

# Truncated Area Under the Curve as a Measure of Relative Extent of Bioavailability: Evaluation Using Experimental Data and Monte Carlo Simulations

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**Purpose.** The use of truncated areas under the curve (AUCs) could be a significant advantage for bioequivalence studies of drugs with long half-lives. The purpose of this study was to evaluate the performance of truncated AUCs as measures of relative extent of bioavailability using a large database of experimental data and Monte Carlo simulations.

**Methods.** The experimental data consisted of 123 single-dose, 2-treatment, crossover studies with at least 18 subjects/study. Monte Carlo techniques were also used to simulate studies that reflected a wide variety of experimental conditions. AUCs were calculated over different time intervals and the standard two one-sided t tests procedure was used to assess bioequivalence.

**Results.** The experimental data showed that conclusions concerning bioequivalence were identical between AUCs truncated at four times the time of peak concentration ( $T_{max}$ ) and AUCs extrapolated to infinity ( $AUC_{inf}$ ) in 120/123 or 97.6% of studies. There was little change in the intra-subject CVs for AUCs truncated at  $3 \cdot T_{max}$  or later. The results of Monte Carlo simulations were generally consistent with the experimental data and showed that AUCs truncated at 72 hours ( $AUC_{0-72}$ ) performed well compared to  $AUC_{inf}$  as measures of bioequivalence for drugs with long half-lives.

**Conclusions.** Based on both the experimental and simulated data, AUCs truncated after the absorption phase perform well as measures of relative extent of bioavailability. Truncated AUCs offer a particular advantage for drugs with long half-lives and these results indicate that it would be reasonable to limit the sample collection period to 72 hours in bioequivalence studies of oral formulations.

**KEY WORDS:** bioequivalence; partial AUCs; truncated AUCs; long half-life; Monte Carlo simulations.

## INTRODUCTION

Bioequivalence of two formulations of a drug with systemic effects is demonstrated when the plasma (or other biological fluid) concentration-time profiles are sufficiently similar. This assessment is currently based on the observed maximum concentration ( $C_{max}$ ), the area under the concentration curve (AUC) from time 0 to the last quantifiable concentration ( $AUC_{lqc}$ ) and the AUC extrapolated to infinity ( $AUC_{inf}$ ). Based on the analysis of log-transformed parameters and the two one-sided t tests procedure, the 90% confidence intervals for the

test (T) to reference (R) ratio of geometric means must be within 80% to 125% in order to meet the current U.S. Food and Drug Administration requirements for average bioequivalence (1).

Generally for single-dose bioequivalence studies, plasma drug concentrations will be determined for at least 2–3 terminal half-lives, or until the  $AUC_{lqc}$  represents 80% or more of the  $AUC_{inf}$ . For drugs with long half-lives such as hydroxychloroquine ( $t_{1/2} > 50$  days), this requires collection of samples for several months; however, it is questionable if collection of samples over such a long period of time is necessary to properly evaluate the relative extent of bioavailability. If differences in bioavailability exist between two oral formulations, they have to become evident while the drug is being released from the dosage form and absorbed. Once absorption is completed, drug disposition should be independent of formulation. Long sample collection periods increase the risk of noncompliance with protocol restrictions by the subjects, increase the likelihood of observing changes in drug disposition over time, increase the cost of studies and increase the risks associated with repeated venipunctures and blood loss. The lower drug concentrations that are expected late after the dose also necessitate the development of more sensitive analytical methods which are often less precise.

The present investigation was undertaken to evaluate the impact of using AUCs truncated at various times using a large database of clinical bioequivalence studies as well as data generated by Monte Carlo simulations.

## MATERIALS AND METHODS

### Experimental Data

Bioequivalence studies performed at Phoenix International and which met the following criteria were included: randomized, crossover, two-treatment, two-period, single-dose studies with at least 18 healthy subjects/study. All studies were approved by an institutional review board and written informed consent was obtained from all subjects. For each study, AUCs estimated using the linear trapezoidal rule were calculated at each sampling time up to  $AUC_{lqc}$ . If drug concentrations were below the lower limit of quantitation (LOQ) at a particular time before the last sampling time, the truncated AUC value was taken as the AUC calculated up to the previous time when the concentration was above the LOQ.  $AUC_{inf}$  was calculated by adding  $AUC_{lqc}$  to the ratio of the last measured concentration and the terminal rate constant ( $K_e$ ).  $K_e$  was determined by linear least-squares regression from the terminal log-linear portion of the concentration-time data.

Analysis of variance (ANOVA) was performed using the natural logarithm ( $\ln$ ) of the parameters. The ANOVA model included factors for sequence, subjects nested within sequence, treatment and period; the ratio of geometric mean AUC and its 90% confidence interval was calculated using the least squares means and the mean square error (MSE) obtained from the ANOVA (1). The number of studies that met the usual 80–125% bioequivalence criterion for AUCs truncated at different times,  $AUC_{lqc}$  and  $AUC_{inf}$  was determined. Concordance results were tabulated to demonstrate agreement/disagreement in conclusions for the different AUC parameters. Time was expressed

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in terms of multiples of Tmax for the reference product to facilitate the comparison between studies with different drugs.

In order to provide a reasonable basis for the variability measures that should be used in Monte Carlo simulations (see below), inter- and intra-subject variances in pharmacokinetic parameters were calculated from the ANOVA. For each study, inter-subject variance was calculated as  $[MS_{\text{subject}}(\text{sequence}) - MSE]/2$ , where  $MS_{\text{subject}}(\text{sequence})$  is the mean square for subjects nested within sequence and MSE was defined previously. MSE was used as the estimate of intra-subject variance. Since they are easier to interpret, coefficients of inter- and intra-subject variation (CVs) for the untransformed parameter are reported and were calculated as follows:

$$CV = \sqrt{e^{\sigma^2} - 10}$$

where  $\sigma^2$  is the inter- or intra-subject variance for the In-transformed parameter (2).

### Simulated Data

Monte Carlo (stochastic) simulations were designed as two-treatment, two-period, two-sequence crossover, single-dose studies with 24 subjects. For each scenario described below, 1000 studies were simulated.

#### Baseline Scenario

A standard 1-compartment model with first-order absorption and elimination was used to simulate the concentration-time data, using the following equation:

$$C(t) = \frac{A \cdot Ka}{Ka - Ke} \cdot (e^{-Ke \cdot t} - e^{-Ka \cdot t})$$

where  $C(t)$  is the drug concentration at time  $t$ ,  $A$  is a scale parameter whose value does not alter the shape of the concentration-time profile,  $Ka$  is the first-order absorption rate constant and  $Ke$  is defined above. It should be noted that  $A = F \cdot D/V$ , where  $F$  is the bioavailability,  $D$  is the dose and  $V$  is the volume of distribution. Since AUC truncation would be particularly important for drugs with relatively long half-lives, the mean pharmacokinetic parameters were selected to produce a concentration-time profile for a drug with a terminal half-life of approximately 100 hr and a maximum concentration at approximately 3 hr. The parameter values used for the simulations are summarized in Table I (the units for  $A$  are arbitrary and were set to mcg/ml).

The parameters  $A$ ,  $Ke$  and  $Ka$  were assumed to follow a lognormal distribution (3). In order to simulate studies that reflect the typical variability observed with experimental data, the simulations were designed to achieve inter- and intra-subject CVs for AUC,  $Ke$  and  $C_{\text{max}}$  equal to the median values observed in the studies described above (Experimental Data). If more than one study was conducted for a given drug, the median CVs for that drug were used in the calculation of the overall median CVs for all studies, in order to avoid putting more weight on a particular drug. Since  $A$  is not directly estimated by noncompartmental analysis, its value was calculated as  $AUC_{\text{inf}} \cdot Ke$ , based on the relationship  $AUC_{\text{inf}} = F \cdot D/CL$  and  $Ke = CL/V$ . Because  $A$  and  $Ke$  are both dependent on  $V$ , there is an expected covariance between these parameters. An estimate of the covariance was obtained from the following equation:

$$\sigma_{\ln AUC_{\text{inf}}}^2 = \sigma_{\ln A}^2 + \sigma_{\ln Ke}^2 - 2 \cdot \text{Cov}(\ln A, \ln Ke)$$

where  $\sigma_{\ln AUC_{\text{inf}}}^2$ ,  $\sigma_{\ln A}^2$  and  $\sigma_{\ln Ke}^2$  were calculated from the experimental data. It should be noted that if the covariance was not taken into account, it was impossible to obtain simulated data with variances for  $\ln A$ ,  $\ln Ke$  and  $\ln AUC_{\text{inf}}$  that were consistent with the experimental data. The covariance was introduced in the simulations by using a bivariate normal distribution for  $\ln A$  and  $\ln Ke$ .

Inter-subject variability in pharmacokinetic parameters ( $A$ ,  $Ke$ ,  $Ka$ ) was first introduced by simulating a given parameter for a particular subject (e.g.,  $Ke_i$ ) from a lognormal distribution with mean  $\mu_{Ke}$  and coefficient of variation  $CV_{Ke\text{-inter}}$ . Intra-subject variability was next introduced by simulating independently the parameters for the two periods for that subject,  $Ke_{i1}$  and  $Ke_{i2}$ , from the lognormal distribution with mean  $Ke_i$  and coefficient of variation  $CV_{Ke\text{-intra}}$ . After repeating the above procedure to also simulate  $A$  and  $Ka$ , the predicted  $C(t)$  for a particular subject and for each study period were calculated using the above equation for the 1-compartment model. The sampling times were 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24, 48, 72, 96, 144, 216, 288 and 408 hr.

Differences between formulations were introduced by using a factor that ranged from 1.0–1.30 and which multiplied the value of  $A$  for the test formulation. This allowed the evaluation of different AUC metrics when there was a true difference in extent of availability that ranged from 0 to 30% between the two formulations.

Measurement or assay variability (which includes variability due to model misspecification as well as variability from the bioanalytical method) was added to each predicted  $C(t)$ . "Observed" concentrations were simulated using a lognormal distribution with a mean equal to  $C(t)$  and a CV that progressively increased from approximately 10% at higher concentrations to 20% at the LOQ, based on the following equation:

$$CV_{\text{assay}} = 0.1 \cdot \frac{(C(t) + \text{LOQ})}{C(t)}$$

where  $CV_{\text{assay}}$  is the measurement variability at a particular  $C(t)$ . The LOQ was set to 1/25 of the predicted mean  $C_{\text{max}}$  of the reference formulation. All concentrations below the LOQ were ignored in the data analysis.

#### Low Assay Sensitivity Scenario

The LOQ was increased to 1/10 (high LOQ) of the predicted mean  $C_{\text{max}}$  for the reference formulation.

#### Highly Variable Drug Scenarios

Additional simulations were performed to evaluate a typical drug with high intra-subject variability. Variance parameters used for simulations were based on the experimental data for studies with an intra-subject CV for  $AUC_{\text{iqc}}$  larger than 25%. The median CVs from these studies were used for simulations and can be found as Model I in Table I. Two additional methods for the simulation of a highly variable drug were also used, based on the approach used by other investigators. In each case, the variance of a different parameter was increased, compared to the value used for the baseline scenario, in order to achieve an intra-subject CV for  $AUC_{\text{inf}}$  of 27% (i.e. the median intra-

Table I. Parameters Used in the Simulations for the 1- and 2-Compartment Models

Parameter	Baseline scenario			Highly variable model I	
	Mean	Intra-subject CV (%)	Inter-subject CV (%)	Intra-subject CV (%)	Inter-subject CV (%)
1-Compartment Model					
Ka (hr <sup>-1</sup> )	2.0	20.0	20.0	20.0	20.0
Ke (hr <sup>-1</sup> )	0.0072	12.7	18.4	26.4	26.9
A (mcg/ml)	250	18.4	21.0	34.6	40.9
correlation (ln A, ln Ke)		0.65 <sup>a</sup>	0.23 <sup>a</sup>	0.61 <sup>a</sup>	0.16 <sup>a</sup>
2-Compartment Model					
Ka (hr <sup>-1</sup> )	2.0	20.0	20.0		
K10 (hr <sup>-1</sup> )	0.05	5.0	15.0		
K12 (hr <sup>-1</sup> )	0.02 (model IV) 0.125 (model V)	5.0	15.0		
K21 (hr <sup>-1</sup> )	0.005 (model IV) 0.03125 (model V)	5.0	15.0		
A (mcg/ml)	500	13.0	20.0		

<sup>a</sup> correlation coefficient.

subject CV observed for the different studies with highly variable drugs). One method (Model II), analogous to the approach used by Bois *et al.*, consisted of increasing the intra-subject CV for A to 29.7% (4). The second method (Model III) consisted of increasing the intra-subject CV for Ke to 26.3%, similar to the approach proposed by Endrenyi and Tothfalusi (5). The covariance between ln A and ln Ke was kept at the same value as the baseline scenario for both Models II and III. The number of subjects was increased to 48 for all simulations of highly variable drugs, in order to have adequate statistical power to meet the 80–125% bioequivalence criterion.

### 2-Compartment Model Scenarios

A 2-compartment model with first-order input and output from the central compartment was used. Two different models were evaluated to reflect drugs with different ratios of elimination/distribution, as described by Bois *et al.* (4). Model IV was characterized by an initial disposition half-life ( $t_{1/2\alpha}$ ) of 10 hr and a terminal disposition half-life ( $t_{1/2\beta}$ ) of 198 hr, while Model V was characterized by a  $t_{1/2\alpha}$  of 4 hr and a  $t_{1/2\beta}$  of 88 hr. The mean and variance parameters used for the simulations can be found in Table I. No covariance was introduced between A and the disposition rate constants since such relationships could not be easily estimated from the experimental data. Sampling times were selected based on the terminal half-life. Thus for Model IV, the sampling times were 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24, 48, 72, 96, 144, 240, 336, 456 and 576 hr while the sampling times for Model V were identical to those used for the 1-compartment model. For Model IV, simulations were done with LOQs of 1 and 10 mcg/ml, allowing quantitation of drug for approximately 3 and less than 1  $t_{1/2\beta}$ , respectively. For model V, quantitation of drug was possible for approximately 5 and 1  $t_{1/2\beta}$  for an LOQ of 1 and 10 mcg/ml, respectively.

### Data Analysis

The pharmacokinetic and statistical analyses were identical to those described above for the experimental data. Power

curves were constructed to report the proportion of the 1000 simulated studies that met the usual 80–125% bioequivalence criterion for each simulation scenario for the following AUC metrics:  $AUC_{0-8}$ ,  $AUC_{0-72}$ ,  $AUC_{lqc}$ , and  $AUC_{inf}$ , where  $AUC_{0-8}$  and  $AUC_{0-72}$  represent the AUC for 8 and 72 hours after drug administration, respectively. A desirable power curve would have a large fraction (ideally 100%) of studies meeting the bioequivalence criterion when the true T/R ratio of bioavailability is within 0.8–1.25 and a small fraction (ideally 0%) of studies meeting the same criterion when the true T/R ratio is outside 0.8–1.25. When the true T/R ratio equals 0.8 or 1.25, 5% of studies should meet the bioequivalence criterion. This is the so-called “consumer risk” set by regulatory agencies and which is a consequence of the 5% level of significance used in the two one-sided t tests.

## RESULTS

### Experimental Data

A total of 123 studies for 48 different drugs were included in the analysis. The median terminal half-life including all studies was 5.0 hr (range: 0.4 to 974 hr) and the median Tmax was 2.0 hr (range: 0.5 to 8 hr). The median intra-subject CVs were 18.4, 14.0 and 12.7%, for A,  $AUC_{inf}$  and Ke, respectively. The median inter-subject CVs were 21.0, 24.7 and 18.4%, for A,  $AUC_{inf}$  and Ke, respectively. A median correlation coefficient of 0.28 was observed between ln A and ln Ke providing further evidence of the need to include a covariance between these parameters in order to produce realistic simulations.

A subset of 25 studies for 11 different drugs was included for determination of the median parameters for a typical highly variable drug. The median intra-subject CVs were 34.6, 27.8 and 26.4%, for A,  $AUC_{inf}$  and Ke, respectively. The median inter-subject CVs were 40.9, 45.9 and 26.9% for A,  $AUC_{inf}$  and Ke, respectively. A median correlation coefficient of 0.27 was observed between ln A and ln Ke.

For most studies, the intra-subject CVs of truncated AUCs were higher at earlier time points and then decreased to reach

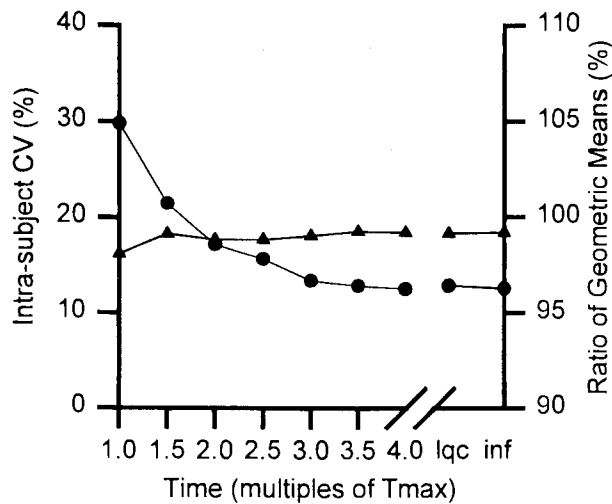


Fig. 1. Intra-subject CV (●) and ratio of geometric means (▲) for AUCs truncated at various times in terms of multiples of Tmax. The symbols represent the median of all studies.

a plateau a few hours after drug administration. This trend is evident from Figure 1 which depicts the median intra-subject CVs for all studies, as a function of multiples of Tmax. There was little change in the intra-subject CVs for AUCs truncated at 3\*Tmax and beyond. Similarly, there was little change in the T/R ratios of geometric means for AUCs truncated at Tmax and beyond (Figure 1). Figure 2 shows the intra-subject CVs of truncated AUCs as a function of time for two separate studies. One profile (desipramine) is typical of most drugs and the other (terfenadine) illustrates a trend for increasing CVs over time, as observed in a few studies.

Concordance and discordance in bioequivalence conclusions based on AUC<sub>inf</sub> and AUCs truncated at different times are shown in Figure 3. Different conclusions were reached in 58/123 (47%) studies at Tmax. All cases of discordance in conclusions at Tmax were from studies that met the bioequivalence criterion for AUC<sub>inf</sub> but were rejected for AUC<sub>1\*Tmax</sub>. It should be emphasized that these differences were largely due

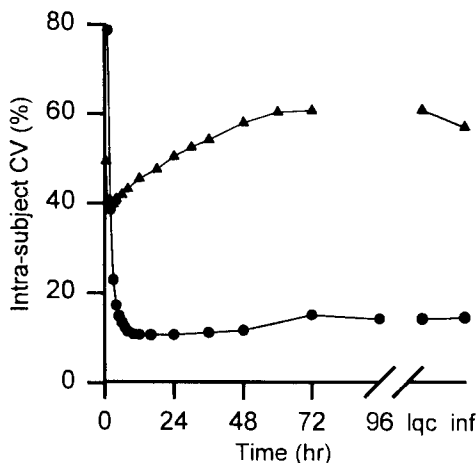


Fig. 2. Intra-subject CV for AUCs truncated at various times for two representative drugs: desipramine (●) and terfenadine (▲).

		AUC <sub>inf</sub>	
		Accept	Reject
AUC <sub>1*Tmax</sub>	Accept	53	0
	Reject	58	12

		AUC <sub>inf</sub>	
		Accept	Reject
AUC <sub>2*Tmax</sub>	Accept	94	1
	Reject	17	11

		AUC <sub>inf</sub>	
		Accept	Reject
AUC <sub>3*Tmax</sub>	Accept	104	1
	Reject	8	10

		AUC <sub>inf</sub>	
		Accept	Reject
AUC <sub>4*Tmax</sub>	Accept	109	1
	Reject	2	11

		AUC <sub>inf</sub>	
		Accept	Reject
AUC <sub>lqc</sub>	Accept	110	1
	Reject	1	11

Fig. 3. Concordance in bioequivalence conclusions between AUCs measured at selected times and AUC<sub>inf</sub>.

to the higher intra-subject CV for AUC<sub>1\*Tmax</sub> (Figure 1) which led to wider 90% confidence intervals that exceeded the bioequivalence criterion. At later times, concordance in conclusions increased considerably between truncated AUCs and AUC<sub>inf</sub> (e.g. 120/123 studies or 97.6% at 4\*Tmax). It is noteworthy that even conclusions based on AUC<sub>lqc</sub> and AUC<sub>inf</sub> differed for 2/123 (1.6%) studies, thus emphasizing that there is no perfect agreement even for AUC metrics that are measured over a long time period.

**Simulated Data**

*Baseline Scenario, 1-Compartment Model*

The 1000 simulated datasets (with inter-subject, intra-subject and assay variability) for the baseline scenario yielded a median C<sub>max</sub> of 267 mcg/ml, T<sub>max</sub> of 3 hr and half-life of 99 hr for the reference formulation. The median intra- and inter-subject CVs for AUC<sub>inf</sub> were 14.0 and 24.7%, respectively,

while the median intra- and inter-subject CVs were 19.0 and 20.1% for  $C_{max}$  and 15.2 and 17.3% for  $K_e$ , respectively. Figure 4A shows the concentration-time profile for the mean (true) parameters while Figure 4B presents the estimates of intra-subject CVs for AUCs truncated at each sampling time for the different 1-compartment model scenarios. For the baseline scenario, there was a rapid decrease in intra-subject CVs within the first 24 hours after the dose, followed by a long period when there was relatively little change in intra-subject CVs. When the LOQ was raised to 1/10 of  $C_{max}$ , the intra-subject CVs were generally similar to those of the baseline scenario, except that they tended to increase slightly during the last few observation times.

Figure 5 shows the probability (*i.e.*, power) of meeting the bioequivalence criterion as a function of the true T/R ratio of bioavailability for  $AUC_{0-8}$ ,  $AUC_{0-72}$ ,  $AUC_{lqc}$  and  $AUC_{inf}$  for both the baseline (A) and high LOQ (B) scenarios. For the baseline scenario, when products differed in extent of bioavail-

ability by  $\leq 5\%$  or  $\geq 20\%$ , the 4 AUC metrics performed similarly, despite the fact that  $AUC_{0-8}$  and  $AUC_{0-72}$  represented only 5 and 39% of  $AUC_{inf}$ , respectively. When differences in performance between truncated AUCs were slightly more evident (e.g., T/R ratios of 1.10 and 1.15), the ordering in terms of power was as follows:  $AUC_{inf} > AUC_{lqc} > AUC_{0-72} > AUC_{0-8}$ . This ordering was a direct consequence of the differences in intra-subject CVs (Figure 4B) since a less variable metric produces more narrow confidence intervals and thus higher probability of meeting the bioequivalence criterion. When the LOQ was raised to 1/10 of  $C_{max}$ , the ordering in terms of power was  $AUC_{inf} > AUC_{0-72} > AUC_{0-8} > AUC_{lqc}$ , again consistent with Figure 4B.

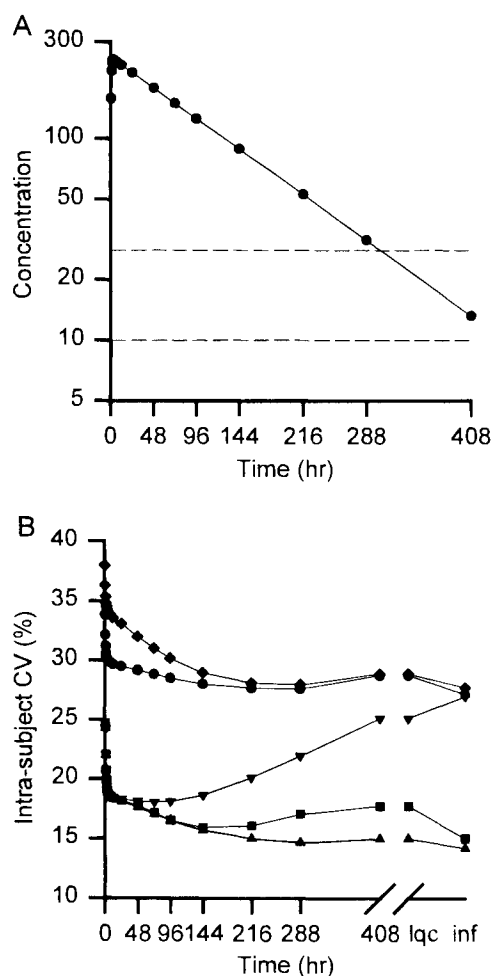
### Highly Variable Drug Scenarios

The highly variable drug Model I resulted in median intra- and inter-subject CVs for  $AUC_{inf}$  of 27.8 and 45.4%, respectively, while the median intra- and inter-subject CVs were 34.0 and 40.0% for  $C_{max}$  and 27.9 and 26.2% for  $K_e$ , respectively. The intra-subject CV *versus* time profiles are shown in Figure 4B for all three highly variable drug scenarios. Although there is an expected shift to higher CVs, the shape of the profiles for the highly variable drug Models I and II is generally similar to that of the baseline scenario (Figure 4B). Conversely, the intra-subject CVs for the highly variable drug Model III progressively increase as a function of time starting at approximately 24 hours. Figures 5C–5E illustrate how the differences in power between the AUC metrics vary depending on the conditions used in the simulations. Thus for the highly variable drug Model I (Figure 5C),  $AUC_{inf}$  had higher power followed by  $AUC_{lqc}$ ,  $AUC_{0-72}$  and  $AUC_{0-8}$ , whereas  $AUC_{inf}$  and  $AUC_{lqc}$  had lower power with Model III (Figure 5E). Finally Model II exhibited only small differences in power between the various AUC metrics (Figure 5D).

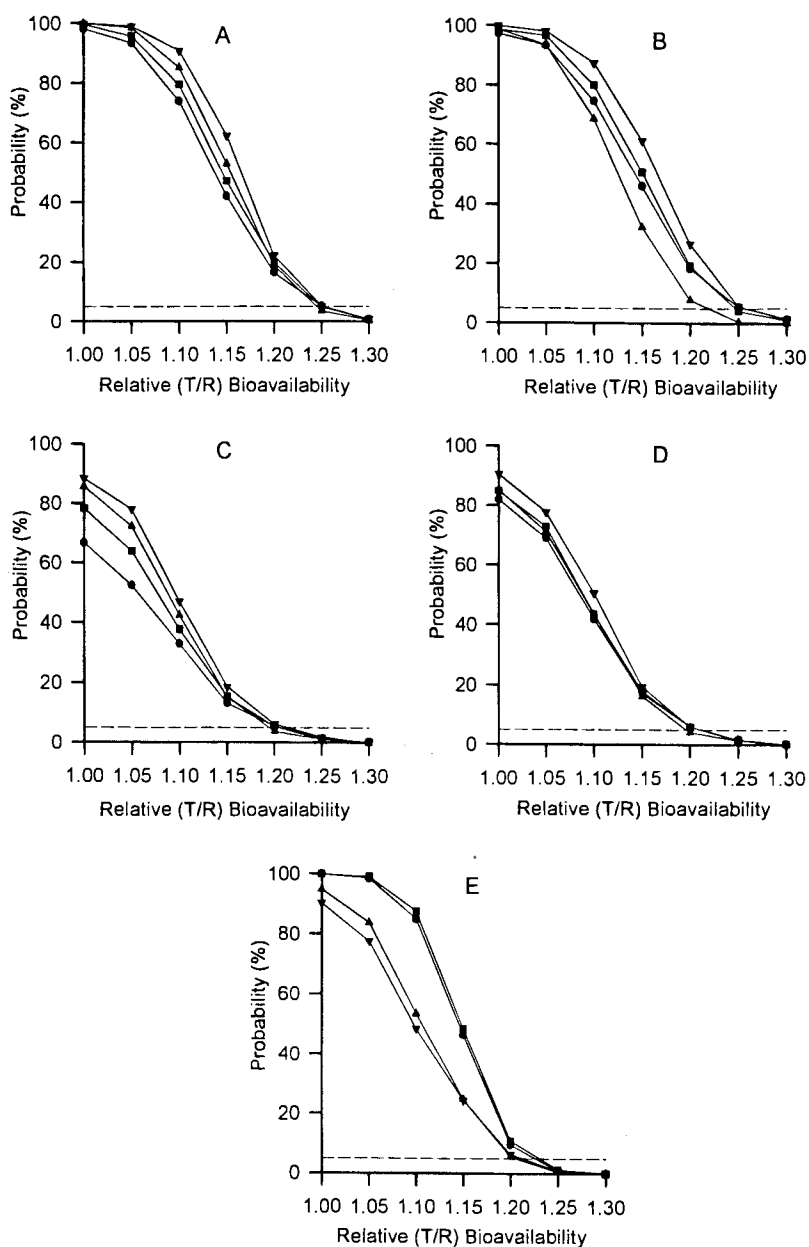
### 2-Compartment Model Scenarios

The 1000 simulated datasets using Model IV resulted in a median  $C_{max}$  of 480 mcg/ml,  $T_{max}$  of 2 hr and terminal half-life of 207 hr for the reference formulation. Model V resulted in a median  $C_{max}$  of 418 mcg/ml,  $T_{max}$  of 1.5 hr and terminal half-life of 92 hr. The concentration-time profiles for the mean (true) parameters are shown in Figure 6A while the intra-subject CVs for the various AUC metrics can be found in Figure 6B. The intra-subject CVs decreased initially after the dose, reached a minimum at approximately 6–8 hours and then increased over time. Figures 7A and B show the power curves for Models IV and V, respectively, while Figures 7C and D show the power curves for the same models but at the higher LOQ. As expected from the intra-subject CVs,  $AUC_{0-8}$  had the highest power followed by  $AUC_{0-72}$ , while  $AUC_{lqc}$  and  $AUC_{inf}$  had lower power.

The higher LOQ caused particular problems for Model IV. The  $t_{1/2\beta}$  was greatly underestimated (median of 13 hr compared to the true value of 198 hours) and was very variable (median intra-subject CV of 50% compared to 27% with the low LOQ) since the concentration data reflected a variable mixture of  $t_{1/2\alpha}$  and  $t_{1/2\beta}$ . Under such circumstances, the inter- and intra-subject variability of  $AUC_{inf}$  increased since a more variable  $t_{1/2\beta}$  was used in the extrapolation to infinity.  $AUC_{0-8}$



**Fig. 4.** (A) Concentration-time profile for the mean (true) parameters for the 1-compartment model. The dashed lines indicate the high and low LOQ. (B) intra-subject CVs for AUCs truncated at various times for the different 1-compartment model scenarios: baseline (▲), high LOQ (■), highly variable drug Model I (◆), highly variable drug Model II (●) and highly variable drug Model III (▼). The symbols for intra-subject CVs reflect the median of 1000 studies.



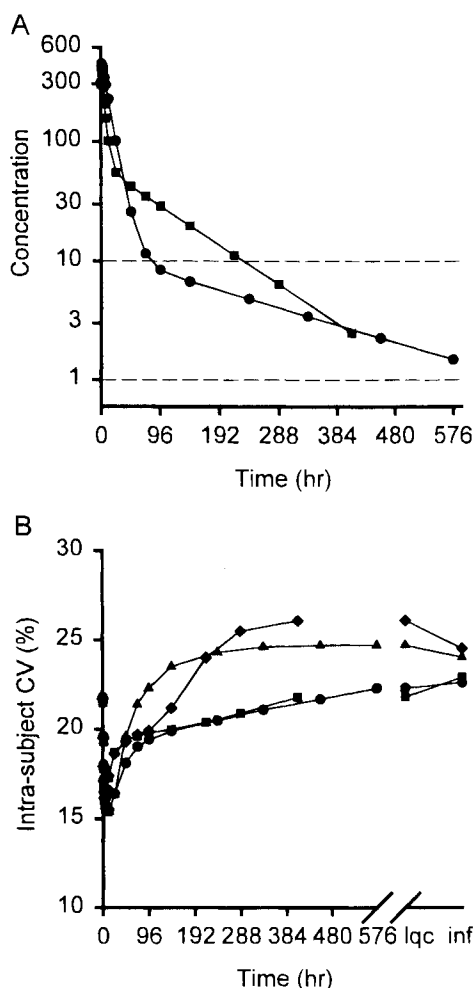
**Fig. 5.** Power curves showing the proportion of the 1000 simulated studies that met the 80–125% bioequivalence criterion as a function of the true T/R ratio of bioavailability. Results are reported for  $AUC_{inf}$  ( $\nabla$ ),  $AUC_{lqc}$  ( $\blacktriangle$ ),  $AUC_{0-72}$  ( $\blacksquare$ ) and  $AUC_{0-8}$  ( $\bullet$ ) for the different I-compartment model scenarios: (A) baseline, (B) high LOQ, (C) highly variable drug Model I, (D) highly variable drug Model II and (E) highly variable drug Model III. The dashed line represents a probability of 5%.

and  $AUC_{0-72}$  performed better even though the terminal disposition phase was often missed completely because of the higher LOQ. This is an important advantage of truncated AUCs because the LOQ is often reached before the  $t_{1/2\beta}$  can be adequately estimated after a single dose of some drugs with multi-compartment characteristics and a long  $t_{1/2\beta}$ .

## DISCUSSION

The parameter  $AUC_{inf}$  is often used to estimate oral/systemic clearance and extent of bioavailability in single dose

experiments. If the AUC is not accurately estimated up to infinite time, the clearance and bioavailability estimates will be biased. Based on these considerations, it is commonly recommended to collect blood samples for at least 2–3 half-lives in order to reduce the fraction of  $AUC_{inf}$  that is calculated by extrapolation beyond the last quantifiable concentration. While these recommendations are generally appropriate, they are less important in the context of a bioequivalence study, particularly for a drug with a long terminal half-life. Bioequivalence studies are performed to compare formulations and thus the focus is on relative changes in AUC (as well as  $C_{max}$ ) between the



**Fig. 6.** (A) Concentration-time profiles for the mean (true) parameters for 2-compartment Models IV (●) and V (■). The dashed lines indicate the high and low LOQ. (B) intra-subject CVs for AUCs truncated at various times for the different 2-compartment model scenarios: Model IV low LOQ (●), Model IV high LOQ (▲), Model V low LOQ (■) and Model V high LOQ (◆). The symbols for intra-subject CVs reflect the median of 1000 studies.

test and reference formulations. If there are differences in rate and/or extent of availability between two oral formulations, they have to become evident relatively early after the dose is administered while the drug is being released from the dosage form and absorbed. Once absorption is completed, the dosage form will no longer have any effect on systemic drug concentrations even though it may be possible to measure the drug in blood/plasma for days, weeks or even months. Based on the gastrointestinal transit time, it would not seem possible for absorption to extend beyond 48–72 hours for oral dosage forms (usually absorption will be completed well before this time). Therefore, collecting blood samples beyond 72 hours should not add any significant new information concerning the bioequivalence of two oral formulations.

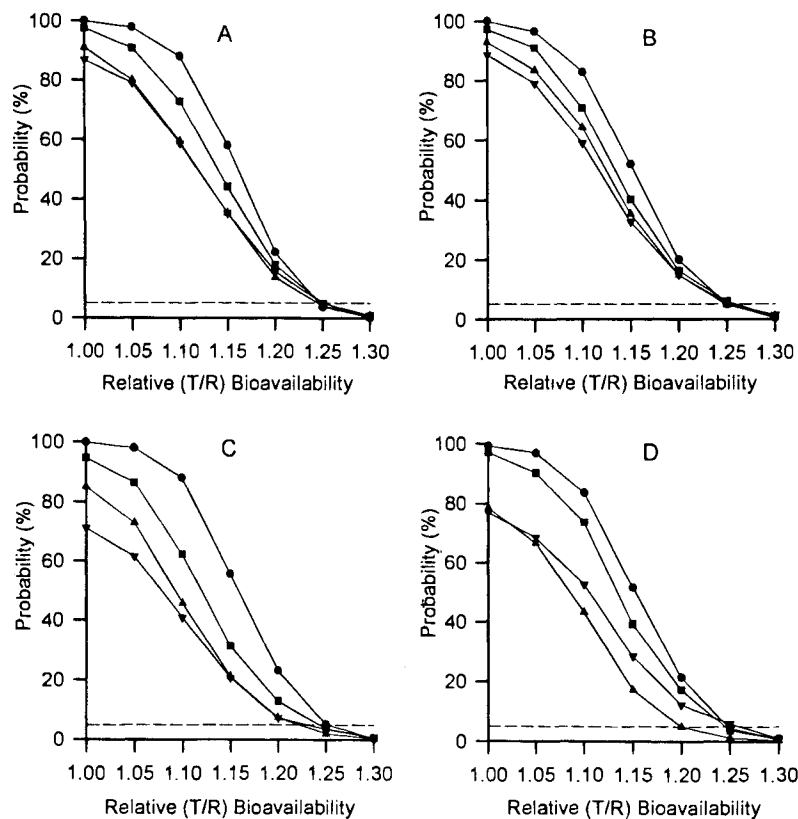
Lovering *et al.* examined the T/R AUC ratios at several blood sampling times after drug administration for different formulations of ten drugs (6). They observed that for most drugs, the ratios of partial or truncated AUCs stabilized a few hours after drug administration and concluded that reliable estimates of bioequivalence could be made with sample collec-

tion extending for 24 hours or less. Midha *et al.* calculated T/R AUC ratios and confidence intervals for 10 bioequivalence studies (24 different analytes) and noted that the bioequivalence conclusions were essentially the same for  $AUC_{lqc}$  and AUC truncated at twice the time required to reach maximum concentration ( $T_{max}$ ) (7,8). In contrast, Martinez *et al.* reviewed data from three bioequivalence studies and recommended using  $AUC_{inf}$  and  $AUC_{lqc}$  rather than truncated AUCs (9). However, their investigation was limited to a small number of drugs and studies. An additional difficulty inherent to the analysis of experimental data is that because it is not known if the two products are truly bioequivalent, concordance/discordance in conclusions using various AUCs does not necessarily indicate that a particular metric gives the correct conclusion.

The above limitation is eliminated when using simulated data because the difference in the bioavailability of the two formulations is known and can be controlled. Using simulations, Martinez *et al.* concluded that the 90% confidence intervals for  $AUC_{TM}$ , defined as the AUC from time 0 to the latest time at which all subjects had measurable concentrations, yielded wider confidence intervals than those based on  $AUC_{inf}$  (9). Only this particular method of AUC truncation was evaluated and these results were challenged by other investigators who performed more extensive simulations based on the same study design (5). Bois *et al.* concluded based on their simulations that a form of AUC truncation (analogous to the  $AUC_{TM}$  described above), produced slightly biased and more variable estimates than those obtained using  $AUC_{lqc}$  (4). Their work focused on several metrics of extent of availability and evaluated only one method for AUC truncation. A limitation of all simulation studies is that their conclusions may depend on the assumptions that are made about the model (*i.e.*, structural pharmacokinetic model and the various sources of variability) and that the simulated data may or may not accurately reflect typical experimental data.

The present investigation was undertaken to determine the impact of shortening the period of sample collection in bioequivalence studies and was based on the examination of a large database of experimental data as well as Monte Carlo simulations. Examination of the experimental data revealed that the T/R ratios of geometric means for truncated AUCs stabilized relatively soon after the dose and changed little beyond  $T_{max}$ . Similarly, there was little change in the intra-subject variability of AUCs truncated at  $3 \cdot T_{max}$  or later. Furthermore, bioequivalence conclusions based on  $AUC_{4 \cdot T_{max}}$  and  $AUC_{inf}$  were identical in 97.6% of studies. This degree of concordance was similar to that between  $AUC_{lqc}$  and  $AUC_{inf}$  (98.4%). Therefore, ratios of AUCs truncated at  $4 \cdot T_{max}$  performed similarly to  $AUC_{inf}$  in terms of point estimate, intra-subject variability and bioequivalence conclusions.

Monte Carlo simulations were used to determine how truncated AUCs performed when there are known differences in bioavailability between formulations. Inter- and intra-subject CVs for the various pharmacokinetic parameters used in the simulations were based on the experimental data in order to assure that the simulated studies were similar to "real" studies. Several different scenarios were used to simulate a wide variety of drugs and experimental conditions. The results demonstrated that the intra-subject CVs of truncated AUCs changed as a function of time and the shape of this relationship depends on the particular simulation scenario. The power to conclude bioequivalence when the formulations are truly bioequivalent is directly affected by the intra-subject CVs since a less variable



**Fig. 7.** Power curves showing the proportion of the 1000 simulated studies that met the 80–125% bioequivalence criterion as a function of the true T/R ratio of bioavailability. Results are reported for  $AUC_{inf}$  ( $\nabla$ ),  $AUC_{lqc}$  ( $\blacktriangle$ ),  $AUC_{0-72}$  ( $\blacksquare$ ) and  $AUC_{0-8}$  ( $\bullet$ ) for the different 2-compartment model scenarios: (A) Model IV low LOQ, (B) Model V low LOQ, (C) Model IV high LOQ and (D) Model V High LOQ. The dashed line represents a probability of 5%.

parameter is more likely to meet the bioequivalence criterion. Generally, the intra-subject CVs were higher for AUCs truncated over the first few hours but then decreased rapidly. For most 1-compartment scenarios, this was followed by a long period when there was relatively little change in intra-subject CVs, consistent with most experimental data (Figures 4B and 1). Conversely, there was a trend for CVs to increase with time for the highly variable drug Model III scenario and for the 2-compartment model scenarios (Figure 4B and 6B). This trend was also observed for some but not most experimental data (Figure 2). These differences in CVs were reflected in the power curves. Thus under some circumstances,  $AUC_{inf}$  had greater power than AUCs truncated at earlier times, whereas for other scenarios truncated AUCs performed better.

Endrenyi and Tothfalusi have demonstrated that the T/R ratio of AUCs truncated one half-life (*i.e.*, 24 hours in their simulations) after the dose had a bias of <2% relative to the true value, even when 3-fold differences in  $K_a$  between formulations were simulated (5). The simulations presented in our investigation did not introduce differences in rate of absorption and consequently the (median of 1000) T/R ratios of geometric means for AUCs truncated at each sampling time were within 1% of the true value for all study scenarios. However, even when simulating a 5-fold lower  $K_a$  for the test formulation compared to the reference formulation, the median bias for the

T/R ratio for  $AUC_{0-72}$  was <3% for all study scenarios. As expected, AUCs truncated sooner after the dose (*e.g.*,  $AUC_{0-8}$ ) were more biased since they were more sensitive to differences in the rate of absorption. Thus, AUCs truncated long enough after the dose should be insensitive to differences in the rate of absorption.

The variations in intra-subject CVs for AUCs truncated at different time intervals after the dose are due to a relatively complex interplay of several sources of variability. Variability in pharmacokinetic parameters, assay variability as well as error in the estimation of the terminal half-life all affect to variable degrees different AUC metrics. AUCs truncated early after the dose are more variable since the added variability due to rate of absorption ( $K_a$ , possible lag time) will have a greater effect on early concentration-time data compared to later ones. Assay variability will also have a greater impact on AUCs truncated early after the dose because only a few concentrations are used in the estimation of such AUCs. The contribution of assay variability will be less as the number of observations increases for AUCs truncated over a longer period of time (*i.e.*, recall that AUCs are essentially weighted means of concentrations and the mean of several concentrations will be less affected by the variability of individual concentrations). Intra-subject variability in absolute bioavailability (*i.e.*, A in our simulations), as expected for a drug with significant and variable first-pass



metabolism, will increase the variability of all AUC metrics to a similar extent. However, intra-subject variability in  $K_e$  (terminal half-life) will have a greater impact on the variability of late concentrations compared to early ones. Similarly, lower concentrations at the end of the concentration-time curve will often approach the LOQ and tend to have larger assay variability. However, late concentrations may account for a relatively small fraction of the AUC and thus will have relatively less effect on the variability of AUCs truncated at later times. The overall effect on the shape of the intra-subject CV *versus* time curve will depend on the relative importance of the different sources of variability. For example, when the intra-subject variability of  $K_e$  (terminal half-life) is relatively high, the intra-subject CVs of truncated AUCs will increase over time, after the early decline, as evidenced by the terfenadine data in Figure 2 and the highly variable drug Model III data in Figure 4B. A similar situation is also evident for the 2-compartment model scenarios (Figure 6B). However, when  $K_e$  variability is relatively low, there will be only relatively minor changes in intra-subject CVs over time after the initial rapid decline (Figure 1, desipramine data in Figure 2 and most scenarios in Figure 4B).

The benefits of AUC truncation are particularly important for drugs with long half-lives. In theory, truncated AUCs should be a good measure of relative extent of bioavailability for all drugs once absorption is completed, regardless of half-life. The results of this study support this point (Figure 3). However, blood sampling schemes are designed before a study is performed and without specific knowledge about the time when absorption will be completed for all subjects in the study. There would be a greater risk that absorption would not be completed if AUCs were truncated relatively soon after the dose, as would be necessary for drugs with short half-lives. Furthermore, there would be only minor advantages to shortening the blood sampling collection period for a drug with a relatively short half-life. This could explain why regulatory agencies have been reluctant to adopt truncated AUCs as a bioequivalence metric for all drugs. The risks and benefits, however, are much different for a drug with a long half-life. For drugs such as hydroxychloroquine, blood samples would need to be collected for months in order to evaluate an "acceptable" fraction of  $AUC_{inf}$  and a parallel study design would typically be used because a crossover design with an appropriate washout period would be impractical. Under such circumstances, use of  $AUC_{0-72}$ , for example, would dramatically shorten the duration of the study from several months to 3 days and there would be very little risk that significant absorption is still ongoing 72 hours after the dose. Although this report has emphasized intra-subject variability and crossover study designs, the results of our simulations demonstrate that truncated AUCs perform equally well for parallel study designs. Midha et al. have also demonstrated the advantage of truncated AUCs in a parallel study design with hydroxychloroquine (10). The shorter blood sampling collection period offers several advantages which were listed previously. From an ethical point of view, one has to question

whether even the small risks of repeated venipunctures and blood loss are justifiable when these samples provide no significant additional information about bioequivalence.

Based on the above considerations, it would be reasonable to restrict the duration of blood sampling to 72 hours for all bioequivalence studies of drugs administered orally. The Health Protection Branch of Canada has issued a draft guidance to use  $AUC_{0-72}$  as the measure of relative extent of bioavailability for drugs with long half-lives. This proposal could serve as a good basis for international harmonization of bioequivalence requirements for drugs with long half-lives.

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