ability to detect differences between formulations (i.e reducing the consumer risk of accepting bioinequivalent formulations)(Fernandez-Teruel et al., 2009a; 2009c)

Brady and Jackson (Braddy & Jackson, 2010) used a model similar to Chen and Jackson models but with non linear metabolism. As in the previous papers the main conclusion was that the parent drug (either AUC or Cmax data) was more sensitive to formulation differences than the metabolite. Apart from their simplicity the main objection of these models was the over parameterization as the first-pass metabolic clearance was modelled as a different and independent parameter than the metabolic systemic clearance.

A second group of papers present simulations based on semi-physiological models (Rosenbaum, 1998; Rosenbaum & Lam, 1997; Tucker et al., 1993)   that solved the over-parameterization issue but they do not included in the simulations the problem of non-linear metabolism. In all the cases, their simulations showed that parent drug and metabolite have the same sensitivity to detect differences in extent of absorption (AUC) when the system is linear, but the Cmax of parent drug is more sensitive to differences in rate of absorption.

The study design (single dose versus steady state studies) has also been addressed by simulation approaches (el-Tahtawy et al., 1994, 1995; 1998; Jackson, 1987, 1989, 2000; Zha & Endrenyi, 1997) with the conclusion that single dose studies are more sensitive to detect differences in absorption rate.

3. BCS-based simulations

The Biopharmaceutic classification system (BCS) has changed the focus of bioequivalence demonstration from plasma levels to the absorption site, as permeability of the intestinal membrane (P), solubility (S) in luminal fluids and in vivo dissolution rate are recognized as the main determinants of rate and extent of absorption. The combination of the two levels of the permeability and solubility factors (High (H) or Low (L) permeability and High or Low solubility) defines the 4 BCS classes (Class 1: HP, HS ; Class 2: HP, LS; Class 3: LP, HS; Class 4: LP, LS) (Amidon et al., 1995; FDA, 2000)

It is generally accepted, and it has been shown through gastrointestinal simulation technology (computer simulations) that for class 1 and 3 formulation impact on extent of absorption is minimal, and regarding absorption rate, the formulation influence is also minimal for class 3 drugs while it could be reflected in Cmax differences for class 1 drugs (Kuentz, 2008). Class 2 drugs having good permeability but low solubility are the candidates showing a great dependence on formulation factors as for these drugs solubility and in vivo dissolution rate are the limiting factors. As BCS classification is relevant for the probabilities of bioequivalence problems related to the formulation, this classification system has been taken into account recently for the simulation approach to the analyte selection discussion (Fernandez-Teruel et al., 2009a; 2009b; 2009c; Navarro-Fontestad et al., 2010)

The authors addressed all the issues mentioned in the previous section that have been discussed in the literature i.e. the intrinsic clearance magnitude, the variability of the analyte, the linearity of the metabolic step and single dose versus steady state designs. In top of that, the four drug BCS classes were simulated in formulations of decreasing quality compared to the reference one. Results were analyzed from the point of view of the analyte giving the right answer to the BE criteria. As BE scenarios were simulated for each drug, it was possible to calculate which analyte detects better the lack of pharmaceutical quality.

The authors explored semi-physiological models of increasing complexity starting with a model considering hepatic first pass effect under linear and non linear conditions, then, adding the intestinal metabolic step and finally considering the existence of two metabolic pathways of different magnitude. The latest addition to those models is the involvement

of intestinal transporters that could eventually lead to a non linearity in the absorption process.

In all the models the scheme for generating the scenarios is depicted in Figure 1 and briefly explained in the next section.
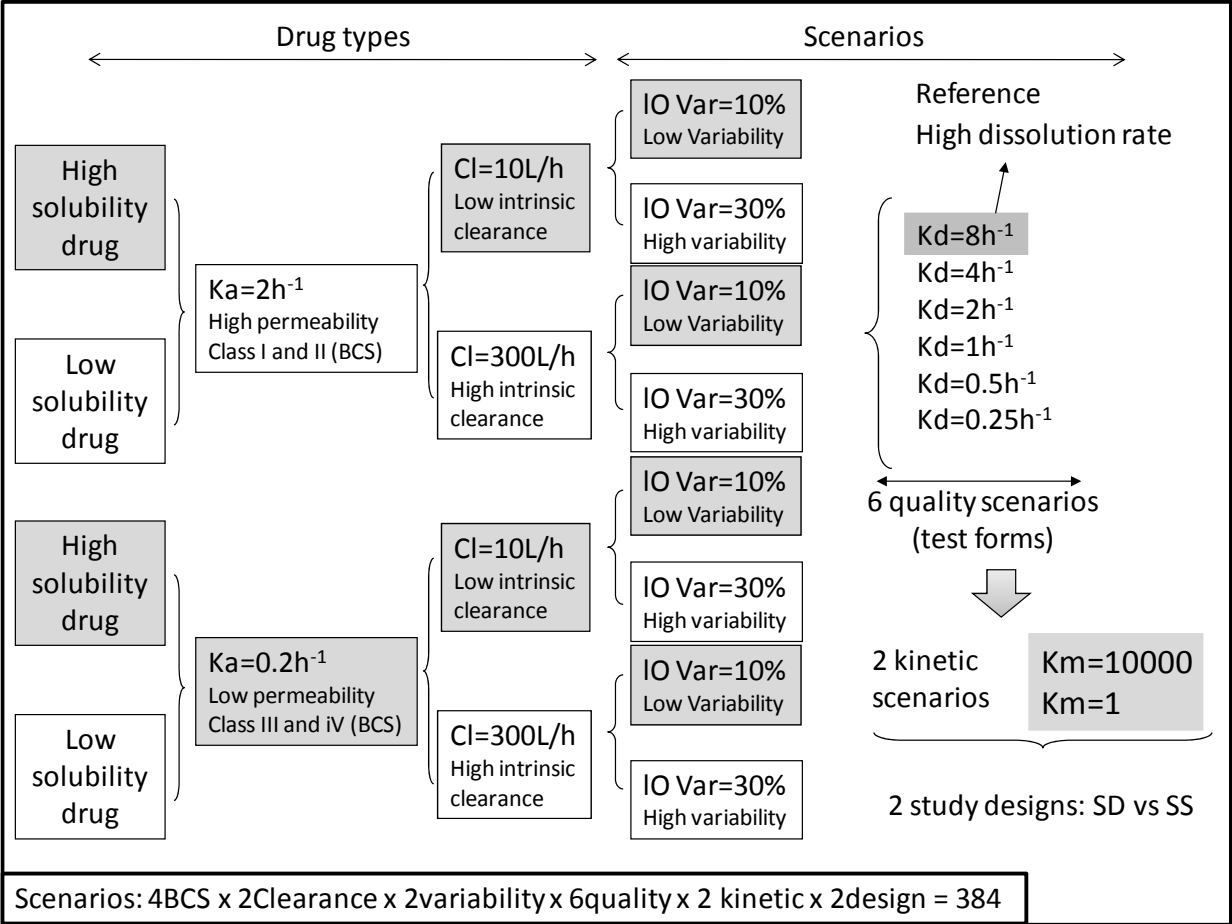
Ka: absorption rate constant; Kd: in vivo dissolution rate constant; IO Var: inter-occasion variability expressed as %; SD: single dose; SS: steady state

Fig. 1. Scenarios and drug types generation scheme for performing the BCS-based simulations. The semiphysiological models of increasing complexity were tested under these assumptions for a given set of pharmacokinetic parameters. (Fernandez-Teruel et al., 2009b, with permission from the authors).

3.1 Description of drug types, study designs and explored scenarios

The aim of these simulations was to define the most sensitive analyte, parent drug or metabolite, for in vivo bioequivalence studies. In this way, several drug types, study design and scenarios are used:

- *Drug types*: simulations can be made for different class of drugs by varying the kinetic parameter values as clearance, permeability, solubility. Simulations have been performed for:

- Four drug classes corresponding to Biopharmaceutical Classification System by combining high and low permeability (Ka) and solubility (S).
- High and low intrinsic hepatic clearance ($Cl_{int,0H}$).
- High and low inter-individual variability in intrinsic hepatic clearance (this point will be explained in detail in the model).
- High and low Michaelis-Menten constant (Km_H): differences between this parameter and liver drug concentrations defines the metabolic pathway saturation so when KmH is small (it takes values around liver drug concentrations) the metabolism becomes non-linear (saturated), but when Km_H is large (it takes values so much greater than liver drug concentrations) the metabolic system remains linear (non-saturated).

- *Study design*: it refers to perform the bioequivalence study after dosing the drug in single dose or in multiple doses. In the case of multiple doses, drug is administered every 8 hours (or a dosing scheme considered) and the bioequivalence study should be performed when steady state is reached.
- *Scenarios*: defining the most sensitive analyte to detect differences in pharmaceutical quality performance requires comparing a reference product with different test products of varying quality. This pharmaceutical quality has been defined in these simulations as similar dissolution rate constant, so good quality has been considered when reference and test products have similar dissolution rate constant value (in vivo in lumen), and six different scenarios were explored by decreasing the value of this parameter from 100% to 3% of reference value.

The combination of all these different factors and levels correspond to a total of 384 bioequivalence scenarios: 32 drug types explored at single dose and steady state, by using 6 different formulations of decreasing quality compared to the reference one. The pharmacokinetic parameters used in Table 1.

3.2 The model implementation

A detailed explanation of the mathematical description of this semi-physiological approach is presented here as well as some examples of the outcomes that could be obtained in order to illustrate how this tool can be applied to particular drugs with known pharmacokinetics parameters in order to not only select the best analyte and study design but also to explore the impact of the quality of the formulation on the outcome of the Bioequivalence trial thus allowing to risk-analysis based decisions. A basic scheme of the model is shown in Figure 2.

The model is a semi-physiological one which includes six compartments: intestinal lumen (C1), liver (C2), systemic compartment (C3), metabolite compartment (C4), solid dosage form (C5) and kidney (C6). Each compartment is represented by Cn, and the processes involved in drug pharmacokinetics are represented by En.

The solid dosage form is administered by oral route, and it dissolves in lumen (E1). Then, the dissolved fraction can be degraded in lumen (E2) or absorbed (E3), but the absorption process duration depends on the intestinal transit time. Once absorbed, the drug is partially metabolized in the liver (E4) and it reaches the systemic plasma compartment, where the drug is rapidly distributed. Finally, the drug is eliminated by both routes: hepatic

metabolism (E4) and renal excretion (E5), while the metabolite formed is eliminated by renal excretion (E6).
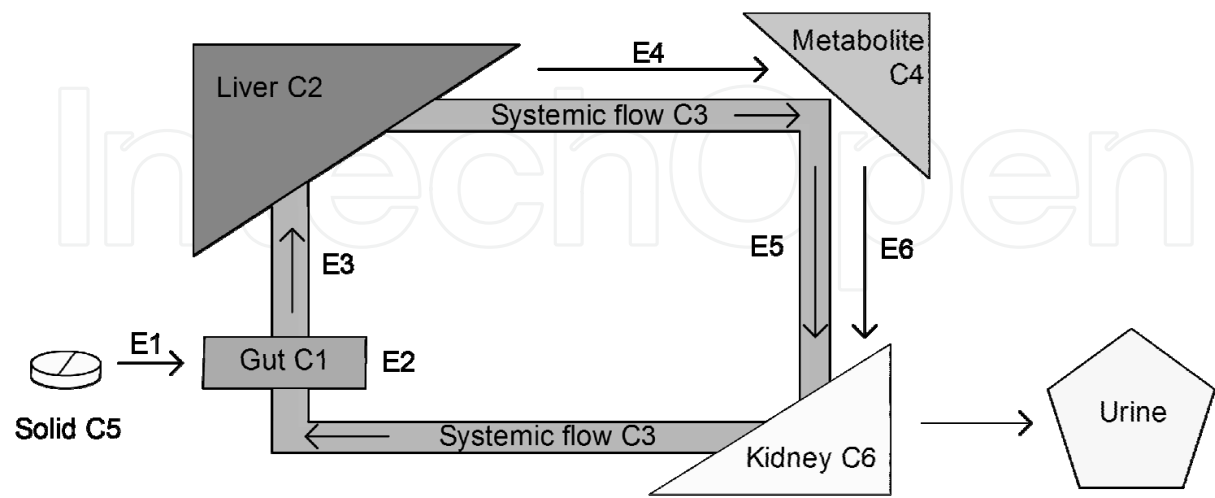

Fig. 2. The basic semi-physiological model used to perform simulations of BE trials for all BCS drugs. These model can be updated with more processes (as intestinal metabolism, or different metabolic routes).

Parameter	Value
Operative absorption time (OAT) (h)	7
Degradation rate in lumen (h−1)	0
Dissolution rate for reference form (h−1 mg−1)	4
Maximum soluble amount (mg)	10
	1000
Intrinsic absorption rate constant of the drug(h−1)	0.2
	2
Intrinsic clearance (L/h)	10
	300
Km intrinsic clearance (mg/L)	1
	10000
Renal clearance of parent drug (L/h)	0.05
Clearance of metabolite (L/h)	20
Hepatic flow (QH) (L/h)	90
Central compartment volume (L)	40
Hepatic volume (L)	1
Metabolite compartment volume (L)	40

Table 1. Pharmacokinetic parameters used in the simulations of Bioequivalence trials

1.   Dissolution in lumen (E1) is considered limited by the solubility:

$$E1 = Kd \cdot A5 \cdot (S - A1) \qquad (1)$$

Where E1 is the dissolution rate, A1and A5 represent the amount of drug in lumen and in solid dosage form respectively and S is the maximum soluble amount. The term Kd should not be interpreted as the first order dissolution rate as it has units of $h^{-1} \bullet mg^{-1}$. This parameterization is equivalent to this second one:

$$E1 = Kdis \cdot A5 \cdot \left(1 - \frac{A1}{S}\right) \qquad (2)$$

where Kdis would represent the typical first order intrinsic dissolution rate constant and its units would be h^{-1}.

2.   The drug dissolved can be degraded in lumen (E2) or absorbed (E3):

$$E2 = K \deg \cdot A1 \qquad (3)$$

Where E2 is the degradation rate, Kdeg is the first order degradation rate constant and A1 the amount of drug in lumen. The luminal degradation was fixed to zero in the simulations, but both the degradation kinetic model and the value of the corresponding parameters can be easily changed to accommodate a degradation step in lumen.

Drug absorption can be implemented as a first order process:

$$E3 = (Ka \cdot A1) \cdot \alpha \qquad (4)$$

Where E3 is the absorption rate, Ka the first order absorption rate constant and A1 the amount of drug in lumen and α is the operator to account for the intestinal transit time. α takes value "1" when the time is less than the intestinal transit time (or operative absorption time OAT in the model) and is set to "0" when time is higher than OAT.

After the OAT the compartment dose C5 was reset to zero, simulating the effect of the intestinal transit and therefore the drug in solid form was not accumulated in the gut for the scenarios of multiple dosage administrations.

Other absorption kinetics can be easily implemented, as an active absorption transport or an efflux mechanism, by adding the corresponding term to the equation.

For example in order to account for an efflux transport mechanism, a new compartment (C_{gut}) should be added, and the equation describing the rate of absorption would be:

$$E3 = (Ka \cdot A1)\alpha - \frac{Vm_E \cdot C_{gut}}{Km_E + C_{gut}} \qquad (5)$$

where Vm_E and Km_E are the Michaelis-Menten parameters and C_{gut} is the concentration in gut wall.

3.   The drug is partially metabolized in the liver (E4) after its absorption:

$$E4 = \phi H \cdot EH \cdot C2 \qquad (6)$$

The hepatic metabolic rate E4 depends on the hepatic blood flow (ΦH), the drug concentration in the liver (C2) and the hepatic extraction ratio (EH).

EH is a parameter dependent on the hepatic blood flow (ΦH), and the intrinsic clearance at concentration C($Cl_{int, CH}$).

$$EH = \frac{Cl_{int,CH}}{Cl_{int,CH} + \phi H} \qquad (7)$$

in which $Cl_{int, CH}$ is

$$Cl_{int,CH} = \frac{Cl_{int,0H} \cdot Km_H}{Km_H + C2} \qquad (8)$$

Thus $Cl_{int, CH}$ is a non-linear function of three parameters: clearance at infinite blood flow and zero hepatic concentration ($Cl_{int, 0H}$), the Michaelis-Menten value (Km_H) and liver drug concentration (C2).

Thanks to this modeling of the hepatic metabolism a wide range of drug types and scenarios can be explored by changing the value of the intrinsic clearance or by changing the value of Km_H. that would lead to linear or non linear conditions depending on the liver concentrations compared to Km_H. In another words first-pass effect was managed as linear using a high value of Km_H and as non linear using a Km_H value around the drug concentration found in liver.

4. Drug is eliminated by hepatic metabolism (E4) and renal excretion (E5):

$$E5 = Clrenal \cdot C3 \qquad (9)$$

Where E5 represents the renal excretion rate. Clrenal is the renal clearance of drug and C3 is the drug concentration in systemic compartment (so it is assumed that systemic concentration equals the concentration in kidney).

As in the other kinetic processes, different excretion mechanism or kinetics (linear-non linear) can be considered and easily implemented.

a. Gut metabolism:(Navarro-Fontestad et al., 2010) In order to describe a first pass metabolic step in small intestinal tissue, similar equations as the ones used for describing hepatic metabolism can be implemented:

$$E = \phi G \cdot EG \cdot C_{gut} \qquad (10)$$

$$EG = \frac{Cl_{int,CG}}{Cl_{int,CG} + \phi G} \qquad (11)$$

$$Cl_{int,CG} = \frac{Cl_{int,0G} \cdot Km_G}{Km_G + C_{gut}} \qquad (12)$$

where 'G' corresponds to 'GUT' parameters, and C_{gut} is the drug concentration in gut compartment. The other parameters having the same meaning than previously explained i.e

E metabolism rate, EG extraction ratio in gut and Cl_{int} intrinsic clearance (in the examples presented in this chapter gut metabolim was not included.)

b. Several metabolic pathways:(Navarro-Fontestad et al., 2010) it can be considered that drug is metabolized by two different routes, leading to different metabolites. The way to implement this model is equivalent to the present one, but it is important to estimate in a good way the extraction ratio, because EH (or 'EG') is different for each metabolite:

$$Cl_{int,0H}^{M1} = \frac{Cl_{int,CH}^{M1} \cdot Km_H^{M1}}{Km_H^{M1} + C2} \tag{13}$$

$$Cl_{int,0H}^{M2} = \frac{Cl_{int,CH}^{M2} \cdot Km_H^{M2}}{Km_H^{M2} + C2} \tag{14}$$

$$EH^{M1} = \frac{Cl_{int,CH}^{M1}}{Cl_{int,CH}^{M1} + Cl_{int,CH}^{M2} + \phi H} \tag{15}$$

$$EH^{M2} = \frac{Cl_{int,CH}^{M2}}{Cl_{int,CH}^{M1} + Cl_{int,CH}^{M2} + \varphi H} \tag{16}$$

where M1 and M2 correspond to parameters (intrinsic clearance, Michaelis-Menten constant or extraction ratio) for metabolite 1 and 2 respectively and the other terms have been already defined.

5. Metabolite formed is eliminated by renal excretion (E6):

$$E6 = Clmet \cdot C4 \tag{17}$$

where E6 represents the excretion rate of the metabolite, Clmet is the renal clearance of metabolite and C4 is the plasma concentration of metabolite.

Metabolite elimination could be also described a sequential phase where the first generation of metabolites is also eliminated by metabolism so a second generation of metabolite(s) is formed.

Once the individual kinetic processes have been described, the next step is to build the equations describing the time-concentration profile in each compartment:

• *Intestinal lumen*: drug is dissolved in lumen (E1) and then it can be degraded in lumen (E2) or absorbed (E3).

$$\frac{dA1}{dt} = Kd \cdot A5 \cdot (S - A1) - K\deg \cdot A1 - (Ka \cdot A1) \cdot \alpha \tag{18}$$

where dA1/dt represents the drug amount change over time in lumen.

• *Liver compartment:* after absorption (E3), drug is partially metabolized in the liver (E4), and it is distributed to systemic compartment.

$$\frac{dA2}{dt} = \left(Ka \cdot A1\right) \cdot \alpha - \phi H \cdot EH \cdot C2 + \phi H \cdot C3 \qquad (19)$$

where dA2/dt represents the drug amount change over time in liver and ΦH•C3 represents distribution from systemic compartment to the liver.

- *Systemic compartment*: Drug is rapidly distributed in systemic compartment, and the elimination of parent drug is renal (E5) and hepatic (E4).

$$\frac{dA3}{dt} = \phi H \cdot FH \cdot C2 - \phi H \cdot C3 - Clrenal \cdot C3 \qquad (20)$$

where dA3/dt represents the drug amount change over time in plasma and ΦH•FH•C2 corresponds to the fraction of drug escaping metabolism in liver (FH=1-EH)

- *Metabolite compartment*: finally, the metabolite formed (E4) is eliminated by renal excretion (E6)

$$\frac{dA4}{dt} = \phi H \cdot EH \cdot C2 - Clmet \cdot C4 \qquad (21)$$

- *Solid dosage form compartment*: Dosage solid form has to be dissolved in lumen (E1) in order to release the drug for absorption. This compartment was added at the end of model, although dissolution form solid form is the first kinetic process, because of model development reasons as in first place the behaviour of the model was checked for a drug solution and then the dissolution from different dosage forms (or formulations) was implemented.

$$\frac{dA5}{dt} = -Kd \cdot A5 \cdot (S - A1) \qquad (22)$$

dA5/dt represents the dissolution from the dosage form and the other terms have been previously defined.

3.3 Description of bioequivalence studies

All bioequivalence studies were evaluated with 2400 simulations per study. The number of healthy volunteers per study was 24, and they were distributed into two groups of 12 volunteers receiving the formulations in a cross-over design. Each volunteer received an oral dose of 100mg of drug products, reference and test in solid dosage form, with a period of a washout between the doses.

A total of 17 samples of both analytes, parent drug and metabolite, were collected for each individual at 0.1, 0.2, 0.4, 0.8,1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 24, 48h after the administration of the drug at single dose. In the case of multiple doses, drug was administered every 8 h until steady state was attained (160 hours) and a total of 10 samples of parent drug and metabolite were collected at 0.1, 0.2, 0.4, 0.8, 1, 2, 4, 6, 8h after the administration of reference or test product.

For the bioequivalence analysis, AUC0-t (calculated by trapezoidal rule) and Cmax were considered: differences between dissolution rates from test and reference products are transformed into AUC and Cmax ratios of both analytes for each drug type and scenario:

$$\text{AUC ratio} = (\text{AUC test})/(\text{AUC reference}) \tag{23}$$

$$\text{Cmax ratio} = (\text{Cmax test})/(\text{Cmax reference}) \tag{24}$$

These results are then presented as bar graphs where each color and group bars represents a different analyte and scenario, respectively.

For the bioequivalence analysis, 90% confidence intervals (90%CI) were calculated for the ratio of AUC_{0-t} and Cmax values for the test and reference dosage forms, using logarithmic transformed data. ANOVA was used to assess the formulation, subject and period effects. Finally, reference and test dosage forms were considered bioequivalent if the 90%CI of AUC_{0-t} and Cmax ratios lay inside 80–125% limits.

On the other hand, the percentage of studies which would conclude bioequivalence using each analyte separately (with this particular study design of 24 subjects) can be estimated and compared to the nominal percentage of failure of 5%. (Type I error: failure is considered when a bioequivalence study states bioequivalence when the products were actually non-equivalent.)

3.4 Individual parameters and data simulation

Parameter values presented in Table 1 correspond to the population parameters values. The individual parameters were generated from these population parameters using an exponential model. Moreover, an inter-occasion variability was added to the individual parameters due to reference and test products are administered in different times:

$$Pi = Pp \cdot e^{\eta IID} \cdot e^{\eta IO1 \cdot O1} \cdot e^{\eta IO2 \cdot O2} \tag{25}$$

where Pp is the population parameter; Pi is the individual parameter; ηIID is the inter-individual variability; ηIO1 is the inter-occasion variability corresponding to first administration (O1) and ηIO2 is the inter-occasion variability corresponding to second administration (O2). O1 and O2 are the identifier variables for occasion 1 and 2

In these simulations, inter-individual variability of 20% was added to all parameters, while an inter-occasion of 10% was fixed in all parameters with the exception of intrinsic hepatic clearance for which a high (30%) or low (10%) level of inter-occasion (or intra-individual) variability was considered.

Finally, the individual plasma concentrations were simulated with the structural model, the individual parameters and a proportional residual error:

$$Cp_i = f\left(Pi, Dose, Time\right)\left(1 + \varepsilon\right) \tag{26}$$

where Cpi is the individual concentration and ε is the residual error.

Other different approach can be used in order to generate population and individual parameters: if it is necessary to add different effects to the parameters, as sequence, period or formulation effects, the population parameters could be generated by using a multiplicative model as:

$$Pp = Pt \cdot Eseq^{seq} \cdot Eper^{per} \cdot Eform^{form} \tag{27}$$

where Pt is the typical parameter; "Eseq", "Eper" and "Eform" are the effects corresponding to the sequence, period and formulation respectively; and "seq", "per" and "form" are the identifiers of sequence, period and formulation respectively.

All these effects can be coded in the model and fixed to zero, in order to be easily modified.

All simulations were performed in NONMEM VI. The control files were edited under Microsoft Excel worksheet and the lines containing the parameters which defined the scenarios were identified. These lines were modified to produce all the scenarios using a Visual Basic (VB) code for Excel. The code included specific commands under 6 layers which were treated as loops for: solubility, absorption, clearance of parent drug, Km, inter-occasion variability in intrinsic hepatic clearance and dissolution rate for test. The VB code created 192 scenarios which were executed under batch processing. The same control file was used for single and multiple dosage simulations as the databases defined this additional layer to simulate the 384 scenarios above declared.

The control file managed the differential equations to simulate the plasma concentrations for test and reference drugs following the conditions defined in Table 1. Additionally the control file calculated the individual AUC and Cmax which were updated for each time. Therefore, the last time contained the final value of AUC and Cmax of each volunteer. All this information was reported in tables after run execution.

The tables generated in each simulation had hundreds of thousands of records and were filtered with SPSS syntax to select the last record of each volunteer which contained the individual Cmax and AUC.

The final step was to capture the 192 filtered tables under MS Excel and calculate using VB programming the AUC and Cmax ratios and ANOVA test for each simulated trial in each scenario. The results were reported into a worksheet of the Excel file with the mean AUC and Cmax ratios for each scenario and the percentage of bioequivalence achieved between test and reference.

4. Results and discussion

Modelling and simulation approaches are useful tools to assess the potential outcome of different scenarios in bioequivalence studies. The aim of these studies was to propose a new semi-physiological model for bioequivalence trial simulations and apply it for different drug classes by considering a basic structural model that can be easily modified to accommodate other kinetic processes or non-linearities in any of them.

In order to present the results in a way easy to understand and useful for regulatory decisions or for optimization of the trial design, the AUC or Cmax ratios were plotted versus the pharmaceutical quality (decreased dissolution rate in vivo in lumen) and relative absorbed fraction. An example of this kind of plots is shown in Figures 2 and 3. These type of figures allow assessing how the decrease of biopharmaceutical quality of test product in each scenario is reflected in the average AUC or Cmax ratios of parent drug or metabolite so it is easily observed which one is more sensitive to the changes in quality.
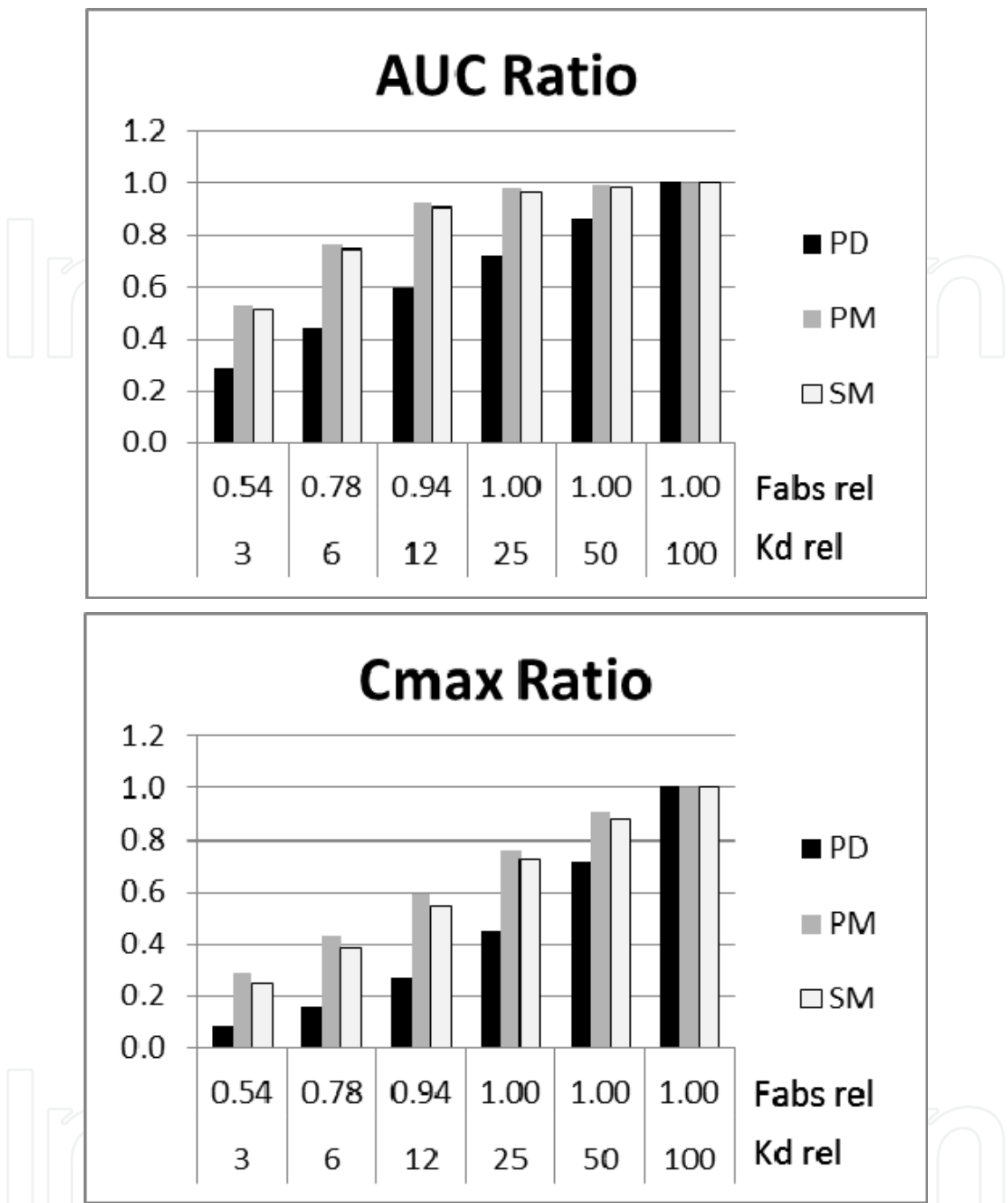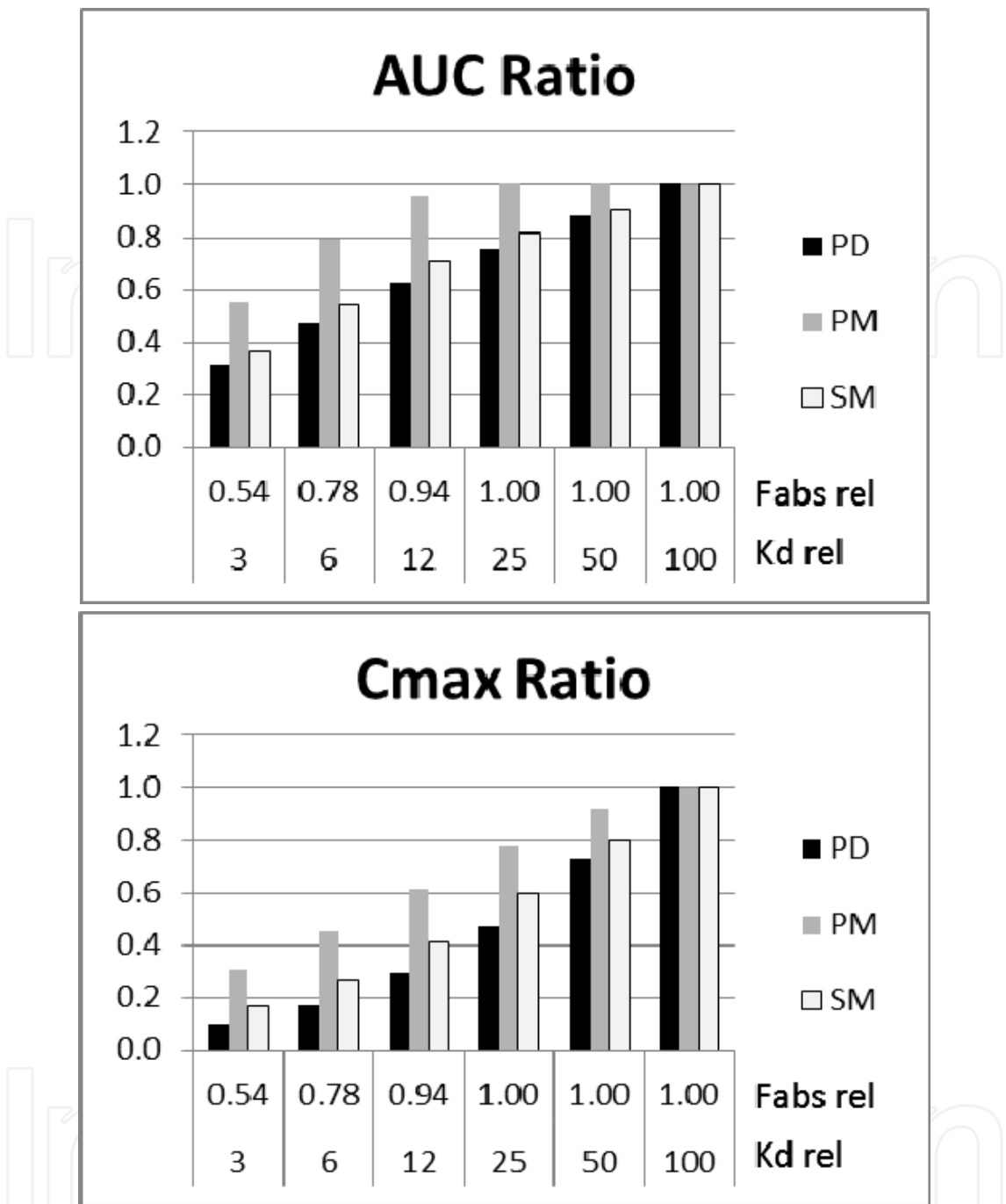
PD: Parent drug; PM: Principal metabolite; SM: Secondary metabolite

Fig. 3. True AUC and Cmax ratios (y axis) versus the relative absorbed fraction (Fabs rel) and the relative dissolution rate constant (Kd rel expressed as %) (x axis) obtained for each scenario. This model corresponds to a class III drug, administered at low dose scheme in single dose, when both metabolic pathways become saturated

In all the simulations performed with these models parent drug is the most sensitive analyte to detect the differences of in vivo dissolution. Some exceptions to this rule have been detected but it would be desirable to check these results with real examples of pharmacokinetic parameters. i.e. with known parameters from particular drugs.

PD: Parent drug; PM: Principal metabolite; SM: Secondary metabolite

Fig. 4. True AUC and Cmax ratios (y axis) versus the relative absorbed fraction (Fabs rel) and the relative dissolution rate constant (Kd rel expressed as %) (x axis) obtained for each scenario. This model corresponds to a class III drug, administered at low dose scheme in single dose, when the principal metabolic pathway becomes saturated.

For instance when a model with pre-systemic metabolism (intestinal and hepatic) was checked (Navarro-Fontestad et al., 2010) it was concluded that, the metabolites (either principal or secondary metabolite) do not show higher sensitivity than the parent drug to detect changes in the pharmaceutical performance, even when pharmacokinetics of the

parent drug is non-linear. In case of non-linear metabolism, higher parent drug sensitivity can be found, as compared with non-linear metabolites. Same conclusion was achieved in the case of linear hepatic metabolism despite of FDA requirements (Fernandez-Teruel et al., 2009c).

In the case of BCS classes with hepatic metabolism under linear conditions the differences in Cmax are detected more sensitively with the parent drug in the single dose study, except in the case of class III drugs with low intrinsic clearance (Fernandez-Teruel et al., 2009c)

In the particular examples represented in Figures 3 and 4 where the participation of an efflux transporter at intestinal level has been included, the parent drug is the most sensitive analyte to detect the lack of pharmaceutical quality in the problem formulation versus the reference one. The presence of an efflux carrier in the structural part o of the model even if it is non-saturated does not change the outcome in relation to previous scenarios.

Regarding the study design (single dose versus steady state) this aspect has been investigated in a model with hepatic metabolism under linear or non linear conditions and considering that a low percent of the dose is eliminated by renal clearance. With these assumptions interestingly, for class III drugs with non-linear pharmacokinetics the steady state design is necessary in addition to the single dose study, as required by EMEA and in contrast to FDA requirements, to compare with the highest sensitivity the Cmax of the **parent drug** not only in case of low intrinsic clearance but also in case of high intrinsic clearance and a small worsening of the in vivo dissolution (relative kd= 0.5). In the case of AUC (class III drugs) the steady state design is more sensitive in case of drugs with low intrinsic clearance but as sensitive as the single dose study when the intrinsic clearance is high (Fernandez-Teruel et al., 2009a)

A particular concern about this result is that following the Biopharmaceutic and Drug Disposition Classification System, BDDCS (Benet et al., 2008; Chen et al., 2010; Wu & Benet, 2005) it would be arguably that such a drug with high solubility, low permeability and highly metabolized (even if at slow rate) is an exception or does not exists. Further simulations with a higher renal clearance of parent drug to allow for a lower percent of the dose being metabolized should be done to clarify if the behavior is the same.

5. Conclusions

A simulation model of Bioequivalence trials have been developed taking into account the biopharmaceutical properties that determine rate and extent of absorption i.e. permeability, solubility and dissolution rate in relation with the human intestinal transit time. This BCS approximation have not been included in the previous simulation exercises found in the literature.

This work illustrates a methodology that could be implemented by the applicant of a marketing authorization of a generic product in order to justify the selected study design and analyte. Once the pharmacokinetic behaviour of the drug under investigation in known it is possible to identify the sensitivity of the different active species in the different study designs. Some structural models have been explored but as it has been explained the model could be adapted to incorporate other kinetic processes and non linear components on them

as well as it could be possible to perform the simulations in saturating or non saturating conditions for each particular non linear step.

The final objective would be to develop customized models for each particular drug in order to justify the selection of the bioequivalence study design and analyte or, when the most sensitive scenario cannot be performed due to analytical or tolerability/safety limitations, to estimate the loss of sensitivity of an alternative design that has to be used pragmatically. In summary, virtual bioequivalence studies may serve as a tool to guide regulatory decisions both for sponsors and Regulatory Agencies.

6. Acknowledgements

7. References

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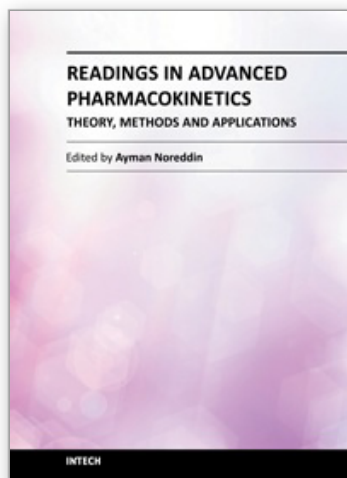
Readings in Advanced Pharmacokinetics - Theory, Methods and Applications

Edited by Dr. Ayman Noreddin

This book, "Readings in Advanced Pharmacokinetics - Theory, Methods and Applications", covers up to date information and practical topics related to the study of drug pharmacokinetics in humans and in animals. The book is designed to offer scientists, clinicians and researchers a choice to logically build their knowledge in pharmacokinetics from basic concepts to advanced applications. This book is organized into two sections. The first section discusses advanced theories that include a wide range of topics; from bioequivalence studies, pharmacogenomics in relation to pharmacokinetics, computer based simulation concepts to drug interactions of herbal medicines and veterinary pharmacokinetics. The second section advances theory to practice offering several examples of methods and applications in advanced pharmacokinetics.

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