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Effect of Different Sampling Schedules on Results of Bioavailability and Bioequivalence Studies: Evaluation by Means of Monte Carlo Simulations

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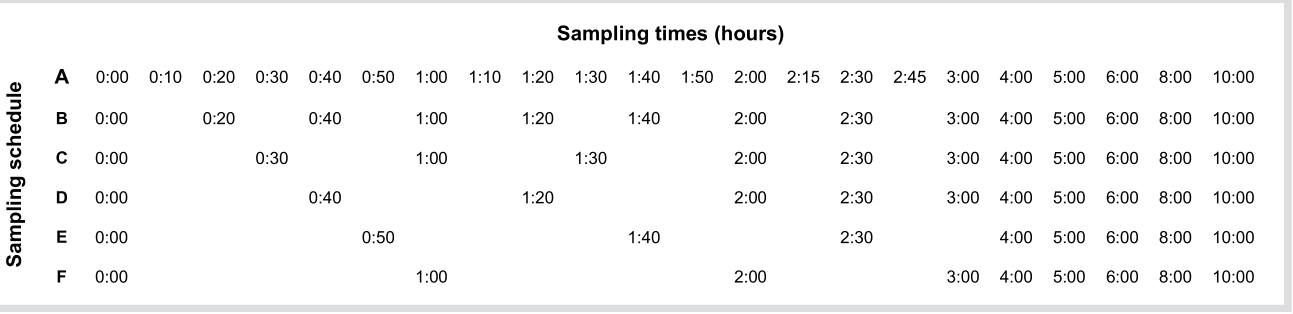
ABSTRACT

Bioavailability and bioequivalence study is one of the most frequently performed investigations in clinical trials. Bioequivalence testing is based on the assumption that 2 drug products will be therapeutically equivalent when they are equivalent in the rate and extent to which the active drug ingredient or therapeutic moiety is absorbed and becomes available at the site of drug action. In recent years there has been a significant growth in published papers that use in silico studies based on mathematical simulations to analyze pharmacokinetic and pharmacodynamic properties of drugs, including bioavailability and bioequivalence aspects. The goal of this study is to evaluate the usefulness of in silico studies as a tool in the planning of bioequivalence, bioavailability and other pharmacokinetic assays, e.g., to determine an appropriate sampling schedule. Monte Carlo simulations were used to define adequate blood sampling schedules for a bioequivalence assay comparing 2 different formulations of cefadroxil oral suspensions. In silico bioequivalence studies comparing different formulation of cefadroxil oral suspensions using various sampling schedules were performed using models. An in vivo study was conducted to confirm in silico results. The results of in silico and in vivo bioequivalence studies demonstrated that schedules with fewer sampling times are as efficient as schedules with larger numbers of sampling times in the assessment of bioequivalence, but only if T_{max} is included as a sampling time. It was also concluded that in silico studies are useful tools in the planning of bioequivalence, bioavailability and other pharmacokinetic in vivo assays.

Introduction

Bioequivalence assays are frequently conducted as a step of the marketing process of generic drug products. They are designed to compare the in vivo performance of different formulations of the same drug and are based on the assumption that 2 drug products will be therapeutically equivalent when they are equivalent in the rate and extent to which the active drug ingredient or therapeutic moiety is absorbed and becomes available at the site of drug action [1]. These assays are conducted by administering at least 2 different formulations (test and reference products) to the study subjects in a crossover design and by collecting blood samples at set time intervals to derive a drug concentration time profile from which pharmacokinetic parameters are obtained [2–5].

In recent years, there has been a significant growth in published scientific papers describing in silico studies based on mathematical simulations to analyze pharmacokinetic and pharmacodynamic properties of drugs, including bioavailability and bioequivalence aspects. In silico studies allow the performance of repeated simulations in short time intervals while changing parameters and/or operational conditions at any time. This enables the understanding of the behavior of a given system, the evaluation of strategies that may change this behavior, predictions and decision making



▶ **Fig. 1** Sampling schedules of in silico bioequivalence studies.

[6–9]. The goal of this study was to evaluate the usefulness of in silico studies in the planning of blood collection on bioequivalence, bioavailability and other pharmacokinetic assays. Monte Carlo simulations were used to define adequate sampling schedules for a bioequivalence study comparing different formulations of cefadroxil oral suspensions. Accuracy in measuring pharmacokinetic parameters directly affects the accuracy of bioequivalence study results. Since the number of blood samples per patient is limited, blood sampling points should be chosen to guarantee that the time concentration profile is adequately defined to allow the calculation of relevant parameters [10]. Inadequate sampling schedules can lead to inconclusive results due to inaccuracy in pharmacokinetic parameter determination. On the other hand, very short intervals may increase the workload of the clinical center, the inconvenience to the study subject, and clinical and analytical costs without increasing study accuracy. As many as 6 240 in silico assays were performed and in silico results were validated through an in vivo bioequivalence assay that included 24 volunteers and compared 4 different cefadroxil formulations.

Methods

Monte Carlo simulation

Cefadroxil was chosen for this research because of its ideal pharmacokinetic and pharmacodynamic characteristics for in silico studies: it follows a one-compartment pharmacokinetic model and shows high bioavailability, low intra- and inter-subject variability, short elimination half-life and wide therapeutic range [11]. Microsoft Office Excel 2003 software was used to simulate drug concentration-time profiles and to calculate 90 % confidence interval (90 % CI) of the ratio of AUC and C_{max} of test and reference products [12, 13]. S-Plus ® 6 for Windows, Student Edition software was employed to generate random values used in simulations.

Simulated plasmatic profiles of cefadroxil were defined through the following equation:

$$C_t = \frac{F \cdot Dose \cdot K_a}{V_d \cdot (K_a - K_{el})} \cdot \left[e^{-K_{el} \cdot t} - e^{-K_a \cdot t} \right] \quad (1)$$

where: C_t = simulated concentration at time t, F = bioavailability, Dose = administered dose, K_{el} = elimination rate constant, K_a = absorption rate constant and V_d = apparent volume of distribution.

▶ **Table 1** Drug content differences * between 4 cefadroxil products used in the in vivo bioequivalence assay.

		TEST			
		D1	**D2**	**D3**	**D4**
REFERENCE	**D1**	0 %	+ 7 %	+ 11 %	+ 16 %
	D2	− 6 %	0 %	+ 4 %	+ 8 %
	D3	− 10 %	− 4 %	0 %	+ 4 %
	D4	− 13 %	− 8 %	− 4 %	0 %

* Differences calculated as 100 × (drug content of test product - drug content of reference product)/drug content reference product; R = Reference Dose, T = Test Dose; D1 = 450 mg, D2 = 480 mg, D3 = 500 mg, D4 = 520 mg

Values of pharmacokinetic parameters K_a, K_{el} and V_d used in Eq. (1) were obtained from previous in vivo cefadroxil bioequivalence studies performed with 24 healthy volunteers in a randomized crossover design, after administration of cefadroxil 500 mg oral suspension (unpublished data). K_a was determined through the method of residuals. K_{el} was calculated by applying a log-linear regression analysis to at least the last 3 quantifiable concentrations of cefadroxil. $AUC_{0\text{-}inf}$ was calculated as $AUC_{0-t} + C_t/K_{el}$, where AUC_{0-t} is the area under the curve from time zero to time of the last measurable cefadroxil concentration calculated using the linear trapezoidal method, and C_t is the last measurable cefadroxil concentration. V_d was obtained as $Dose/K_{el} \cdot AUC_{0-inf}$ [14].

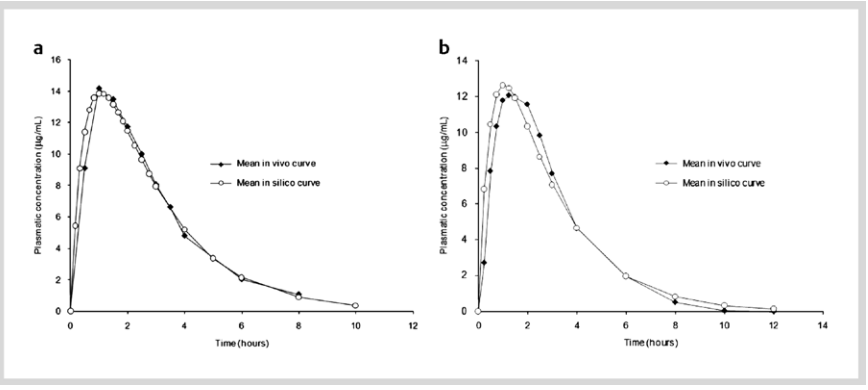
The reference product for the in silico assays was a 500 mg dose of cefadroxil oral suspension. 10 different doses, ranging from − 10 % to + 20 % of 500 mg (450 mg to 600 mg), were used to mimic test products with different bioavailabilities. 6 sampling schedules (A, B, C, D, E and F) with a number of blood samples between 9 and 22 distributed from time 0 h to time 10 h after cefadroxil administration were tested (▶ **Fig. 1**). Bioequivalence was concluded if 90 % CI of the ratio of AUC_{0-t} and C_{max} of test and reference products lay within 80 % and 125 % [12, 13]. 2 simulation models were used, one based on minimum and maximum values of pharmacokinetic parameters and one based on intra- and inter-subject variability.

In silico assays based on maximum and minimum values of pharmacokinetic parameters (in silico model 1)

Maximum and minimum values of each pharmacokinetic parameter (K_a, K_{el} and V_d) were identified and random values were obtained uniformly for each maximum and minimum interval. Ran-

Sampling schedule	Sampling times (hours)																					
M	0:00	0:10	0:20	0:30	0:40	0:50	1:00	1:10	1:20	1:30	1:40	1:50	2:00	2:15	2:30	2:45	3:00	4:00	5:00	6:00	7:00	8:00
N	0:00		0:20		0:40		1:00		1:20		1:40		2:00		2:30		3:00	4:00	5:00	6:00	7:00	8:00
O	0:00			0:30			1:00			1:30			2:00		2:30		3:00	4:00	5:00	6:00	7:00	8:00
P	0:00				0:40				1:20				2:00				3:00	4:00	5:00	6:00	7:00	8:00
Q	0:00					0:50					1:40				2:30			4:00	5:00	6:00	7:00	8:00
R	0:00						1:00						2:00				3:00	4:00	5:00	6:00	7:00	8:00

▶ **Fig. 2** Sampling schedules of in vivo bioequivalence study.



▶ **Fig. 3** Mean in vivo plasmatic concentration time profile obtained after oral administration of cefadroxil 500 mg to 24 healthy volunteers and mean in silico plasmatic concentration time profile for the reference dose of cefadroxil (500 mg) obtained by in silico model 1 **a** and in silico model 2 **b**.

dom values of K_a, K_{el} and V_d were combined to generate simulated plasmatic concentration time profiles. Each K_a, K_{el} and V_d value was used only once. Simulated plasma concentration time profiles for the reference product (500 mg dose) which showed C_{max} less than 10 µg/ml and/or higher than 18 µg/ml were excluded, since this is the range described in the literature for cefadroxil C_{max} after oral administration of 500 mg of the reference product [15]. Bioequivalence between reference and test products was evaluated using each sampling schedule (A, B, C, D, E and F). Each in silico bioequivalence study comparing a test product (dose ranging from 450–600 mg) to the reference product (500 mg dose) included 12 subjects, which means 12 simulated curves for the test product and 12 simulated curves for the reference product. 54 bioequivalence assays were conducted for each test product and for each sampling schedule totaling 3 240 in silico assays.

In silico assays based on intra- and inter-subject variability (in silico model 2)

In this case, intrinsic variability of administered dose and pharmacokinetic parameters were included in the simulation procedure [16]. A normal distribution around the administered dose value with a variation of 2 % was assumed:

$$D_{ij} = D_i + \eta_j \quad (2)$$

where D_{ij} = dose of the ith treatment, administered to the jth individual, D_i = claimed dose for the ith treatment, and η_j = content variability among doses (2 %).

For the pharmacokinetic parameters (K_a, K_{el} and V_d), a log-normal distribution around the average value was assumed:

$$P_{ij} = P_i \times e^{\eta_j} \times e^{\eta_{ij}} \quad (3)$$

where P_{ij} = pharmacokinetic parameter of the jth individual upon receiving the ith treatment, P_i = average population parameter for the ith treatment, η_j and η_{ij} = inter- and intra-subject variability, respectively.

Furthermore, an assay error was considered and assigned to each concentration determined through Eq. (1) to account for the variability arising from all procedures involved in the determination of plasmatic drug concentration, including variability in sampling schedule, possible drug degradation following samples handling and intrinsic errors of quantifying procedure. The assay error corresponds to a 10 % variation of the mean concentration determined at time t, and has normal distribution with an average of zero:

$$(C_{ij}(t))_{obs} = C_{ij}(t) + \eta_{assay} \quad (4)$$

where $(C_{ij}(t))_{obs}$ = concentration defined through Eq. (1), plus the assay error; $C_{ij}(t)$ = concentration defined through Eq. (1), and η_{assay} = assay error.

In silico bioequivalence tests comparing reference and test products were conducted for 12 volunteers, considering a 2 sequence, crossover, randomized trial. 50 bioequivalence assays were conducted for each combination of reference (500 mg dose) and test

▶ **Table 2** Percentage of bioequivalent results obtained from in silico model 1 bioequivalence studies between the reference 500 mg dose and test doses from 450 mg to 600 mg using different sampling schedules (S.S.).

S.S.	Bioequivalent results (%)																			
	450 mg		460 mg		465 mg		475 mg		525 mg		535 mg		540 mg		550 mg		575 mg		600 mg	
	C_{max}	AUC	C_{max}	AUC	C_{max}	AUC	C_{max}	AUC	C_{max}	AUC	C_{max}	AUC	C_{max}	AUC	C_{max}	AUC	C_{max}	AUC	C_{max}	AUC
A	32	37	48	52	57	56	65	68	80	80	70	74	70	70	61	63	52	35	17	18
B	33	37	48	52	57	56	65	67	80	81	72	72	70	70	61	63	52	35	17	15
C	33	33	48	52	57	54	63	63	80	80	72	72	70	70	61	63	54	35	18	15
D	41	33	50	50	59	54	68	61	80	80	72	72	72	69	63	61	52	33	17	17
E	35	33	52	52	56	52	65	63	81	80	70	70	65	68	61	61	52	33	17	15
F	31	30	39	46	54	50	61	63	81	74	72	68	67	65	59	57	52	31	15	15

▶ **Table 3** Percentage of bioequivalent results obtained from in silico model 2 bioequivalence studies between the reference 500 mg dose and test doses from 450 mg to 600 mg using different sampling schedules (S.S.).

S.S.	Bioequivalent results (%)																			
	450 mg		460 mg		465 mg		475 mg		525 mg		535 mg		540 mg		550 mg		575 mg		600 mg	
	C_{max}	AUC	C_{max}	AUC	C_{max}	AUC	C_{max}	AUC	C_{max}	AUC	C_{max}	AUC	C_{max}	AUC	C_{max}	AUC	C_{max}	AUC	C_{max}	AUC
A	52	38	76	46	76	52	80	66	92	68	92	58	88	56	72	56	56	34	22	20
B	48	38	62	46	72	52	78	68	90	60	88	60	82	56	76	52	54	28	20	18
C	52	38	62	44	66	50	74	72	88	62	86	60	84	54	74	52	46	32	26	20
D	50	42	62	50	64	54	82	66	84	64	82	56	76	54	66	50	48	30	14	16
E	48	38	68	46	68	50	70	66	80	60	82	64	76	56	60	48	46	26	26	16
F	48	34	62	40	72	48	64	66	82	58	82	56	80	54	60	50	42	22	20	12

▶ **Table 4** 90 % confidence interval (90 % CI) of the ratio of AUC and C_{max} of test and reference products considering sampling schedule M of the in vivo bioequivalence study.

		90 % Confidence Interval (%)			
Reference	**Test**	**D1 (450 mg)**	**D2 (480 mg)**	**D3 (500 mg)**	**D4 (520 mg)**
D1 (450 mg)	C_{max}	–	101–112	105–117	107–119
	AUC	–	105–113	110–119	118–127
D2 (480 mg)	C_{max}	89–99	101–110	98–109	100–111
	AUC	88–95	–	101–110	108–117
D3 (500 mg)	C_{max}	86–95	91–101	–	97–107
	AUC	85–90	91–99	–	102–111
D4 (520 mg)	C_{max}	84–93	90–100	93–104	–
	AUC	78–85	86–93	90–98	–

▶ **Table 5** 90 % confidence interval (90 % CI) of the ratio of AUC and C_{max} of test and reference products considering sampling schedule N of the in vivo bioequivalence study.

		90 % Confidence Interval (%)			
Reference	**Test**	**D1 (450 mg)**	**D2 (480 mg)**	**D3 (500 mg)**	**D4 (520 mg)**
D1 (450 mg)	C_{max}	–	101–113	106–118	106–118
	AUC	–	105–114	110–120	118–127
D2 (480 mg)	C_{max}	88–99	–	99–110	99–111
	AUC	88–95	–	101–109	107–116
D3 (500 mg)	C_{max}	85–95	91–101	–	95–106
	AUC	83–90	91–99	–	102–111
D4 (520 mg)	C_{max}	84–94	90–101	94–105	–
	AUC	78–85	86–93	90–98	–

▶ **Table 6** 90 % confidence interval (90 % CI) of the ratio of AUC and C_{max} of test and reference products considering sampling schedule O of the in vivo bioequivalence study.

		90 % Confidence Interval (%)			
Reference	**Test**	**D1 (450 mg)**	**D2 (480 mg)**	**D3 (500 mg)**	**D4 (520 mg)**
D1 (450 mg)	C_{max}	–	104–116	108–120	109–121
	AUC	–	106–114	111–120	118–127
D2 (480 mg)	C_{max}	86–96	–	98–109	100–111
	AUC	88–95	–	101–110	108–116
D3 (500 mg)	C_{max}	83–93	92–102	–	96–107
	AUC	83–90	91–99	–	102–110
D4 (520 mg)	C_{max}	82–91	90–100	93–104	–
	AUC	78–84	86–93	90–98	–

▶ **Table 7** 90 % confidence interval (90 % CI) of the ratio of AUC and C_{max} of test and reference products considering sampling schedule P of the in vivo bioequivalence study.

		90 % Confidence Interval			
Reference	**Test**	**D1 (450 mg)**	**D2 (480 mg)**	**D3 (500 mg)**	**D4 (520 mg)**
D1 (450 mg)	C_{max}	–	89–127	95–136	95–136
	AUC	–	105–114	111–121	119–129
D2 (480 mg)	C_{max}	78–112	–	89–128	89–127
	AUC	88–95	–	102–111	108–118
D3 (500 mg)	C_{max}	73–105	78–112	–	83–119
	AUC	82–90	90–98	–	102–111
D4 (520 mg)	C_{max}	74–105	78–112	84–120	–
	AUC	78–84	85–92	90–98	–

▶ **Table 8** 90 % confidence interval (90 % CI) of the ratio of AUC and C_{max} of test and reference products considering sampling schedule Q of the in vivo bioequivalence study.

		90 % Confidence Interval			
Reference	**Test**	**D1 (450 mg)**	**D2 (480 mg)**	**D3 (500 mg)**	**D4 (520 mg)**
D1 (450 mg)	C_{max}	–	98–110	100–113	103–117
	AUC	–	105–114	110–119	118–128
D2 (480 mg)	C_{max}	90–102	–	96–109	99–112
	AUC	88–95	–	100–109	108–117
D3 (500 mg)	C_{max}	89–100	92–104	–	97–110
	AUC	84–91	92–99	–	103–112
D4 (520 mg)	C_{max}	86–97	89–101	91–103	–
	AUC	78–85	85–93	89–97	–

▶ **Table 9** 90 % confidence interval (90 % CI) of the ratio of AUC and C_{max} of test and reference products considering sampling schedule R of the in vivo bioequivalence study.

		90 % Confidence Interval			
Reference	**Test**	**D1 (450 mg)**	**D2 (480 mg)**	**D3 (500 mg)**	**D4 (520 mg)**
D1 (450 mg)	C_{max}	–	103–117	104–117	107–121
	AUC	–	107–116	113–123	120–130
D2 (480 mg)	C_{max}	86–97	–	94–107	98–110
	AUC	86–94	–	101–110	108–117
D3 (500 mg)	C_{max}	85–96	93–106	–	97–110
	AUC	82–89	91–99	–	102–111
D4 (520 mg)	C_{max}	82–93	90–102	91–103	–
	AUC	77–83	85–93	90–98	–

product (dose ranging from 450 to 600 mg) and for each sampling schedule (A, B, C, D, E and F) totaling 3 000 in silico tests.

In vivo bioequivalence study

4 products with different bioavailabilities were emulated using different volumes of a cefadroxil oral suspension to produce doses of 450 mg (D1), 480 mg (D2), 500 mg (D3) or 520 mg (D4), mimicking differences in bioavailability ranging from −13 % to +16 % (▶ **Table 1**), and were compared in the in vivo bioequivalence assay.

The clinical protocol was approved by the local Ethical Committee. 24 healthy male volunteers were included in an open-label, randomized, 4-way crossover study with a washout period of 3 days. Drugs were administered with 200 ml of water after a 10h fasting period, which continued for an additional 4 h after drug administration. Subjects were provided with standard meals at 4 h and 7 h after drug administration in each treatment group. Venous blood samples (4 ml) were collected following sampling schedule M (▶ **Fig. 2**). Plasma was immediately separated by centrifugation at 2 200 g for 10 min, transferred to properly labeled tubes and stored at − 20 °C until cefadroxil quantification through a validated high performance liquid chromatographic method [17]. Bioequivalence was concluded if 90 % CI of the ratio of AUC_{0-t} and C_{max} of test and reference products lay within 80 % and 125 %. The influence of sampling schedule on the results of bioequivalence studies was evaluated by comparing bioequivalence results between 4 products of cefadroxil following 6 sampling schedules (Schedules M, N, O, P, Q and R, ▶ **Fig. 2**).

Results

In vivo and in silico concentration time profiles are depicted in ▶ **Fig. 3**. ▶ **Table 2**, **3** summarize in silico bioequivalence assays results for different test doses and different sampling schedules, while in vivo bioequivalence assay results are shown in ▶ **Table 4–9**.

Discussion

Monte Carlo simulation methods are frequently employed to explore the influence of several factors in bioequivalence tests [2, 16–23].

The mathematical models proposed in this paper offer some advantages over other simulation models [10, 19, 20, 22, 24], e.g., the use of accessible software, the possibility of changes on data entry by the user, the possibility of carrying data to other programs, the ability to receive data from other programs and the possibility to perform a large number of tests. In silico and in vivo data showed good correlation. The in silico model 1, based on maximum and minimum values, was simpler and faster to use when compared to in silico model 2, based on intra- and inter-subject variability, since it does not require error determination for each pharmacokinetic parameter and for each cefadroxil plasmatic concentration. Bioequivalence studies simulated with in silico model 1 showed similar percentages of bioequivalent results for both pharmacokinetic parameters C_{max} and AUC_{0-t} for each test product and sampling schedule. Conversely, studies simulated with in silico model 2 showed higher percentages of bioequivalent results for C_{max} than for AUC_{0-t}. Midha and colleagues [25] conducted a bioequivalence assay comparing 2 doses with a 20 % difference between them and

found similar results, since AUC_{0-t} 90 % CI showed a more significant difference between doses than C_{max} 90 % CI. A possible explanation for this fact is that C_{max} depends on only one measure while AUC_{0-t} depends on a set of measures. In silico bioequivalence study results showed very similar results for all sampling schedules (A, B C, D, E and F) indicating that the exclusion of some sampling points does not influence bioequivalence study results and conclusions. This was also observed for in vivo bioequivalence study results using sampling schedules M, N, O, Q and R: bioinequivalence was always observed between products D1 and D4 for the pharmacokinetic parameter AUC_{0-t}. These data support the argument by Kong and Gonin [10] that decreases in sampling intervals do not represent a real gain in results accuracy. However, this was not the case for sampling schedule P; bioequivalence studies conducted according to this schedule presented 90 % CI for C_{max} outside the recommended 80 % to 125 % limits for most reference vs. test comparisons, including those with dose content differences as low as 4 %, where bioinequivalence would not be expected. This schedule is the only one that does not provide sampling at the time of C_{max} (T_{max}) of cefadroxil, which is approximately 1 h, affecting accuracy of C_{max} determination. Consequently, a high intra-individual variation coefficient was observed for C_{max}, which does not represent the real variability of cefadroxil, and a low statistical test power was obtained for this pharmacokinetic parameter. Nevertheless, 90 % CI for AUC_{0-t} were similar to those obtained for other schedules.

Conclusion

It can be concluded that schedules with fewer sampling times are as efficient as schedules with a larger number of sampling times in the assessment of bioequivalence, but only if T_{max} is included as a sampling time. It can also be concluded that in silico studies using Monte Carlo simulations are a useful tool in the planning of bioequivalence, bioavailability and other pharmacokinetic in vivo assays.

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Conflict of Interest

The authors have no conflict of interest to declare in connection with the contents of this manuscript.

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