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Research paper

An alternative single dose parameter to avoid the need for steady-state studies on oral extended-release drug products

Paulo Paixão [a,b,*], Luís F. Gouveia [a], José A.G. Morais [a,b]

[a] iMed.UL, Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal
[b] INFARMED – National Authority of Medicines and Health Products, Lisbon, Portugal

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ABSTRACT

Use of single and multiple-dose studies is required to establish the bioequivalence between two extended-release oral dosage forms under the current European Guidelines. However, FDA is less strict in this regard and only requires a single-dose study. The objective of this work is to use a computer simulation in order to test the two approaches. Three pharmacokinetic models, representing different release mechanisms, were considered, and Monte Carlo simulations with intra- and inter-individual variabilities were performed. Five different bioequivalence protocols were used and a new pharmacokinetic metric – C_τ, the concentration at the end of the intended dosing interval obtained in the single-dose study – is proposed in order to avoid the need for steady-state studies while keeping the ability to detect differences between formulations. Results have shown that the European requirements are more capable to discriminate between two potentially different formulations but at the cost of the multiple-dose study and with an increased number of subjects when compared to the FDA requirements. However, the use of C_{max} and AUC_{0-t} obtained on a single-dose study with the added C_τ metric equals the discriminatory ability of the current EMA requirements, without the need of a multiple-dose study. This proposed approach results in the reduction in the number of studies and volunteers enrolled in clinical bioequivalence trials, without compromising the quality assurance of a new extended-release oral formulation.

1. Introduction

Oral extended-release (ER) drug products are dosage forms designed to release drug in a modified manner in order to achieve prolonged and constant profiles, with reduced fluctuations in drug plasma concentrations, frequently resulting in a dosage regimen with lower frequency of administration and thereby potentially improving patient compliance [1]. Since continued advances in pharmaceutical sciences have given rise to new technological processes for controlled release formulations, challenges have been faced by industry and regulatory scientists to ensure pharmaceutical, biological and therapeutic equivalences [2] and in that context the European Medicine Agency (EMA) recently released a concept paper on the need to revise the note for guidance on modified release, oral and transdermal dosage forms [3]. In that paper, various discussions have been addressed on topics like primary pharmacokinetic parameters, strength(s) to be used, requirements for food interaction studies and requirements for steady-state studies, to name a few.

It is widely accepted that single-dose studies are generally more sensitive than multiple-dose studies to differences in the rate of drug substance absorption, and therefore, the requirements for the use of steady-state studies have been reduced on the new EMA guideline for bioequivalence on oral immediate release drug products [4,5]. The same rational supports the FDA decision to currently not require multiple-dose studies for the determination of bioequivalence between innovator and generic MR products [6]. However, under the European regulations, its use for the purpose of bioequivalence has been mandatory for these type of drug products [7].

Due to the special kinetic characteristics of the MR products, it has been pointed out that the pharmacokinetic parameters traditionally used for IR products (C_{max} and AUC) may not always be sufficient to guarantee therapeutic equivalence [8]. For example, for IR products, treatment comparison of AUC values can be adequately defined by truncated profiles of long half-life ($t½$) drugs, as long as absorption is complete. However, evaluation of the terminal part of the pharmacokinetic profile is far more complex when the long $t½$ is reflective of prolonged absorption. In these situations, the use of multiple-dose studies and the evaluation of bioequivalence at trough plasma concentration in steady state (C^{ss}_{trough}) may result in an increased ability to detect differences in product performance.

* Corresponding author. iMed.UL, Faculdade de Farmácia, Universidade de Lisboa, A. Prof. Gama Pinto, 1649-003 Lisboa, Portugal. Tel.: +351 21 794 64 44/00/72; fax: +351 21 794 64 70.
E-mail address: ppaixao@ff.ul.pt (P. Paixão).

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According to the pharmacokinetic principles, C^{ss}_{trough} is equal to the concentration at the end of the proposing dosing interval (C_τ) obtained on single dose administration multiplied by the accumulation factor (*R*). *R* by itself may be calculated by the ratio of $AUC^{ss}_{0-\tau}$ by $AUC_{0-\tau}$ obtained on single dose administration. Since under the assumption of additivity and linearity $AUC^{ss}_{0-\tau}$ is equal to $AUC_{0-\infty}$ obtained on single dose administration, it is expected, at least with linear pharmacokinetics drugs, that the information that C^{ss}_{trough} contains should also be present within C_τ. In this context, the purpose of this paper is to establish (1) the need for multiple-dose studies and (2) the possibility of waiving them with a new primary pharmacokinetic parameter, namely C_τ obtained on single dose administration.

2. Methods

2.1. Model equations

In order to simulate the expected plasma profiles after the administration of different types of ER products, three different types of integrated equations were used. These differ on the release mechanism but, in common, only monocompartment distribution and first-order elimination kinetics were considered. The first model, Eqs. (1)–(4), was used to simulate the plasma profiles after the administration of the more simple and conservative ER formulations, like the matrix type. In these cases, it has been shown that drug release may be approximated by multiple-exponential functions [9] with C_{im} the plasma concentration arising from the immediate release portion of the formulation, C_f the plasma concentration resulting from the intermediate release portion of the formulation and C_s the plasma concentration obtained from the slower release of drug from the formulation,

$$C_{im} = \frac{F_{oral} \cdot k_a \cdot f_{im} \cdot D}{(k_a - k_{el}) \cdot V_d} \cdot \left[e^{\left(-k_{el} \cdot (t - t_{lag})\right)} - e^{\left(-k_a \cdot (t - t_{lag})\right)} \right] \quad (1)$$

$$C_f = \frac{F_{oral} \cdot k_a \cdot k_f \cdot k_f \cdot D}{V_d} \cdot \left[\frac{e^{\left(-k_f \cdot (t - t_{lag})\right)}}{(k_a - k_f)(k_{el} - k_f)} + \frac{e^{\left(-k_a \cdot (t - t_{lag})\right)}}{(k_f - k_a)(k_{el} - k_a)} + \frac{e^{\left(-k_{el} \cdot (t - t_{lag})\right)}}{(k_f - k_{el})(k_a - k_{el})} \right] \quad (2)$$

$$C_s = \frac{F_{oral} \cdot k_a \cdot k_s \cdot k_s \cdot D}{V_d} \cdot \left[\frac{e^{\left(-k_s \cdot (t - t_{lag})\right)}}{(k_a - k_s)(k_{el} - k_s)} + \frac{e^{\left(-k_a \cdot (t - t_{lag})\right)}}{(k_s - k_a)(k_{el} - k_a)} + \frac{e^{\left(-k_{el} \cdot (t - t_{lag})\right)}}{(k_s - k_{el})(k_a - k_{el})} \right] \quad (3)$$

where D is the dose, F_{oral} is the absolute oral bioavailability of the drug, k_a is the immediate release absorption rate constant, k_f is the intermediate release absorption rate constant, k_s is the slower release absorption rate constant, f_a is the fraction of the dose released at the faster rate, f_f is the fraction of the dose released at the intermediate rate, f_s is the fraction of the dose released at the slower rate equal to $1 - f_a - f_f$, t_{lag} is the lag time, V_d is the drug volume of distribution, and k_{el} is the first-order elimination rate constant. In this case, the final plasma concentration is the sum of the previous integrated equations that individually considered the effect of three first-order release constants with increasingly lower values,

$$C_p = C_{im} + C_f + C_s \quad (4)$$

The second model, Eqs. (5)–(7), was used to simulate the plasma profiles after the administration of a controlled released formulation, like the osmotic pump. In this case, it is assumed that the formulation releases the drug with zero-order kinetics for most of the available dose, and in the end, a residual dose is released with a first-order rate. C_{perf} considers the plasma drug concentration arising from the zero-order portion of the formulation and C_{fo} the plasma concentration resulting from the final first-order portion of the formulation,

$$\begin{cases} C_{perf} = F_{oral} \cdot \frac{k_0}{k_{el} \cdot V_d} \left(1 - e^{\left(-k_{el} \cdot (t - t_{lag})\right)}\right) & t \le (T_p - t_{lag}) \\ C_{perf} = F_{oral} \cdot \frac{k_0}{k_{el} \cdot V_d} \left(1 - e^{\left(-k_{el} \cdot (T_p)\right)}\right) \cdot e^{\left(-k_{el} \cdot (t - T_p - t_{lag})\right)} & t > (T_p + t_{lag}) \end{cases} \quad (5)$$

$$C_{fo} = \frac{F_{oral} \cdot K_{end} \cdot D_{rem}}{V_d \cdot (k_{end} = k_{el})} \cdot e^{\left(-k_{el} \cdot (t - T_p - t_{lag})\right)} - e^{\left(-k_{end} \cdot (t - T_p - t_{lag})\right)} t > (T_p + t_{lag}) \quad (6)$$

where F_{oral} is the oral absolute bioavailability of the drug, k_0 is the zero-order release rate, T_p is the end of the zero-order part of the formulation equal to $(D - D_{rem})/k_0$, where D is the dose of the formulation, and D_{rem} the remaining amount of drug released in a first-order rate, k_{end} is the final first-order release rate constant, t_{lag} is the lag time, V_d is the drug volume of distribution, and k_{el} is the first-order elimination rate constant. Again, the final plasma concentration is the sum of the previous integrated equations that individually considered the effect of the two different release mechanisms,

$$C_p = C_{perf} + C_{fo} \quad (7)$$

The third and final model, Eqs. (8)–(11), was used to simulate the plasma profiles of a biphasic system consisting of a controlled released formulation, like the osmotic pump but with an initial immediate release portion. In this case, it is assumed that the formulation releases the drug with both a fraction of the dose in an immediate release with first-order kinetics and another fraction of the dose with zero-order kinetics. Again, at the end, a residual dose is released with a first-order rate. C_{im} is the plasma concentration arising from the immediate release portion of the formulation, C_{perf} considers the plasma drug concentration arising from the zero-order portion of the formulation before and after the release of the drug and C_{fo} the plasma concentration resulting from the final first-order portion of the formulation,

$$C_{im} = \frac{F_{oral} \cdot f_{ka} \cdot k_a \cdot D}{V_d \cdot (k_a - k_{el})} \cdot \left(e^{\left(-k_{el} \cdot (t - t_{lag})\right)} - e^{\left(-k_a \cdot (t - t_{lag})\right)}\right) \quad t > t_{lag} \quad (8)$$

$$\begin{cases} C_{perf} = F_{oral} \cdot \frac{k_0}{k_{el} \cdot V_d} \left(1 - e^{\left(-k_{el} \cdot (t - t_{lag})\right)}\right) & t \le (T_p - t_{lag}) \\ C_{perf} = F_{oral} \cdot \frac{k_0}{k_{el} \cdot V_d} \left(1 - e^{\left(-k_{el} \cdot (t_p)\right)}\right) \cdot e^{\left(-k_{el} \cdot (t - T_p - t_{lag})\right)} & t > (T_p + t_{lag}) \end{cases} \quad (9)$$

$$C_{fo} = \frac{F_{oral} \cdot k_a \cdot D_{rem}}{V_d \cdot (k_a - k_{el})} \cdot \left(e^{\left(-k_{el} \cdot (t - T_p - t_{lag})\right)} - e^{\left(-k_a \cdot (t - T_p - t_{lag})\right)}\right) t > (T_p + t_{lag}) \quad (10)$$

where F_{oral} is the oral absolute bioavailability of the drug, k_0 is the zero-order release rate, T_p is the end of the zero-order part of the formulation equal to $((1 - f_{ka}) \cdot D - D_{rem})/k_0$, being D the dose of the formulation, f_{ka} the fraction of the dose released initially with a first-order rate and D_{rem} the remaining amount of drug released at the end with a first-order rate, k_a is the initial first-order release rate constant, k_{end} is the final first-order release rate constant, t_{lag} is the lag time, V_d is the drug volume of distribution, and k_{el} is the first-order elimination rate constant. Again, the final plasma concentration is the sum of the previously integrated equations that individually took into account the effect of the three different release mechanisms,

$$C_p = C_{im} + C_{perf} + C_{fo} \quad (11)$$

Eqs. (4), (7), and (11) were used to simulate the single dose administration. For the multi-dose administration, constant intervals and uniform dose were used, the plasma profiles being built based on the superposition principle and on the linearity in the rate constants.

2.2. Simulation of reference product

For each of the three different models, a reference drug product, based on previously described formulations [9], was simulated with the constant values presented in Table 1. For each model, two situations were simulated (without and with t_{lag}), resulting in six different scenarios.

2.3. Pharmacokinetic parameters

For each simulated profile, various pharmacokinetic (PK) parameters were calculated namely, after the single dose administration, C_{max} the maximal plasma concentration; AUC_{0-t} the area under the concentration–time curve from time 0 to 48 h calculated by the trapezoidal rule; and the C_τ the plasma concentration at time 12 h. After multiple doses and at steady state: C^{ss}_{max} the maximum plasma concentration at steady state; $AUC^{ss}_{0-\tau}$ the area under the concentration–time curve from time 0 to 12 h at steady state calculated by the trapezoidal rule; and the C^{ss}_{trough} the plasma concentration at time 12 h at steady state.

2.4. Ability to detect differences between the formulations

In order to test the need for multiple-dose studies, five different protocols for BE assessment including different sets of PK parameters were considered. In protocols 1 and 2, both single and multiple dose PK parameters were used differing in the inclusion of C^{ss}_{trough} by protocol 2. They test the ability, under the current European Guideline, to detect different formulations as well as the usefulness of this latter PK parameter. Protocols 3 and 4 use single dose PK parameters only, according to the FDA bioequivalence approach (protocol 3) and also the ability of the proposed alternative PK metric (C_τ) to detect the same differences between the formulations (protocol 4). Finally, protocol 5 simply evaluates a multiple-dose study for comparison purposes. Complete description of the PK parameters under these five protocols is presented in the table within Fig. 2.

In order to simulate potentially different formulations, a proportional error was added to the different formulation parameters, accordingly to equations depicted under paragraph 2.7 – Calculations, with coefficients of variation of 0.10, 0.15 and 0.20. A total of 250 different formulations were simulated for each CV case using in-house developed Microsoft Excel® 2007 VBA procedures for process automation. Each of the 250 simulated potentially different test formulations for the 3 models, with and without t_{lag}, was compared to the corresponding reference formulation by analysing the ratio of the test to reference PK parameter. Each formulation was considered significantly different from the reference formulation if the ratio of the test to the reference was outside the currently accepted bioequivalence 0.80–1.25 interval in any of the corresponding protocol PK parameters.

Table 1
Model parameters for each of the three different pharmacokinetic models.

Model 1			Model 2			Model 3		
D	100	mg	D	100	mg	D	100	mg
Formulation parameters								
k_a	3.4	h^{-1}	T_0	10.6	h	k_a	1	h^{-1}
f_{im}	0.05		k_0	9	mg/h	f_{ka}	0.05	
k_f	0.45	h^{-1}	k_d	0.6	h^{-1}	T_0	10.3	H
f_f	0.35		D_{rem}	5	mg	K_0	8.8	mg/h
k_s	0.1	h^{-1}	t_{lag}	1.2	h	k_d	0.6	h^{-1}
f_s	0.6					D_{rem}	5	mg
t_{lag}	0.5	h				t_{lag}	1.2	h
Physiologic parameters								
V_d	27	L	V_d	27	L	V_d	27	L
k_{el}	0.41	h^{-1}	k_{el}	0.3	h^{-1}	k_{el}	0.3	h^{-1}
F_{oral}	0.8		F_{oral}	0.8		F_{oral}	0.8	

2.5. Relationship between the formulation and the pharmacokinetic parameters

In order to assess the influence of the formulation parameters in the pharmacokinetic parameters, a partial least-squares (PLS) regression was undertaken, using the software Statgraphics Centurion XV version 15.1.02 (StatPoint Inc., USA). For these models, the previously simulated formulation parameters with a CV of 20% for the models with t_{lag} were used as inputs, and the pharmacokinetic parameters calculated based on the simulated concentration profiles were used as outputs. To allow the interpretation of the regression coefficients, both the input and the output variables were normalised by auto-scaling [10]. The number of significant components was optimised by using a leave-40-out approach. The minimum number of components was considered based on the average Prediction r^2 (Q^2) calculated with the PRESS residuals and was used in the calculation of the standardised coefficients of the PLS regression models.

2.6. Likelihood of declaring two identical products as bioequivalent

Using the current standards for bioequivalence, and according to the Shuirmann's statistical method [11], the number of subjects included in the clinical study may influence the outcome for bioequivalence if it does not ensure sufficient statistical power to account for the variability present on the analysed data. In order to estimate the number of subjects needed for a sound conclusion of bioequivalence in the different protocols, all scenarios were evaluated individually with 250 simulated bioequivalence crossover studies. A range of 12–48 volunteers per study were generated by a Monte Carlo simulation approach using in-house developed Microsoft Excel® 2007 VBA procedures. Each volunteer received a dose of the drug in test and reference formulations, assuming a complete washout between the two formulations administration. Samples were collected at 0, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 18, 24, 36 and 48 h after a single dose administration of the drug and at 0, 1, 2, 3, 4, 5, 6, 7, 8, 10 and 12 h after reaching steady state. Since the purpose of these studies was to evaluate the number of subjects needed to show bioequivalence between the two formulations in the different proposed scenarios, both reference and test formulations were considered equal, with the same mean parameters. However, in order to consider the typical variability, a set of individual parameters was created for each volunteer in order to include both the inter-subject and intra-subject variability. Again, individual formulation parameters were created based on a proportional error with a CV of 0.10 for all formulation parameters. For the physiologic parameters, both inter- and intra-subject errors were considered based on an exponential error model. Inter-subjects variability was included with a CV of 0.25 for V_d and k_{el} and a CV of 0.15 for F_{oral}. Intra-subject variability was included with a CV of 0.15 for V_d and k_{el} and a CV of 0.05 for F_{oral}. Finally, the individual plasma concentrations over time were simulated with the structural model, the individual parameters and a proportional experimental error. A CV of 0.1 was used to reflect the sampling and quantification errors. For simplicity, neither sequence nor period variability was

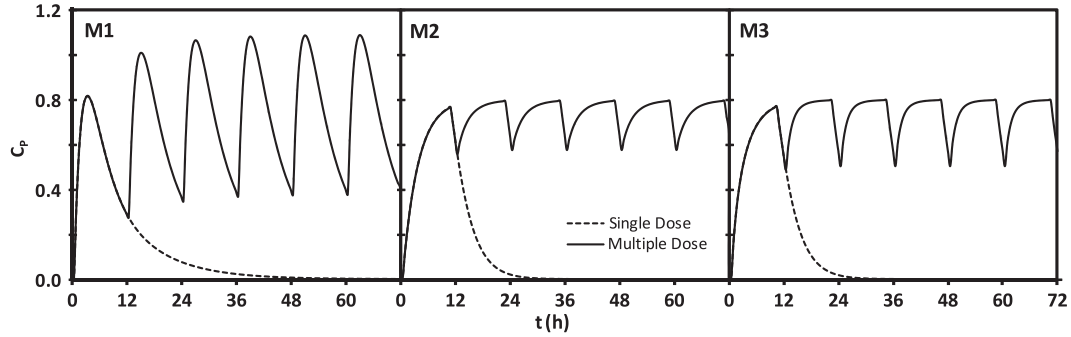

Fig. 1. Simulated plasma profiles for the reference formulation using the three proposed models after single and multiple doses. Similar profiles are obtained with a time-shift equal to t_{lag} when this parameter is considered.

		P1	P2	P3	P4	P5
SD	C_{max}	✓	✓	✓	✓	-
	AUC_{0-t}	✓	✓	✓	✓	-
	C_{12h}	-	-	-	✓	-
MD	C^{ss}_{max}	✓	✓	-	-	✓
	$AUC^{ss}_{0-\tau}$	✓	✓	-	-	✓
	C^{ss}_{trough}	-	✓	-	-	-

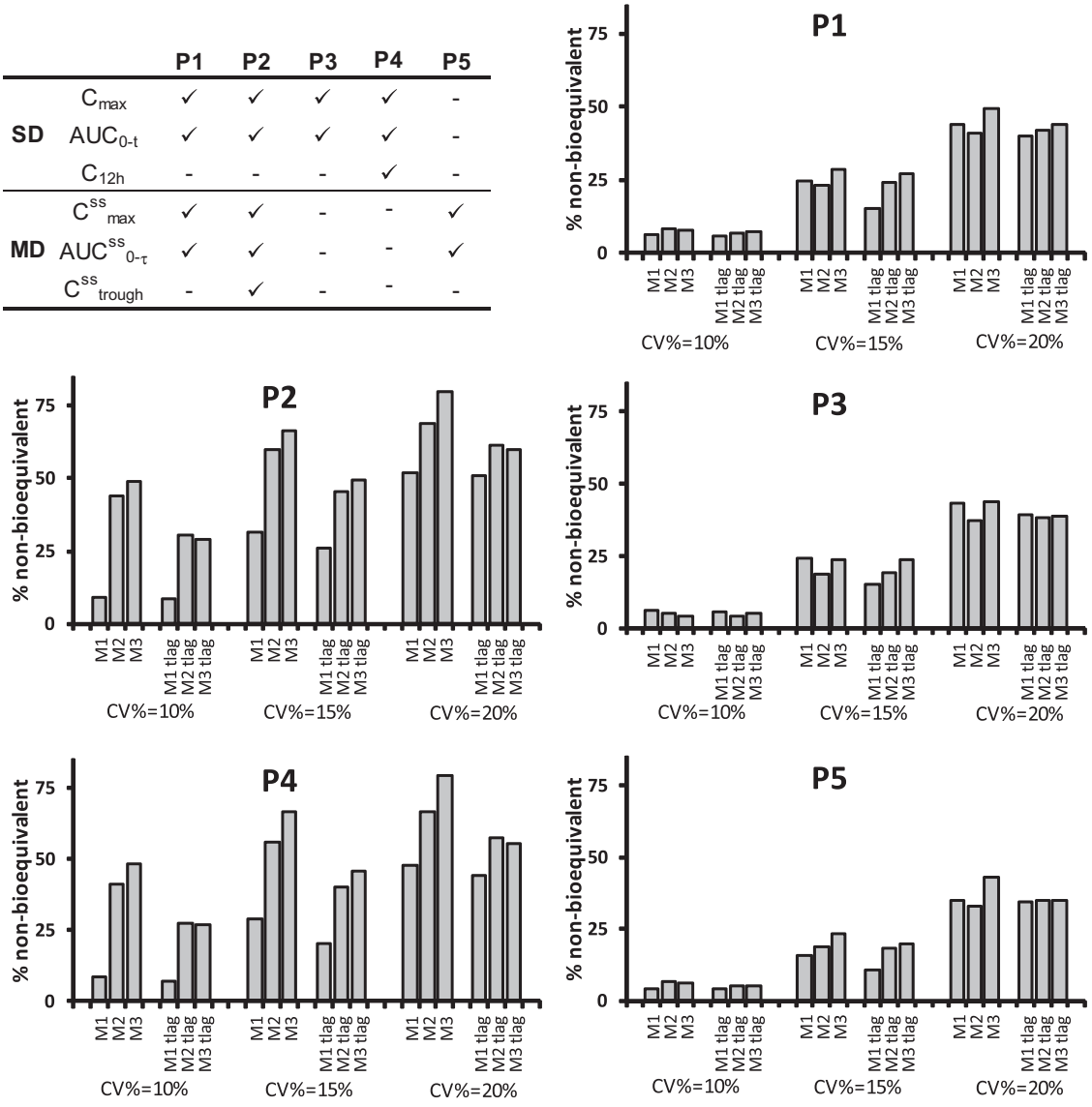

Fig. 2. Percentage of test formulations identified as different from the reference in the 3 studied models (M1, M2 and M3), with and without t_{lag}, by the five protocols. CV% indicate the amount of variability that was included in the formulation parameters during the Monte Carlo simulation. Table on the top-left corner indicates the metrics used for bioequivalence assessment in each of the 5 protocols.

included. Bioequivalence between formulations was determined by calculating the 90% confidence intervals (90% CI) for the ratio (test/reference) of the relevant population pharmacokinetic parameters, using logarithmic transformed data. The dosage forms were considered bioequivalent if the 90% CI of the considered pharmacokinetic parameters ratios lay inside the acceptance interval of 80–125%. An ANOVA was used to assess the formulation, subject and period effects.

2.7. Computations

The software procedures needed to generate the data and to assess BE of the simulated studies described were developed under Microsoft Excel 2007 VBA(R) environment and compiled in an add-in.

The random number generation was performed by using the Excel 2007 VBA function RND() that produces pseudo-uniform random numbers in the range [0,1[with a 10^{13} calls period [12]. The normal random numbers were generated by using the Box–Muller transform algorithm [13], see the following equation.

$$NormalRnd(\mu,\sigma) = \mu + \sigma \cdot [\sqrt{-2\log r_1} \cdot \cos(2\pi r_2)]. \quad (12)$$

where r_1 and r_2 are random numbers drawn from an uniform distribution in the interval [0,1], μ and σ are the average and standard deviation of the desired normal random distribution. Although other computationally more efficient algorithms do exist to generate normal random numbers, the Box–Muller transform was chosen as it is suitable to the intended purpose and it was already compiled in a library, developed by the authors.

The proportional and exponential errors used in the simulations were generated by using Eqs. (13) and (14), in which x is the estimate, and cv is the desired coefficient of variation for the estimate error.

$$\varepsilon_{pro} = x \cdot NormalRnd(1, cv) \quad (13)$$

$$\varepsilon_{exp} = x \cdot [e^{NormalRnd(0,cv)} - 1] \quad (14)$$

These errors were added to the estimates (for each subject/period/sequence), which were then stored in separate files for later checking/debugging purposes. The final data ("time × Conc" profiles) were generated by adding a sampling and analytical proportional error to each of the samples concentration.

3. Results

3.1. Model equations and parameters

As can be seen in Fig. 1, the considered models and their corresponding parameters resulted in different pharmacokinetic profiles that intend to simulate different types of possible formulations. In common, all these formulations were simulated in order to be administered in a twice a day drug regime. In all cases, more than 20% of the first dose is considered to remain in the body at the time of the second administration ($AUC_{0-\tau}$ < 80% of $AUC_{0-\infty}$), representing a situation where drug accumulates in a multiple-dose regime that justifies the BE study under multi-dose administrations. Additionally, it is observed that by the time of the sixth administration (60 h after the first one), steady state is already achieved in all the considered scenarios.

3.2. Ability to detect differences between the formulations

Due to the high number of formulation parameters and their interaction, a Monte Carlo approach was used in order to create potentially different formulations. These were simulated with the inclusion of variability only in the formulation parameters, resulting in an increased probability to obtain a different formulation from the initial one when the introduced variability has a larger value. Since no variability was introduced in the physiologic parameters, the decision on the bioequivalence between the simulated formulations and the reference one was simply based on the point determination of the ratio of the simulated to the reference PK parameters. The percentage of non-bioequivalent formulations as identified by the different protocols is presented in Fig. 2. As expected, there is an increase in non-bioequivalence with the increase in the variability of the formulation parameters. P2 (the current EMA guideline approach) was the protocol that consistently identified a higher number of different formulations but, in general, P4 (our proposed approach) presented similar results. P1 and P3 protocols were similar between themselves and less competent in identifying differences in the formulations, especially for the PK models 2 and 3. P5 was always inferior to P3.

3.3. Relationship between the formulation and the pharmacokinetic parameters

PLS regression was performed as described in the Methods section. For the first pharmacokinetic model, the relationship between the formulation parameters and the calculated pharmacokinetic parameters was based on regression models using 3–5 components (explaining from 0.461 to 0.739 of the input variability) and presented an average R^2 of 0.990 (range 0.999–0.977) and an average Q^2 of 0.989 (0.999–0.976). For the second pharmacokinetic model data, the regression models were based on 2 to 4 components (explaining from 0.409 to 0.797 of the input variability) and presented an average R^2 of 0.883 (0.999–0.702) and an average Q^2 of 0.877 (0.999–0.686). For the third pharmacokinetic model data, the regression models used 3–5 components (explaining from 0.438 to 0.715 of the input variability) and presented an average R^2 of 0.879 (0.999–0.713) and an average Q^2 of 0.872 (0.999–0.694).

In order to assess the influence of each formulation parameter on the various pharmacokinetic parameters, the regression standardised coefficients were determined and are presented on Fig. 3.

3.4. Likelihood of declaring two identical products as bioequivalent

The results obtained from the simulated bioequivalence studies, with equal test and reference formulations but with both formulation and physiologic variabilities included as described in the

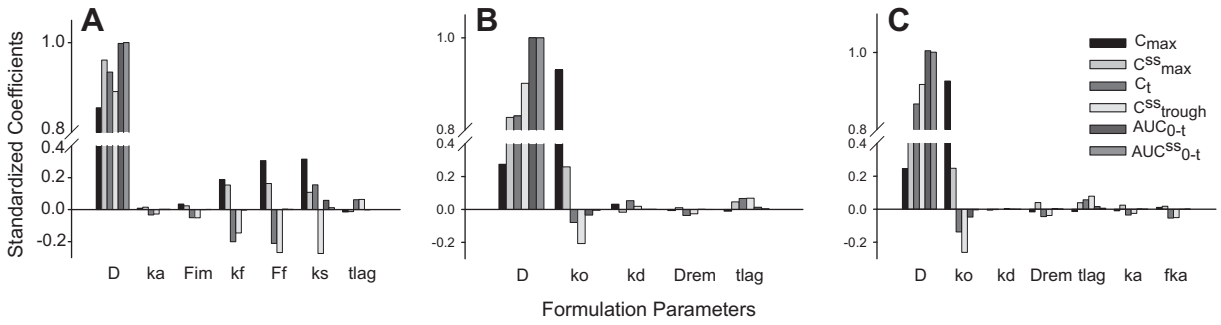

Fig. 3. Standardised coefficients vs. formulation parameters for the PLS regression models built to predict the PK parameters. Results for the PK model 1, Eq. (4), with t_{lag} are depicted in graphic (A), for the PK model 2, Eq. (7) with t_{lag} in graphic (B) and for PK model 3, Eq. (11), with t_{lag} in graphic (C).

methods, are presented in Fig. 4. As can be seen, regardless of the PK model used, the number of subjects needed in order to achieve a power of 80%, typically used in real life bioequivalence studies, is larger when using both C_τ and C^{ss}_{trough}. This is also confirmed by the approximated estimation of the intra-individual error using $CV\%_{intra} = 100 \times \sqrt{e^{MSE} - 1}$, which for C_τ and C^{ss}_{trough} is approximately 30–40% and for the remaining PK parameters is approximately 20–30% in all models.

Based on these findings and taking Fig. 4A as an example, two equal formulations would need a single-dose study with approximately 20 subjects and a multiple-dose study with around 30 subjects to show bioequivalence under the P2 protocol (current EMA requirement), but only a single-dose study with 30 subjects to show bioequivalence under the P4 protocol (our proposal). Therefore, a major reduction in both the overall number of subjects and the study length as well as in the experimental work could be achieved without loss of ability to detect non-bioequivalent formulations.

4. Discussion

According to the current EMA guideline [7], the establishing of bioequivalence between prolonged release oral drug products should be made on the basis of single- and multiple-dose studies which are designed to demonstrate for the test product the same claimed prolonged release characteristics as the reference product with equivalent performance, the inexistence of dose dumping and comparable food effect on the *in vivo* performance for both

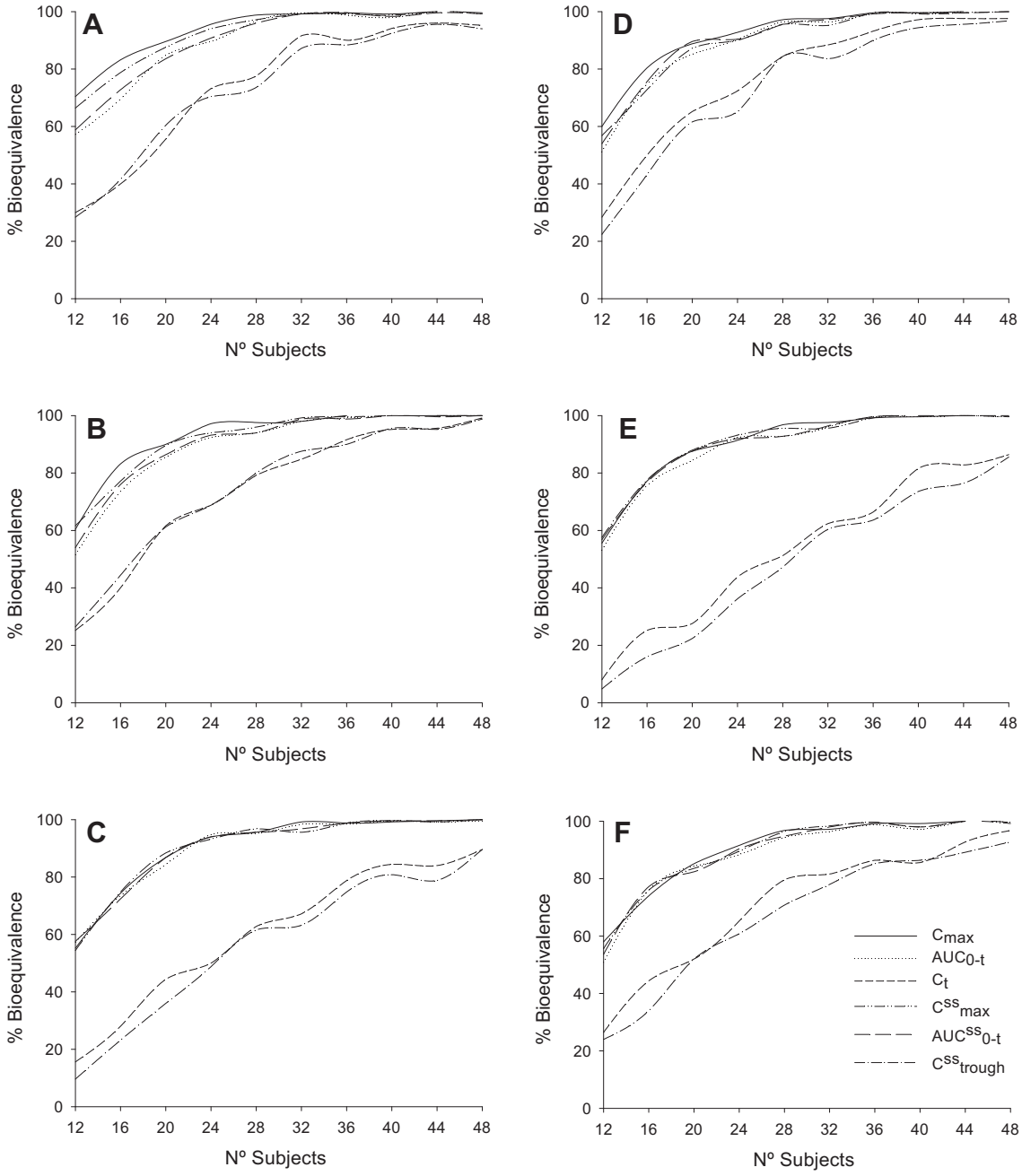

Fig. 4. Fraction of studies demonstrating bioequivalence vs. number of included subjects for the different PK models with added variability. Results for the PK model 1, Eq. (4), without and with t_{lag} are depicted in graphic (A and B), for the PK model 2, Eq. (7), without and with t_{lag} in graphic (C and D) and for PK model 3, Eq. (11), without and with t_{lag} in graphic (E and F), respectively. A total of 250 simulated studies were performed for each individual situation.

formulations. For that purpose, in the simplest cases, at least two single-dose studies (under fasting and fed condition) as well as a multiple-dose study are needed in order to demonstrate bioequivalence. P2 protocol represents this type of requirements (without the consideration of the food-effect study). According to the FDA requirements [6], only two single-dose fasting and fed studies are required for the same purpose. P3 protocol represents again this type of requirements. When comparing the simulations within these two protocols (Fig. 2), it is shown that the EMA requirements have a higher ability to identify differences between the two formulations.

In immediate release oral dosage forms, multiple-dose studies are generally not accepted, as it is recognised that this study design is less able to detect differences between formulations in C_{max} [5]. FDA makes a similar statement in the case of modified-release oral drug products [6]. The results obtained from our simulations also support these statements, as protocol P5 presented the lowest ability to detect differences among formulations in all the studied scenarios. Furthermore, multiple-dose studies performed on healthy subjects may also present some safety issues, due to the higher concentrations and the longer contact of the subject with the drug, which may render the study unethical to perform. Use of patients for bioequivalence studies may also be questionable due to ethical reasons as well [14]. In this line of thinking, although the superiority in detecting differences in two formulations for the P2 over the P3 protocol, it is a much desirable goal to reduce the use of multiple-dose studies without the significant loss of discriminative power.

This increased ability does not seem to arise from the fact that multiple-dose studies are used, since protocols P1 and P3 are basically producing the same type of results, but are due to the fact that the latter phase of release from the two formulations is also being tested when requiring bioequivalence in C^{ss}_{trough}, which can be further observed in Fig. 3. All parameters are influenced in great extent by the dose. This is a well known characteristic of C_{max}, for example, and has led to the proposal of alternative indirect metrics of rate of absorption like the C_{max}/AUC ratio [15]. It is also observed that the kinetic parameters that influence C_{max} and C^{ss}_{max} are basically the same, but with greater extent for C_{max}. The major parameters influencing C^{ss}_{trough} are, however, different from the ones influencing C_{max}, justifying the superiority of the P2 protocol when compared to the P3 one. The same conclusions may also be addressed to the PK parameter C_τ. In this case, in all the models, C_τ seems to be influenced by the same formulation parameters as C^{ss}_{trough} (Fig. 3) with global similar intensities, and this fact may indicate that the two parameters are representing the same information. This conclusion is also drawn when comparing the discriminative ability of the P2 and P4 Bioequivalence protocols. As can be seen in Fig. 2, the two protocols are producing basically the same results with the P2 protocol resulting in only 3.7% (average) more non-equivalences in the 20% CV simulations when compared to the P4 protocol. These results indicate that it may be possible, using only one single-dose study and the added C_τ parameter, to obtain the same conclusions as the current requirements on bioequivalence for the EMA.

Intra-subject variability is a major constraint in bioequivalence, as it implies a larger number of subjects in order to achieve sufficient power to demonstrate the similarity between the two formulations. Due to its inherent variability, C^{ss}_{trough} is no longer required in cases where multiple-dose studies are presented for immediate release oral drug products [5]. Based on data from a published multiple-dose bioequivalence study with two extended-release formulations [16], an intra-subject CV of 15% was calculated for C^{ss}_{max} and $AUC^{ss}_{0-\tau}$, whereas a value of 34% was calculated for C^{ss}_{trough}. Our results with the simulated bioequivalence trials (Fig. 4) are in agreement with these data and show that the EMA requirement for bioequivalence is more demanding in terms of the number of required subjects when compared to the FDA requirement. In the same line, the proposed PK metric C_τ is also presenting larger variability than C_{max} and AUC_{0-t} on single dose, which would also require a larger number of subjects, but with the waiving of the multiple-dose study.

In the presented simulations, some assumptions were made, namely constant intervals of dosing, uniform doses, as well as linear pharmacokinetics, which allowed the use of the superposition principle. In the first two cases, these are considerations that are standardised in the clinical bioequivalence trials and can be considered valid in the present situation. However, there are a large number of drugs to be known to present non-linear pharmacokinetic processes on absorption, distribution and metabolisation for which these models may not, in principle, be applied. The relevance of these hypothetical non-linear processes is judged by considering the dose effect on C_{max} and AUC [17]. Being aware that every drug can have non-linear pharmacokinetics at extreme doses, and considering that this non-linearity can be a confounding factor for the establishment of the bioequivalence, current guidelines require that the studies be performed ideally in the linear range of the dose vs. drug concentrations relationship. In this context, the findings of the present work can be applied for the majority of the drugs in the market. It is also important to mention that these simulations were performed on the consideration that a significant accumulation of drug is observed in the multiple-dose regime, and as such, C_τ is quantifiable and both pharmacokinetic and clinically relevant. There are, however, various drug products, like MR formulations of methylphenidate [18] or zolpidem [2] to name a few, where that is not the case. In these cases, C_τ and C^{ss}_{trough} are residual and close to the LLOQ for the analytical methods, making them both irrelevant and difficult to evaluate in a bioequivalence trial.

5. Conclusion

The simulations presented in this paper show that the inclusion of additional pharmacokinetic parameters increases the ability to identify differences between formulations. Nevertheless, when these additional parameters are only obtained with an extra cost in terms of more bioequivalence studies, increased length studies and/or number of subjects, one should weigh the benefit obtained vs. the extra cost. In face of these results, the protocols P2 and P4 clearly outperform the remaining ones, and P2 shows only a marginally higher ability to detect differences between two extended-release formulations when compared to P4 with the extra cost of performing multiple-dose studies. It is our belief that, at least under the simulated conditions, the benefit obtained from performing multiple-dose studies is too small to compensate the extra effort expended. By using C_{max}, AUC_{0-t} and additionally C_τ, the evaluation of bioequivalence between two ER formulations can be made simply by using single dose data, with similar discriminatory characteristics of the current standards for the EMA guidelines. These findings suggest that a reduction in the number of studies and volunteers enrolled on clinical bioequivalence trials to this purpose is possible without loss of discriminative power.

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