Evaluation of Truncated Areas in the Assessment of Bioequivalence of Immediate Release Formulations of Drugs with Long Half-Lives and of Cmax with Different Dissolution Rates

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Received February 5, 1999; accepted February 23, 1999

Purpose. To Evaluate truncated AUC in place of AUCt or extrapolated AUCinf, for drugs with long half-lives and to study the relationship between Cmax and *in vitro* dissolution rates.

Methods. Monte-Carlo simulations were conducted using actual mean plasma concentrations of five long half-life drug products. The simulations were based on a catenary pharmacokinetic system in which the drug disposition in the body was represented by a one-or two-compartment model, characterizing the observed mean profiles. The influence of dramatic changes in the *in vitro* dissolution rate constant 'k_d', was simulated in scenarios consisting of 20 crossover trials with 24 subjects per trial, comparing a fast dissolving reference and a hypothetical, slow dissolving test formulation.

Results. The AUC's truncated after the completion of distribution phase were found surrogate to the AUCt or AUCinf measures. Except for Phenylbutazone, the Cmax measure was insensitive to the changes in the *in vitro* dissolution rate. The Cmax measure was found to be useful in the bioequivalence assessment since it reflected both the rate and extent of absorption. (Cmax/AUCt) measure was specific to absorption rate.

Conclusions. For the bioequivalence determination of long half-life drug products, (1) the use of truncated AUC's after completion of the distribution phase instead of AUCinf, appears feasible. (2) Cmax measure may be insensitive to input rate changes, if the absorption rate is not constrained by the input rate in relation to the distribution or elimination rate.(3) (Cmax/AUCt) may be more specific to 'k_a' differences, but Cmax reflects differences in both rate and extent of absorption.

KEY WORDS: truncated AUC; long half-life; *in vitro* dissolution rate; Monte-Carlo simulations.

INTRODUCTION

Drugs with terminal elimination half-lives of 72 hours or more, may be considered long half-life drugs. The pharmacokinetics of long half-life drugs has been studied and reported in the literature(1–2). Traditionally, bioequivalence of two orally administered formulations is concluded with respect to the extent and rate of absorption. This is characterized by area under the curve (AUC) and the maximum observed concentration (Cmax)(3) respectively. A typical 2-way crossover bioequivalence study involving immediate release, long half-life drug formulations faces numerous problems. These include prolonged blood sampling times, a long washout period, possible non-compliance of the study subjects to the protocol, possible imbalance in sequences for statistical analysis, time-dependent variability in the drug disposition in two study phases, possibility of toxicity due to long exposure, accumulation in a particular tissue, drug dependency, and finally (due to all these), the increased cost of the study. For these bioequivalence studies, the frequently asked question is whether the long duration of blood sampling, which is based on the terminal half-life, is necessary. It could be argued that since the formulation has left the gastrointestinal tract generally in less than 48 hours, and the absorption process related to the formulation is invariably over following one elimination half-life in time, the additional sampling merely leads to information about disposition. In situations where estimation of terminal rate constant is compromised, extrapolation of AUC to infinity leads to higher variability and wider confidence intervals. It has been of interest in the past(4–6) whether the pharmacokinetic measure AUCinf is necessary to demonstrate bioequivalence or it could be replaced by AUC's truncated after the absorption and distribution phases are complete.

AUC is a robust estimator of the extent of absorption, while Cmax is a surrogate and imperfect measure of the rate. One reason for this is the insensitivity of Cmax to the differences in input rate, which may result from the differences in dissolution when dissolution is the rate-limiting step for the gastrointestinal absorption. The other reasons include, the Cmax is frequently equated with the largest measured concentration rather than the one estimated from the nonlinear regression analysis of the concentration time data, Cmax estimation from concentration time data may be flawed due to inappropriate sampling protocol (such as too few samples or improper spacing), or the differences in the input rate might arise from the differences in the absorption site milieu instead of the dissolution rates, influencing the membrane permeability. In this context, another question which may be asked is, assuming sampling scheme is adequate, and given that the elimination is the governing process(7), whether a poorly dissolving long half-life drug formulation will lead to a substantial difference in the input to affect the Cmax and bioequivalence.

A computer simulation study was conducted using 'Monte-Carlo' technique for the evaluation of a) truncated areas, in place of AUCt or extrapolated AUCinf and b) the relationship between Cmax and in-vitro dissolution rates. Bioequivalence scenarios of five immediate release long half-life drug formulations with two dramatically different in-vitro dissolution rates were simulated based on the actual study data.

METHODS

The simulation study was carried out through the following steps:

1. From actual mean *in vitro* percent dissolved-time and *in vivo* plasma concentration-time data, initial best fitting estimates for 'k_d', the first order dissolution rate constant, and 'k_{el}' or 'β', the first order terminal rate constant, were obtained.

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[3] The views expressed in this paper are professional opinions and perspectives of the authors and do not represent the official policy of the U.S. Food and Drug Administration at this time.

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0724-8741/99/0600-0939$16.00/0

Other parameters such as volume of distribution and bioavailability fraction were obtained from the literature (8).

2. By keeping the 'k_d' and 'k_{el}' or 'β' as constants and by mixing and matching the parameters, the mean curves were modeled, using a catenary pharmacokinetic system in which disposition in the body was represented by either a one- or two-compartment model. The final best-fit estimates of the pharmacokinetic model parameters were obtained using non-linear regression.

3. Realistic inter- and intra-individual variances were put on the pharmacokinetic model parameters. The assay error was assumed to be negligible for both formulations.

4. 'Monte-Carlo' simulations were conducted to generate pharmacokinetic measures for a reference and a hypothetical test formulation, with different in-vitro 'k_d's.

5. For the two formulations, bioequivalence was assessed, with respect to AUC's truncated at various sample points, AUCt, AUCinf, Cmax, Cmax/AUCt and Cmax/AUCinf, based on the two one-sided test criterion.

Assumptions

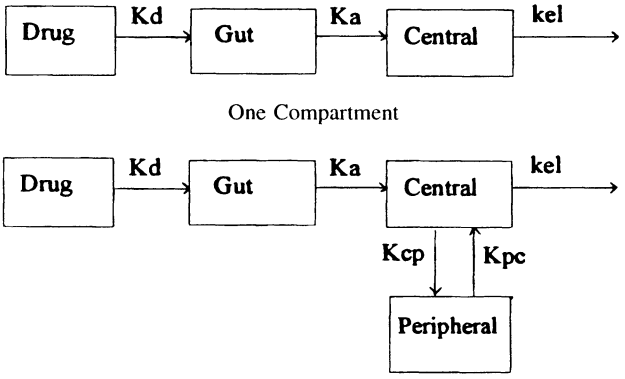
Study Related: i) *in vitro* and *in vivo* dissolutions are first order processes, ii) *in vivo* dissolution is reflective of the *in vitro* dissolution, iii) study does not have a flip-flop situation and iv) study population does not have outliers.

Simulation Related: i) the pharmacokinetic parameters follow a normal distribution in the study population, ii) the parameter variability was truncated to three standard deviations.

Five long half-life drugs were selected based on different physico-chemical and pharmacokinetic characteristics (Table 1). For drugs other than nortriptyline HCl, the bioavailability fraction 'F' was assumed to be 0.99 (8). For nortriptyline HCl, 'F' was assumed to be 0.5 (8). The dissolution rate constant 'k_d' and the terminal rate constant 'k_{el}' or 'β', were obtained from the actual mean dissolution and plasma level time profiles ('in-house' data). The dissolution data had been generated, as per the USP dissolution specifications (9). The mean *in vitro* dissolution data was fit using SAS NLIN (10) program to yield the best estimate of dissolution rate constant 'k_d'. A first order exponential function,

$$X_{(t)} = X_{(infinity)}*(1 - e^{-Kd*(t)}),$$

with $X_{(t)}$ the percent dissolved at time 't', $X_{(infinity)}$ the percent dissolved at time infinity (assumed to be 100%), 'k_d' the dissolution rate constant and t, time in hr, was used to fit the actual mean dissolution data. The terminal portion of the observed mean ln (Cp) vs. time data was regressed to yield the best estimate of the terminal elimination rate constant, based on the maximum correlation coefficient R^2 value. At least four data points were used for the regression. For some other pharmacokinetic parameters such as volume of distribution, parameters of a 70 kg normal man (8) were used as the initial estimates in model fitting. Using the estimates of 'k_d' and 'k_{el}' or 'β' as constants, catenary pharmacokinetic systems (11) involving one or two compartments (as shown in the following schemes) were fitted to the actual mean plasma level data using non-linear regression (12). For piroxicam and ethosuximide, a system with one-compartment disposition model characteristic was found ideal while for nortriptyline hydrochloride, tamoxifen and phenylbutazone a two-compartment disposition model system with central elimination was found ideal, based on the standard 'goodness of fit' criterion like AIC (13).



One Compartment

Two Compartment

Scheme

The final estimates for the pharmacokinetic measures with the corresponding intra- and inter-individual %CV are given in Table 2. The inter-individual variances, were from the actual data where possible, and from the literature otherwise. The intra-individual variances though different from some other reports (18,19), were realistic and similar to a typical crossover trial. For each trial, the reference (R) formulation profiles were simulated using the best fitted value of 'k_d' from the actual

Table 1. Selected Long Half-Life Drugs and the Pharmacokinetic Parameter Relationships

Drug	Acid or base, pKa	k_d (R, T)	k_a	α	k_d/k_a	k_{el} or β	k_a/α, k_d/α*	k_a/k_{el} or k_a/β	k_d/k_{el} or k_d/β (R, T)
Phenylbutazone	Acid, 5.1	4.094, 0.205	1.354	0.224	3.024, 0.151	0.010	6.1, 0.915*	135.4	409.4, 20.5
Piroxicam	Acid, 4.5	3.933, 0.197	3.228	—	1.218, 0.061	0.010	—	322.8	393.3, 19.7
Nortriptyline	Base, 9.7	7.5, 0.376	0.195	0.039	38.6, 1.928	0.004	5.0	48.6	1881.8, 94
Ethosuximide	Acid, 9.3	8.1, 0.406	20.3	—	0.399, 0.020	0.012	—	1692.5	676.2, 33.8
Tamoxifen	Non-ionic	8.9, 0.447	0.256	0.255	34.9, 1.746	0.0089	1.004	32.0	1117.4, 55.9

Note: R represents Reference formulation simulation, i.e, 'k_d' = original k_d from the actual data. T represents Test formulation simulation, i.e, 'k_d' = 5% of the original 'k_d'. k_a = absorption rate constant, expressed as (1/hr). k_d = in-vitro dissolution rate constant, expressed as (1/hr). α = hybrid distribution rate constant, expressed as (1/hr). k_{el} or β = terminal rate of elimination for a one-or two-compartment model, expressed as (1/hr). Please refer to the model schemes.

Table 2. Pharmacokinetic Parameter (mean) Estimates (Initial Values and Inter-and Intra-Individual Variability)

Parameter	PHE	I_1, I_2	NOR	I_1, I_2	TAM	I_1, I_2	ETH	I_1, I_2	PIR	I_1, I_2
Dose, [mg]	100	2, --	75	2, --	20	2, --	250	2, --	20	2, --
F	0.99	1, 0.01	0.5	1, 1	0.99	1, 0.01	0.99	1, 0.01	0.99	1, 0.01
k_a [hr−1]	1.354	30, 10	0.195	30, 10	0.256	30, 10	20.3	30, 10	3.228	30, 10
k_d [hr−1]	4.094	12.4	7.5	17	8.9	3.9	8.1	10	3.933	18
β [hr−1]	0.01	25, 2	0.004	36, 2	0.008	20, 2	—	—	—	—
V_d [liters]	4.922	5, 5	1096.5	22, 5	262.9	22, 5	25.6	22, 5	9.642	20, 5
α [hr−1]	0.224	5, 5	0.039	5, 5	0.255	5, 5	—	—	—	—
k_{pc} [hr−1]	0.133	5, 5	0.009	5, 5	0.058	5, 5	—	—	—	—
k_{10}, k_{el} [hr−1]	0.017	5, 5	0.017	5, 5	0.036	5, 5	0.012	5, 5	0.01	5, 5
k_{cp}[hr−1]	0.084	5, 5	0.017	5, 5	0.017	5, 5	—	—	—	—

Note: k_{pc}, k_{cp} = Inter-compartmental rate constants for peripheral to central and central to peripheral. I_1, I_2 = Inter-Individual and Intra-Individual variability expressed as %Coefficient of Variation. PHE = Phenylbutazone. NOR = Nortryptaline. TAM = Tamoxifen. ETH = Ethosuximide. PIR = Piroxicam.

mean dissolution data. The hypothetical, poorly dissolving test (T) formulation profiles were simulated by reducing the reference formulation 'k_d' by 95%. The two formulations thus were different only with respect to their in-vitro dissolution rates.

For each of the five drugs, 20 trials with 24 subjects per trial, were simulated, using a two period, two treatment, randomized, balanced, crossover design. The simulations were conducted using a LOTUS 123 add-in software, @RISK (14). A Latin-Hypercube sampling technique was used to generate individual subject concentration-time profiles. For each individual subject, the usual pharmacokinetic measures such as area under the curve until the last measurable sample point t, AUC_t (calculated by trapezoidal rule), maximum observed concentration Cmax, and area under the curve until the infinite time AUC_{inf} calculated as $\{AUC_t + C_t/k_{el}$ or $\beta\}$ were estimated for both phases. In addition, various truncated AUC measures corresponding to the actual sampling times were generated. After logarithmic transformation, the measures were evaluated using analysis of variance in SAS (15), with sequence, subject nested within sequence, period and treatments as ANOVA factors. The bioequivalence of the two formulations with different dissolution rates was assessed using a two one-sided test criterion (16).

RESULTS

The observed mean and simulated plasma drug profiles of the two formulations are shown in Fig. 1. The 90% confidence intervals corresponding to various pharmacokinetic measures for the 20 trials are given in Fig. 2. It could be seen that even after a 95% reduction in the *in vitro* dissolution rate constant, for piroxicam, ethosuximide and nortriptyline HCl, the 90% confidence intervals met the 80–125% regulatory bioequivalence acceptance criteria, for all bioequivalence measures, for all 20 trials. For tamoxifen, 18 of the 20 trials met the bioequivalence criteria for all measures. Two trials failed the 80–125% bioequivalence criterion for lnCmax (CI's: 79.6–92.1, 78.8–91), but passed for the ln(Cmax/AUCt) (CI's: 82–91.5, 82.7–91.8) and ln(Cmax/AUCinf) (CI's: 81.9–91.6, 82.5–91.7) measures. In all twenty phenylbutazone trials, lnCmax as well as ln(Cmax/AUCt) and ln(Cmax/AUCinf) failed the 80–125% criterion. For all studied drugs, the truncated AUC measures, $lnAUC_{72}$, $lnAUC_{73}$, $lnAUC_{96}$, $lnAUC_{97}$, $lnAUC_{120}$, $lnAUC_{145}$ and $lnAUC_{168}$ passed the 80–125% criterion comfortably similar to the $lnAUC_t$ and $lnAUC_{inf}$ measures.

DISCUSSION

The simulations were aimed at effectively evaluating the metric for comparative extents of absorption and to deal with the source for assessing the relative rates of absorption. Towards the first goal, for a given drug the measure AUC_t was found to be more robust compared to the Cmax or AUC_{inf}. This observation is similar to Bois (17–18). The truncated AUC measures AUC_{72}, AUC_{73}, AUC_{96}, AUC_{97}, AUC_{120}, AUC_{145}, and AUC_{168} easily passed the two one-sided test criterion. The truncated AUC_{48} measure, though far removed from the Tmax values, resembled the partial AUC in cases such as piroxicam and nortriptyline. For the studied drugs, the 'k_a/k_{el}' and 'k_d/k_{el}' (or the corresponding 'k_a/β' and 'k_d/β') ratios were large (Table 1). Consequently, absorption was essentially complete early within the profiles, and it had generally but not always little effect on AUC's truncated after distribution was complete. Based on our results, it therefore could be argued that, for the studied drugs, after completion of the distribution phase, prolonged blood draws may not be necessary. This conclusion is supported by others (19–22). Regarding the truncation time, however the authors advise caution. It is inappropriate to measure the terminal slope for less than one or two half-lives. Also, many times, it is seen that long half-life drugs form pharmacologically active metabolites with long half-life (e.g., anti-psychotic drugs). In these evaluations, we have not considered pharmacokinetics of the active metabolites. Under these circumstances, if the truncations result in passing the 90% confidence intervals of the parent drug and failure of the 90% confidence intervals of the metabolite, or vice versa, the bioequivalence evaluation would be difficult. Initially, insensitivity of the long half-life drug Cmax to the *in vitro* dissolution rate was expected since it was thought that the elimination is primarily governing the absorption metric rather than the dissolution (input). While *in vitro* dissolution tests would have picked up the differences in other four products, only for phenylbutazone the dissolution differences did show bio-inequivalence. For drugs exhibiting a one-compartment disposition characteristic, i.e., piroxicam and ethosuximide, the 'k_a/k_{el}' ratio was in favor of the numerator. After reduction of 'k_d' for the

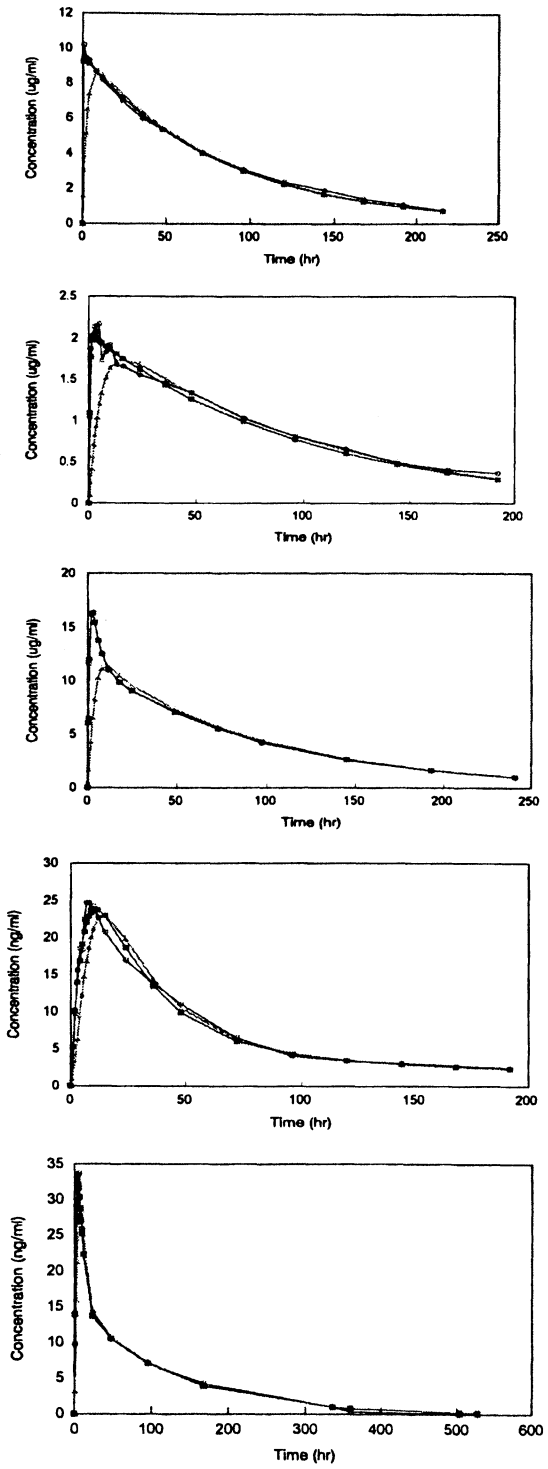


Fig. 1. Mean observed and simulation plasma profiles (top to bottom): ethosuximide, piroxicam, phenylbutazone, nortriptyline HCl and tamoxifen. --*-- observed mean plasma profile; --■-- mean plasma profile of the reference formulation with k_d from the actual *in vitro* results, considered = 100%; --▲-- mean plasma profile of the test formulation with k_d considered as 5% of the reference formulation.

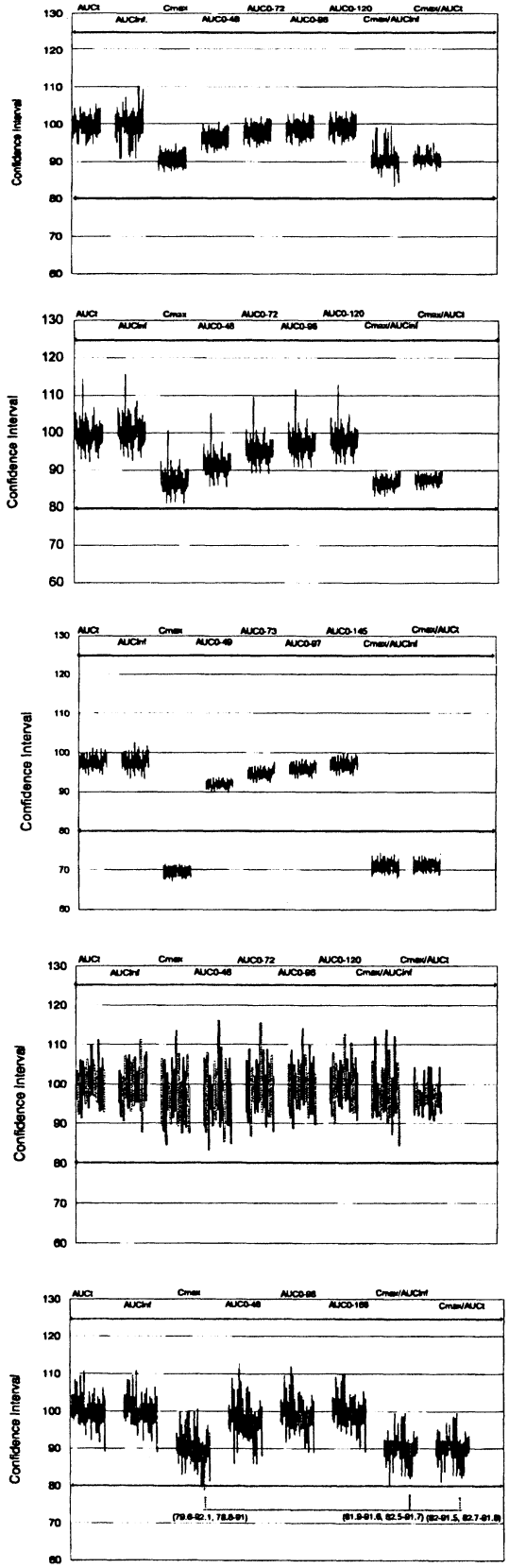


Fig. 2. 90% confidence/intervals of various in-transformed pharmacokinetics measures from the 20 simulated bioequivalence trials, each with 24 subjects, from top to bottom: ethosuximide, piroxicam, phenylbutazone, nortriptyline HCl and tamoxifen. Each bar represents confidence interval of a bioequivalence trial.

Test formulation, the ratio of 'k_d/k_{el}' which now influenced the Cmax, was also in favor of the numerator, thus not affecting the Cmax. For the drugs showing a two-compartment disposition characteristic, Cmax was influenced more by absorption and distribution, rather than absorption and elimination. For nortriptyline and to some extent tamoxifen, the insensitivity of Cmax to the *in vitro* dissolution rate was expected, since the absorption rate rather than dissolution-rate was 'input-rate' limiting. With the ratio of 'k_a/α' (α, is the distribution hybrid rate constant) being close to 1.0, two tamoxifen bioequivalence trials failed the Cmax measure. For phenylbutazone, the situation was unique. The 'k_d' change from Reference to Test, shifted the Cmax dependency from 'k_a/α' the Reference to 'k_d/α' for the Test, with a dramatic reduction from 6.045 to 0.0915 (implying that for the Reference, Cmax is distribution-rate limited and for the Test, it is input-rate limited), leading to a significant Cmax change. This suggests that the Cmax measure with a two-compartment body disposition, may be sensitive to *in vitro* 'k_d' if 'k_a' is constrained by 'k_d' in relation to the distribution hybrid rate constant 'α'. Also, based on the results, it could be concluded that phenylbutazone, whose two-compartment disposition is unique with a small distribution volume, may be a possible candidate for *in vitro in vivo* correlation. Since the Cmax/AUCt ratio, which is independent of the amount of the drug absorbed, and reflects the quotient of absorption and elimination rate constants, also failed the two one-sided test criterion, this implies that the observed Cmax changes may be predominantly due to *in vivo* absorption rate changes secondary to changes in the *in vivo* input rate, rather than changes in the extent of input and absorption. For tamoxifen, on the other hand, the passing of Cmax/AUCt and failure of Cmax measure in two trials, suggests that the Cmax failure may be due to the reduced extent of drug input and absorption, affected by the altered dissolution rate, (i.e., reduced fraction of the dose absorbed up to Cmax), rather than the altered rate of absorption. Though, it is not the intent of the paper to delve in to the controversial (23–24) issue of secondary metrics of bioequivalence, based on the confidence intervals, the Cmax/AUCt measure appears specific to 'k_a'. In bioequivalence testing however, since we invariably cannot separate 'k_a' from 'F', the Cmax measure, which reflects both 'rate' as well as 'extent' of absorption, is also useful.

ACKNOWLEDGMENTS

The authors would like to acknowledge the following FDA and outside experts for their valuable suggestions: 1) Dr. Roger L. Williams, 2) Dr. Andre Jackson, 3) Dr. Moo Park, 4) Mr. Donald J. Schuirman, and 5) Dr. Thomas N. Tozer. The authors also acknowledge the reviewers for excellent comments and suggestions.

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