Without Extrapolation, C_{max}/AUC is an Effective Metric in Investigations of Bioequivalence

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INTRODUCTION

Bois et al. (1,2) recently compared, extensively and in detail, performances of several metrics which can be applied for evaluating relative rates and extents of drug absorption in investigations of bioequivalence. The consequences were studied for single administrations of drugs which followed linear kinetics.

Bois et al. (1) found that the area under the curve (AUC) contrasting plasma concentration with time was the most reliable metric for evaluating the extent of absorption provided that it was measured until the last quantifiable concentration (lqc) without extrapolation. The maximum concentration (C_{max}) was similarly reliable; however, it was sensitive also to rate.

A clear conclusion preferring a metric as an index of comparative absorption rates was not reached (2). C_{max} was found to be insensitive. In addition, the metric was also nonspecific (4) to the assessment of absorption rates since it reflected also the extent of absorption (as well as the rates of disposition processes).

C_{max}/AUC was recommended (5) as a metric of enhanced specificity since it was independent of the extent of absorption. This feature was confirmed in subsequent studies (6,7). Moreover, C_{max}/AUC was demonstrated to have smaller variation than C_{max} itself (7,8).

However, Bois et al. (2) found that, using AUC extrapolated to a time of infinity (AUC_{inf}), C_{max}/AUC_{inf} was, with various scenarios involving two-compartmental models, sensitive to measurement errors, and thereby yielded high producer risks. On the other hand, AUC_{inf} exhibited similarly poor behaviour as a metric assessing the extent of absorption (1). By contrast, AUC measured until the last quantifiable concentration (AUC_{lqc}) was found to be a satisfactory metric for this purpose (1).

Therefore, the present communication aims to evaluate whether C_{max}/AUC_{lqc} shares the deficiency of C_{max}/AUC_{inf} as an effective metric evaluating absorption rates. It will be further explored whether the effectiveness of the assessment could be maintained if the observations are terminated earlier, i.e., only partial AUCs are measured. This approach would be in line with recent suggestions of Midha et al. (9).

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METHODS

Simulation of Bioequivalence Trials

In order to obtain a comparable baseline, the procedures of Bois et al. (1,2) were generally followed. Therefore, only deviations from their methodology and some of the principal features of the procedures will be presented.

Monte Carlo simulations of 2-way crossover trials were performed in order to evaluate the features of 4 metrics assessing the equivalence of absorption rates. Under all conditions, 300 trials were simulated with 24 subjects in each. Comparison of the results from 3 batches of 100 simulations showed close similarity, while the results of 100, 200 and 300 simulations demonstrated satisfactory convergence; in addition, contrasts with the results of Bois et al. (1,2), whenever available, suggested reasonable agreement. The subjects were randomly allocated to the two sequences of drug administration.

Either one- or two-compartmental distribution kinetics was assumed in the various simulations. The mean model parameters, listed in Table I, characterized typical features of the drug in the population. With two compartments, two sets of parameters were considered which differed in the ratio of rate constants (k_{21}/k_{10} of either 2.5 or 0.4) for release from the peripheral compartment (k_{21}) and elimination from the central compartment (k_{10} = CL/V, the ratio of clearance and apparent volume of distribution).

Except when otherwise indicated, simulated measurements were obtained at times of 0, 0.25, 0.5, 1, 1.5 and 2 hr following drug administration, and every 2 hours thereafter up to 16 hr. The observational (assay) error had a coefficient of variation (CV) of 10%, with truncation at ±3CV, in addition to a constant limit of quantitation (LQ). LQ was generally 1% of the theoretical maximum concentration of the reference drug product, identically for all subjects in a simulation.

Pharmacokinetic Models and Experimental Conditions

Following again Bois et al. (1,2) several conditions were considered: The *baseline conditions* were discussed above. The population means of the parameters and their inter- and intraindividual variations are presented in Table I. Arbitrarily, an oral dose of 500 mg was assumed.

For the condition of *low sensitivity* LQ was set at 10% (instead of 1%) of the theoretical, maximum concentration of the reference drug product. Consequently, concentrations could be measured only for a short time.

With the model assuming *zero-order absorption* (instead of a first-order process), the infusion time (the duration of the input) was fixed at $2/k_a$, where k_a = 1.39 hr^{-1} was the equivalent first-order absorption rate constant (Table I). The coefficient of variation of the infusion time was identical to that of k_a at the baseline condition.

For the model including a random *lag time*, a population mean of 1 hr was assumed. The lag time was considered to

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Table I. Population Means and Variations of Pharmacokinetic Parameters in the Simulations

Parameter[a]	Population mean[b]		CV (%)	
	1-compt.[d]	2-compt.[d]	Inter[e]	Intra[e]
Volume of distribution, V (L/kg)	1	1	10	10
Clearance, CL (L/(hrxkg))	0.347	0.50	20	20
Absorption rate constant, k_a (hr^{-1})	1.39	2.0	20	20
Bioavailability, F	0.5	0.5	11.5[c]	5.8[c]
Central to peripheral distribution rate constant, k_{12} (hr^{-1})	—	0.2 (Model I) 1.25 (Model II)	20	20
Peripheral to central distribution rate constant, k_{21} (hr^{-1})	—	0.05 (Model I) 0.3125 (Model II)	20	20

[a] Bioavailability was assumed to follow a uniform distribution. The other parameters were simulated by normal distributions which were truncated at ±3 standard deviations.
[b] The means for intraindividual distributions were the previously sampled values of the parameters for a given individual.
[c] The given CVs limited the sampled bioavailabilities to the mean ± 0.1.
[d] 1- and 2-compartment models.
[e] Inter- and intraindividual variation.

follow a normal distribution with a CV of 50%. The distribution was truncated at ±2CV.

Under the "flip-flop" condition of *low absorption/elimination ratio*, the ratio of the corresponding rate constants was 0.25 (instead of 4), i.e., $k_a = 0.0867\ hr^{-1}$. The simulated sampling times were: 0, 1, 2, 4, 6, 8, 12, 16, 20, 24, 32, 40 and 48 hours.

In the condition of *low bioavailability*, corresponding to high first-pass elimination, the extent of absorption (F) was assumed to follow a uniform distribution with a population mean of 0.1 (instead of 0.5) with a range of ±0.05 (instead of ±0.1).

Following again Bois et al. (1,2), two models were considered with *two-compartment distribution kinetics* (Table I). The models assumed first-order absorption into and elimination from the central compartment. With both models, the ratio of rate constants for uptake to and release from the peripheral compartment was $k_{12}/k_{21} = 4$ and $k_{10} = CL/V = 0.34\ hr^{-1}$. For both models, two levels of analytical sensitivity were considered: LQ was set at either 1 or 10% of the theoretical maximum concentration of the reference drug product.

Metrics Assessing Absorption Rates

Most of the metrics investigated by Bois et al. (2) were not reanalysed. Only C_{max} and C_{max}/AUC_{inf} were retained in order to provide a baseline for comparisons. C_{max} was the largest simulated concentration for a drug product. AUC was calculated by the trapezoidal rule within the range of available measurements. Extrapolation to the time of infinity was based on the slope fitted by linear regression to the last 4 logarithmic concentrations provided that the last reading exceeded LQ.

AUC_{lqc} was evaluated within the range of observations. A concentration was regarded to be zero if its value was less than LQ. AUC_{10} was measured for up to 10 hours (or up to 24 hours with the "flip-flop" condition); the choice of 10 hours, about half way between T_{max} and the duration of the experiments, was arbitrary. AUC_{lqc} and AUC_{10} were then substituted to calculate the ratio-metric C_{max}/AUC.

Power Curves

The simulations were extended toward a wider range of differences between the absorption rate constants of the two drug products. The intention was to observe the proportion of simulated crossover trials in which equivalence of the investigated metric is declared. Power curves present the relationship between the probability of accepting equivalence and the increasing difference between contrasted kinetic features, the absorption rate constants. In computer simulations, the fraction of trials signalling equivalence estimates the probability.

The assessments of bioequivalence applied the principle of the two one-sided tests procedure (10). In its implementation, for a statement of bioequivalence, the 90% confidence limits of logarithmically calculated individual ratios of metrics were expected to be between log 0.80 and log 1.25. This corresponded to the internationally harmonized criterion (3) for the equivalence of AUCs. The procedure also reflected the expectation of FDA for stating the equivalence of C_{max} values.

Ideally, when metrics for the contrasted drug products differ by less than the regulatory expectation (e.g., 25% difference in C_{max}) then the power curves should indicate their equivalence, whereas at large differences inequivalence should be declared. In the presence of errors and variations, the distinctions are less decisive. At least, it is then expected that at the preset (e.g. 25%) difference in the metric, only a small, e.g. 5% of the trials would indicate equivalence; this is the risk of the consumer, the patient. On the other hand, 100% of the trials should signal equivalence when the metrics for the contrasted formulations are in fact identical. A lower observed percentage reflects the risk of the producer for observing the inequivalence of the formulations when they are truly equivalent.

RESULTS

One-Compartment Model

Figure 1 presents power curves obtained under 6 conditions. The curves recorded under the baseline condition (Fig. 1A) confirm earlier observations (2,4–7) that C_{max} as well as C_{max}/AUC reflect insensitively the absorption rate constant: with the simulated measurement errors and parameter variations, about 50–150% difference in k_a was required to elicit a 25% difference in the metrics.

The relationship among power curves obtained for the 4 investigated parameters remained always the same. C_{max} yielded the least powerful decision on the acceptance of bioequivalence. In particular, when the two products were truly equivalent and the ratio of absorption rate constants was 1.0 then C_{max} yielded the smallest proportion of actually accepting bioequivalence and, therefore, the highest producer risk.

C_{max}/AUC_{inf}, applying extrapolation to a time of infinity, was consistently more powerful than C_{max}. Except under the condition of low bioavailability when C_{max} exhibited very low sensitivity (see below; Fig. 1E), the consumer risks yielded by C_{max}/AUC_{inf} and C_{max} were very similar.

The power curves for C_{max} and C_{max}/AUC_{inf} observed in this study were generally similar to those presented by Bois et al. (2). The rightward shift of the curve with zero-order absorption (Fig. 1F) is less pronounced in the present investigation than in their work; however, the impression of the shift in their study is based on a single, far-removed condition. Bois et al. (2) did not present power curves for the conditions of low assay sensitivity and low absorption.

This feature was shared also by C_{max}/AUC_{lqc}: the consumer risks (indicated by the k_a-ratio at which 5% of the trials were accepted) were very similar to those yielded by C_{max}/AUC_{inf} and, with the exception already noted, C_{max}. However, C_{max}/AUC_{lqc} showed higher power at the smaller k_a-ratios and generally a somewhat smaller producer risk than C_{max}/AUC_{inf}.

The power curve of C_{max}/AUC_{10} was in all cases to the right of the other curves. As a result, this metric exhibited lower producer and higher consumer risk than the others.

The power curves obtained under conditions of low sensitivity (Fig. 1B) and random lag time (Fig. 1D) were similar to those seen with the baseline condition (Fig. 1A). With low bioavailability (Fig. 1E), the power curves for the ratio metrics were similar to those obtained in the baseline condition. However, C_{max} itself showed very low sensitivity. The curves obtained with the "flip-flop" condition (Fig. 1C) demonstrated the acceptance of equivalence at smaller k_a-ratios, and those recorded with zero-order absorption (Fig. 1F) at higher k_a-ratios than the curves obtained under the baseline condition.

Two-Compartment Models

Figure 2 illustrates power curves with 2-compartmental models under 4 conditions. The curves for C_{max} and C_{max}/AUC_{inf} essentially agreed with those presented by Bois et al. (2).

The curves for C_{max} were reasonably independent of the analytical sensitivity, i.e. the level of LQ (contrasts of Fig. 2A with 2B, and Fig. 2C with 2D). The curves with Model II were shifted to the left, and yielded higher producer risks, than those recorded with Model I.

C_{max}/AUC_{inf} showed, at 3 of the 4 conditions, very low sensitivity for determining the equivalence of absorption rates. The observed producer risks were even somewhat higher than those reported by Bois et al. (2).

In contrast, C_{max}/AUC_{lqc} exhibited, at low assay sensitivity (Fig. 2B and 2D), similar power to that shown by C_{max}. At high assay sensitivity (Figs. 2A and 2C), C_{max}/AUC_{lqc} was actually more powerful than C_{max}, and yielded smaller producer risk. The consumer risk was in all cases similar for the two metrics.

The curves for C_{max}/AUC_{10} were close to those characterizing C_{max}/AUC_{lqc} but were generally shifted to the right.

DISCUSSION

The principal conclusion of the present study is that the effectiveness of C_{max}/AUC for assessing comparative absorption rates is at least high as that of C_{max} provided that partial and not extrapolated AUC is used in the calculations.

Thus, the reservations of Bois et al. (2) about the effectiveness of the ratio C_{max}/AUC as a metric assessing comparative absorption rates, for drugs exhibiting two-compartmental kinetics, are limited to the case when the extrapolated AUC_{inf} is used in the computations. In fact, the ratio metric becomes generally more powerful than C_{max} when AUC_{lqc} is applied in the calculations. Moreover, C_{max}/AUC_{lqc} has consistently smaller variation than C_{max}.

The bias and precision of the 4 metrics were evaluated. The metrics were, under all conditions, unbiased. The standard deviations of C_{max}/AUC_{lqc} were always, and those of C_{max}/AUC_{10} almost always, lower than the observed variations of both C_{max} and C_{max}/AUC_{inf}.

The effectiveness of C_{max}/AUC_{lqc}, as a metric evaluating absorption rates, is satisfying but not unexpected. It mirrors the characteristics of AUC_{lqc} as a metric for the extent of absorption especially when contrasted for two-compartmental models with the insensitivity of the extrapolated AUC_{inf} (1).

The performance of C_{max}/AUC_{10} is interesting and in some ways promising. The results of the simulations parallel the experimental evidence recently obtained by Midha et al. (9). They found that partial AUCs were effective metrics for evaluating the extent of absorption and, placed within the ratio C_{max}/AUC, also for the rate of absorption. Midha et al. (9) noted that limiting the duration of bioequivalence trials would save substantial effort and would enable the more precise estimation of C_{max}. The present results and those of Midha et al. (9) suggest that further explorations of the application of partial AUCs will be valuable.

It could be useful to restate the merits and limitations of the ratio metric C_{max}/AUC. The most important advantage of C_{max}/AUC (in any of its representations) is that it is independent of the extent of absorption (5–7). In contrast, C_{max} itself fully reflects, along with all measured concentrations, the extent of absorption (1,5) and is thereby a very nonspecific metric for the assessment of absorption rates (4–7,11). In fact, Bois et al. (1), based on their simulations, found C_{max} to be one of the most reliable metrics for this purpose.

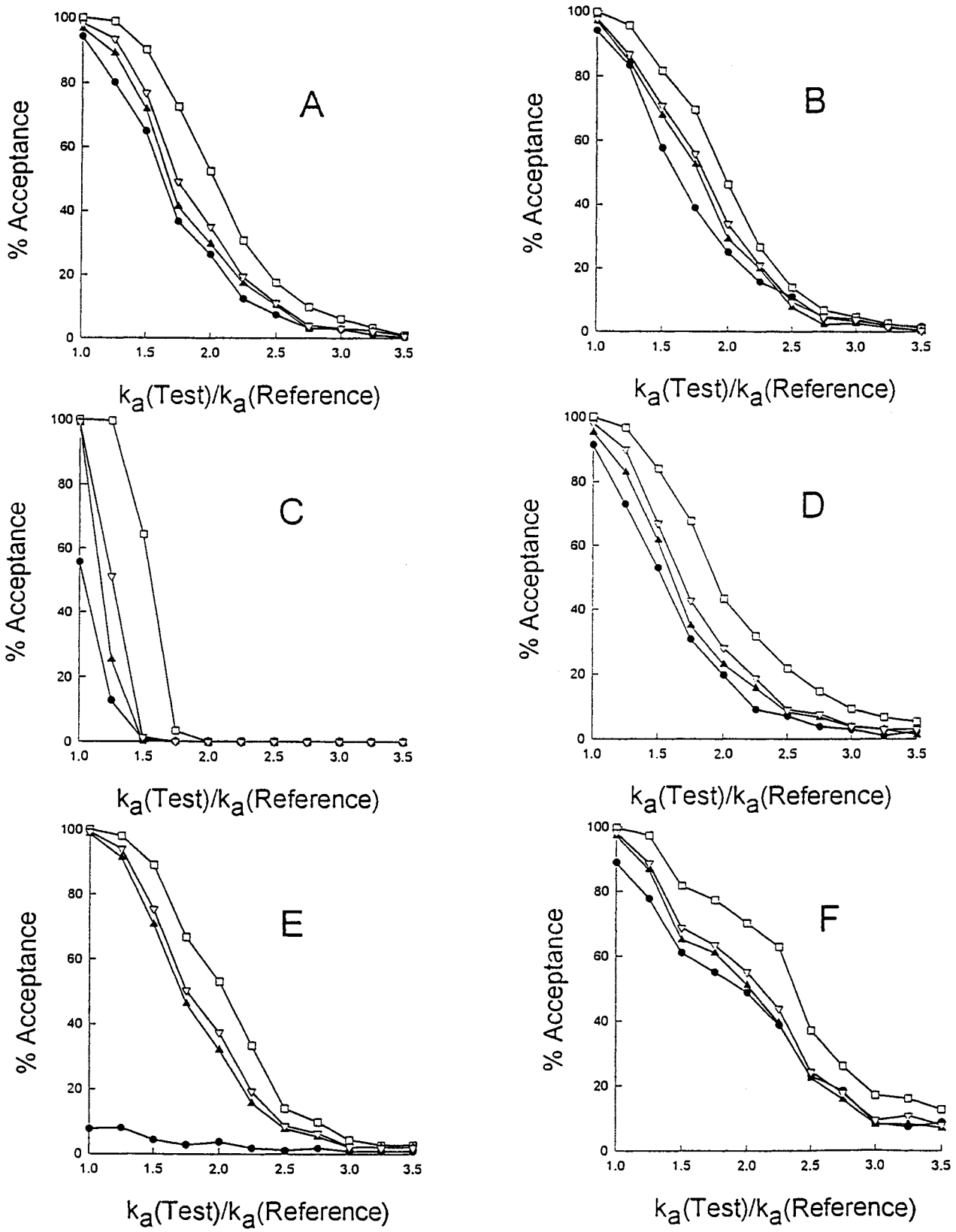

Fig. 1. Power curves for determining the equivalence of absorption rates by 4 metrics in the presence of 6 one-compartmental model conditions. The vertical axis shows the percentage of simulated crossover trials in which the equivalence of a metric by the two one-sided tests procedure was accepted. The horizontal axis displays the true ratio of absorption rate constants of the two formulations. Filled circles: C_{max}; filled triangles: C_{max}/AUC_{inf}; open triangles: C_{max}/AUC_{lqc}; open squares: C_{max}/AUC_{10}. A: Baseline condition; B: low sensitivity, LQ = 0.1 $C_{max,R}$; C: "flip-flop" condition, low absorption/elimination ratio of rate constants; D: random lag time; E: low bioavailability, F = 0.1; F: zero-order absorption.

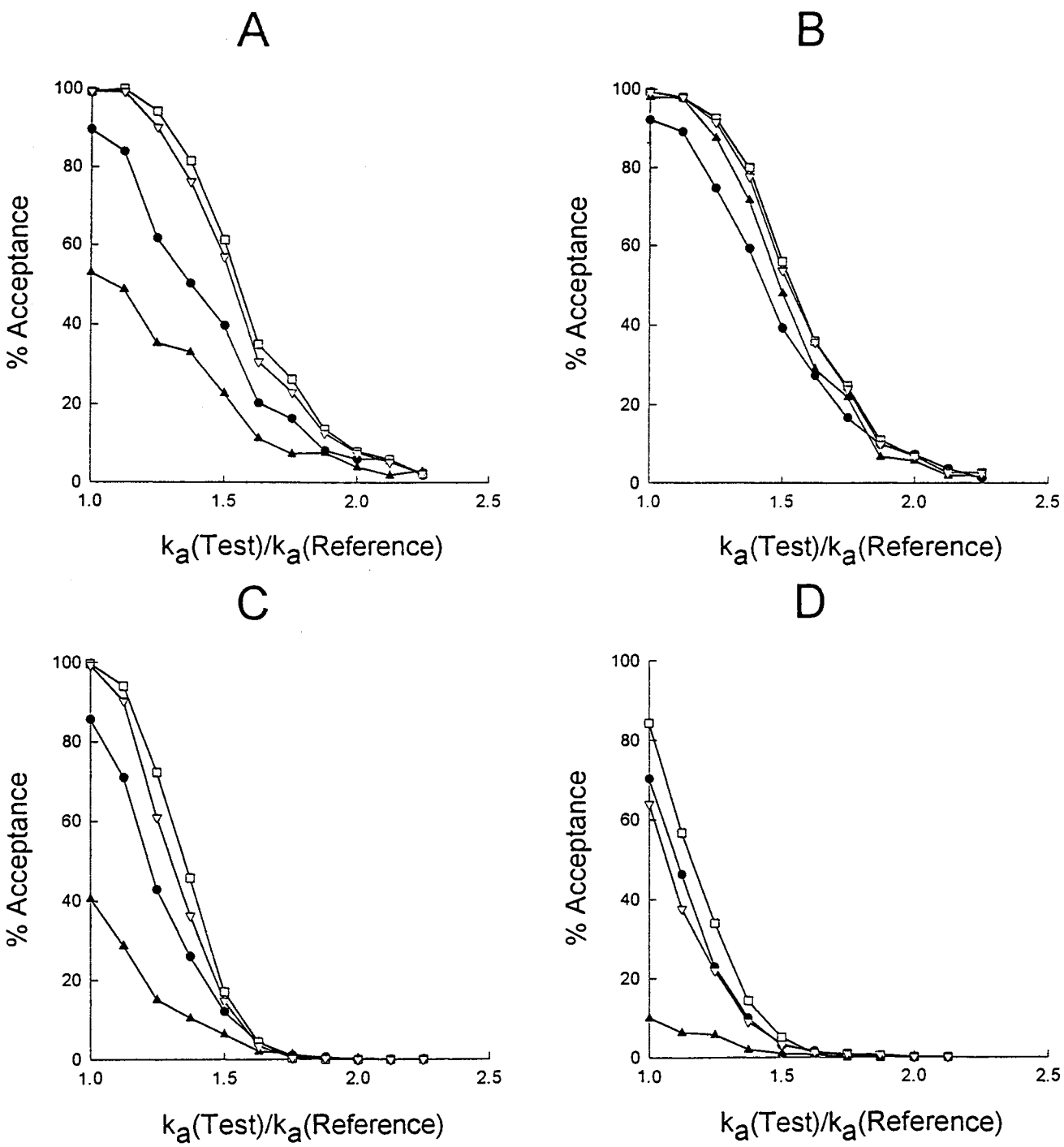

Fig. 2. Power curves for determining the equivalence of absorption rates by 4 metrics in the presence of 4 two-compartmental conditions. The vertical axis shows the percentage of simulated crossover trials in which the equivalence of a metric by the two one-sided tests was accepted. The horizontal axis displays the true ratio of absorption rate constants of the two formulations. Filled circles: C_{max}; filled triangles: C_{max}/AUC_{inf}; open triangles: C_{max}/AUC_{1qc}; open squares: C_{max}/AUC_{10}; A: Model I (large ratio of elimination/distribution rate constants), high assay sensitivity (LQ = 0.01 $C_{max,R}$); B: Model I, low assay sensitivity (LQ = 0.1 $C_{max,R}$); C: Model II (small ratio of elimination/distribution rate constants), high assay sensitivity; D: Model II, low assay sensitivity.

As a secondary advantage, the ratio metric has smaller variation than C_{max} (7,8). As demonstrated by the results presented in this communication and also those of Midha et al. (9), the insertion of partial AUCs at least maintains this property.

C_{max}/AUC shares some of the deficiencies of C_{max} as a metric for absorption rates, even if some of the disadvantages are reduced. For example, while C_{max}/AUC is not affected by differences and variations in the extent of absorption, as does C_{max}, it still reflects effects of rates of disposition processes. The kinetic sensitivity of the ratio metric is still low. The variation of C_{max}/AUC is generally lower than

that of C_{max}, especially when partial AUCs are utilized, but higher than that of AUC (7,8).

Altogether, however, the ratio metric C_{max}/AUC characterizes comparative absorption rates much more effectively than does C_{max}, provided that AUC measured until the last quantifiable concentration (AUC_{lqc}) is applied in the computations.

CONCLUSIONS

There is increasing evidence both from simulations (2,5–7) and the analysis of crossover trials (7,9) that the ratio metric C_{max}/AUC should be strongly preferred to C_{max} for the assessment of comparative absorption rates following the administration of single oral doses. The ratio metric does not reflect differences between extents of absorption and is, consequently, more specific than C_{max} (4,5). Moreover, the ratio metric tends to have smaller variation and higher power for the regulatory decision on bioequivalence than C_{max} (7–9).

Results of the present communication strongly confirm the latter statement provided that the extrapolated AUC_{inf} is not used in the calculations. Instead, the AUC applied in the computations should be limited to the range of actual measurements. Observations obtained until the last quantifiable concentration yield a ratio metric, C_{max}/AUC_{lqc}, which is generally superior to both C_{max} and C_{max}/AUC_{inf} for assessing the equivalence of absorption rates. The use of partial areas in C_{max}/AUC is promising.

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