Evaluation of a Cmin and a Normalized Cmin Method for the Confirmation of Steady-State in Bioequivalence Studies

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Purpose. Two methods to confirm attainment of steady-state conditions in multiple-dose bioequivalence studies are described and evaluated: (1) the Cmin method and (2) the Area Below the Cmin plasma-concentration-versus-time-curve method (ABCM method).

Methods. *Cmin Method*—After repetitive drug administration to presumed steady-state, successive trough, or Cmin, values are evaluated to determine if they are equal. *ABCM Method*—The ABCM of successive doses from dose two to presumed steady-state [ABCM(ss)] are divided by the ABCM for the first dose, ABCM(t), to give ABCM(ss)/ABCM(t)=R, which describes the increase in ABCM(n) with successive doses. The quantity, R, is then divided by an accumulation ratio to render the value independent of intra-subject clearance differences. Monte Carlo simulations were done to test the effects of data error and slow-clearing subpopulations on the method's performance. Data from multiple-dose bioequivalence studies were evaluated using confidence intervals for both methods to determine how well each predicted steady-state for immediate-release and controlled-release drug products.

Results/Conclusions. The Cmin method more accurately predicted the attainment of steady-state conditions for immediate-release formulations compared to the ABCM method. Conversely, the ABCM procedure more accurately predicted the attainment of steady-state conditions for controlled-release formulations compared to the Cmin method. The simulation results were further supported by the experimental data.

KEY WORDS: bioequivalence; steady-state; area below the Cmin curve.

INTRODUCTION

The determination of extent of absorption for bioequivalence studies following multiple dosing (MD) is accomplished by comparing test versus reference AUC(0-τ) values, which, in order to be valid, requires that all study subjects reach steady-state (SS) conditions. To verify that SS has been achieved blood samples are taken just prior to each drug administration (i.e., at Cmin) with intensive blood sampling during one dosing interval at SS (1) to characterize drug absorption.

The main problems in determining attainment of SS conditions by all subjects in MD studies are:

1. the inability to identify *a priori* those subjects with significantly slower than average clearances that will not reach steady state in the number of dosing half-lives calculated using average subject's clearance.

2. lack of an established method to render the data independent of these slow clearance subjects once they are identified during study analysis.

3. the absence of clear criteria and rationale as to the number of Cmin values to collect and evaluate.

4. a suitable statistic to compare subjects within a treatment group to determine if "on average" they have reached steady-state.

Currently, several statistical procedures have been most often used to "confirm" steady-state (2,3). However, the absence of a clear statistical endpoint (preferably based upon confidence intervals) has resulted in a largely subjective and empirical interpretation of these data. The purpose of this paper will be to introduce two methods for the determination of steady-state using only MD data (e.g., Cmin from the first dose and Cmins from the dose believed to be at steady-state).

THEORETICAL

Case A: Tau = 24 hours

For any drug whose kinetics are linear, plasma levels will increase after repeated dosing as drug accumulates. Plotting Cmin values, one obtains a rising baseline, Figure 1. And,

$$Cmin_{(n)} > Cmin_{(n-1)} \quad (1)$$

where n = dose number.

At steady-state:

$$Cmin_{(n)} = Cmin_{(n-1)} \quad (2)$$

The ratio of these Cmin values should equal 1.0 at steady-state.

If $Cmin_{(n)}$ and $Cmin_{(n-1)}$, during MD, are used to define the sides of a trapezoid with base = τ (dosing interval), the area below $Cmin_{(n)}$ and $Cmin_{(n-1)}$ (ABCM) can be estimated using the trapezoidal rule.

$$ABCM(n) = (\tau/2)*[Cmin_{(n)} + Cmin_{(n-1)}] \quad (3)$$

and at steady-state these Cmin values would be defined as:

$$ABCM(ss) = (\tau/2)*[2*Cmin_{(ss)}] \quad (4)$$

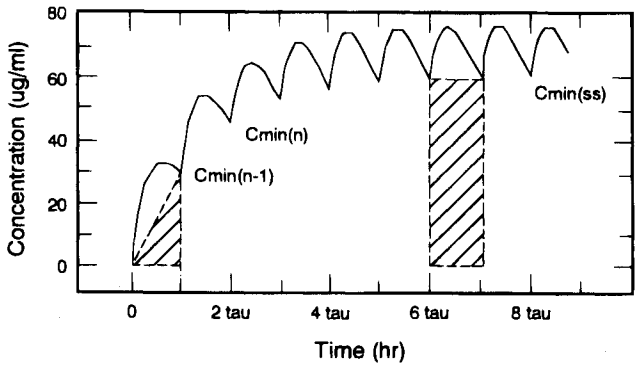

Fig. 1. Simulated data showing ABCM(t)-triangulated, area below Cmin(1), and ABCM(ss), area below Cmin(ss) following multiple dosing in the one-compartment model.

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The ABCM(t) for the first dosing interval can be described as a right triangle (Figure 1) with side = $Cmin_{(1)}$.

$$ABCM(t) = \frac{Cmin(1)}{2} * \tau \quad (5)$$

Then the ratio of:

$$ABCM(ss)/ABCM(t) = 2* \frac{Cmin_{(ss)}}{Cmin(1)} = R \quad (6)$$

which defines the ratio of the areas below the curve between the first dose and a dose at steady-state for any drug product.

If the absorption and elimination of a product under consideration can be described by a sum of exponentials, then Equation 6 can be rewritten as:

$$ABCM(ss)/ABCM(t) = 2[\{e^{-K\tau}/(1 - e^{-K\tau})\} - \{e^{-Ka\tau}/(1 - e^{-Ka\tau})\}]*1/(e^{-K\tau} - e^{-Ka\tau}) = R \quad (7)$$

If the product under consideration is also an immediate-release formulation, then a special case exists whenever Ka >> K. As the exponential term containing Ka approaches 0, Equation 7 would become:

$$ABCM(ss)/ABCM(t) = 2/(1 - e^{-k\tau}) = Rir \quad (8)$$

The relationship, R, in Equation 6 can be normalized by the accumulation ratio (R2) while Rir can be normalized by dividing by (R3). These accumulation ratios have been defined by a previous author (4):

$$R2 = Cmin(ss)/Cmin(1) \quad (9)$$

$$R3 = 1/(1 - e^{-k\tau}) \quad (10)$$

The normalized relationship for Equations 6 and 8 using R2 and R3, respectively, gives the final normalized ratios for Equations 6 and 8 in Equations 11 and 12.

$$[ABCM(ss)/ABCM(t)/(R2)] = 2 = Rn \quad (11)$$

$$[ABCM(ss)/ABCM(t)/(R3)] = 2 = Rn_{(IR)} \quad (12)$$

Case B: Tau Less than 24 hours

Whenever the dosing interval τ is less than 24 hours and Cmin values are collected at the "same time of day" to avoid potential problems with diurnal variation (1), $m\tau$ intervals will have to be combined where $m = 24/\tau$.

Therefore, an ABCM can be defined based upon the new $m\tau$ interval.

$$ABCM_{(m)} = 1/2*(m\tau)*[Cmin_{(n)} + Cmin_{(n+m)}] \quad (13)$$

and

$Cmin_{(n)}$ = Cmin at time τ

$Cmin_{(n+m)}$ = Cmin at time $m\tau$

Using the same procedures as for ABCM(ss) in Case A results in $ABCM(ss)_{(m)}$ being defined as:

$$ABCM(ss)_{(m)} = \tfrac{1}{2}*(m\tau)*[Cmin(ss)_{(n)} + Cmin(ss)_{(n+m)}] \quad (14)$$

Then the following ratio is obtained:

$$ABCM(ss)_{(m)}/ABCM(t) = \frac{m*[Cmin(ss)_{(n)} + Cmin(ss)_{(n+m)}]}{Cmin(1)} = R_{(m)} \quad (15)$$

Expression of Equation 15 in the exponential form of Equation 7 and assuming Ka >> K for an immediate release formulation gives:

$$ABCM(ss)_{(m)}/ABCM(t) = 2m/(1 - e^{-k\tau}) = Rir_{(m)} \quad (16)$$

Normalization of Equations 15 and 16 by R2 and R3, respectively, gives the final normalized Equations 17 and 18.

$$[ABCM(ss)_{(m)}/ABCM(t)/(R2)] = 2m = Rn_{(m)} \quad (17)$$

$$[ABCM(ss)_{(m)}/ABCM(t)/(R3)] = 2m = Rn_{(ir)(m)} \quad (18)$$

Equations 11 and 12 (Case A, τ = 24 hrs) define the relationships between ABCM values for the steady-state and first dose for any formulation normalized by accumulation factor R2, and the special case for immediate-release formulations with Ka >> K normalized by accumulation factor R3. The same relationships when $\tau < 24$ hrs are described by Equations 17 and 18.

METHODS

Monte Carlo Simulations

The simulations were all done with random error (based upon a normal distribution) added to the model parameters and did not contain any error related to a diurnal effect. Therefore, all analysis of simulations were based upon the measured tau interval instead of the mtau.

Scenario I—Baseline-Immediate-Release

These simulations were done assuming a one-compartment model with first-order absorption and elimination to determine the effect of intrasubject error in Ka, clearance (CL), and volume of distribution (V) on the proposed Cmin and ABCM methods. An additional simulation was done to establish the "true" fraction of steady-state (F_{ss}) attained at 3.5 dosing half-lives, Area-Inf method (5). Parameters for the simulations were: Ka_T = 0.48 hr^{-1}, Ka_R = 0.60 hr^{-1}, CL = 7.5 l/hr, V = 130 l. The dose was 500 mg (F = 1 and dosing every 6 hours) for each simulation with blood sampling every 6 hours to 42 hours post-dose and hourly sampling from 42–48 hours. Stochastic variation was introduced as previously described in Ka, CL and V (6). The effect of changes in intrasubject variability for Ka, CL, and V on the confirmation of steady-state were studied. The Cmin and ABCM methods, applied to the same Cmins, were compared after doses 1–7 to determine which procedure had the higher probability of concluding steady-state versus the likelihood of the same decision using the simulated F_{ss} (e.g., 90%) criterion. Intrasubject variability values. were:

A. Ka = 40%, CL = 40%, V = 14%-(High)

B. Ka = 20%, CL = 20%, V = 7%-(Medium)

C. Ka = 10%, CL = 10%, V = 3%-(Low)

Simulations were performed using SAS on a Compac 5133 personal computer.

Scenario II—Immediate-Release—Subpopulation Simulations

The objective of these simulations was to investigate what effect, if any, the presence of intrasubject variation in subpopulations with a decreased clearance would have on the performance characteristics of the Cmin and ABCM methods. The simulations in Scenario I using high and low levels of variability were repeated to include subpopulations with decreases in clearance of 10%, 20% and 40% from the baseline value of 7.5 1/hr. The decreased clearances were present in either 5%, 10%, or 20% of the simulated 24 study subjects. Each 24-subject bioequivalence study was repeated 1000 times. These results were evaluated at the end of three dosing half-lives. No corrections for the change in subpopulation clearance were made for F_{ss}.

Scenario III—Baseline-Controlled Release

These simulations were done assuming a one-compartment model with first-order absorption and elimination to determine the effects of various levels of added intrasubject error in Ka, CL, and V on the ability of the Cmin and ABCM methods to predict SS for a controlled-release product. The two simulations were performed with various Ka's and dosing intervals (Tau) to determine the resultant effects (i.e., dosing in other than the post-absorptive phase) on the accurate estimation of R2 in relation to the fraction of steady-state achieved. Mean parameters for the simulations were:

	Ka_T	Ka_R	Clearance (CL)	Volume of distribution (V)	Tau
A)	$0.1150\ hr^{-1}$	$0.0924\ hr^{-1}$	1.73 1/hr	10 L	12 hr
B)	$0.0433\ hr^{-1}$	$0.0346\ hr^{-1}$	1.73 1/hr	10 L	24 hr

The dose was 100 mg (F = 1) for each simulation with blood sampling every 12 hours (for A) to 72 hours post-dose, then with hourly sampling from 72–96 hours. For Simulation B, sampling was every 24 hours to 168 hours post-dose, then with hourly sampling from 168 to 192 hours. Parameter distributions were the same as for the immediate-release simulations.

Scenario IV—Controlled Release-Subpopulation Simulations

The high and low variability level simulations in Scenario III were repeated to include subpopulations with decreases in clearance of 10%, 20% and 40% from the baseline value of 1.73 1/hr. The simulations were done the same as those for immediate-release Scenario II. The Cmin and ABCM techniques were evaluated for 7 doses to determine which procedure had the higher probability of correctly concluding attainment of steady-state conditions.

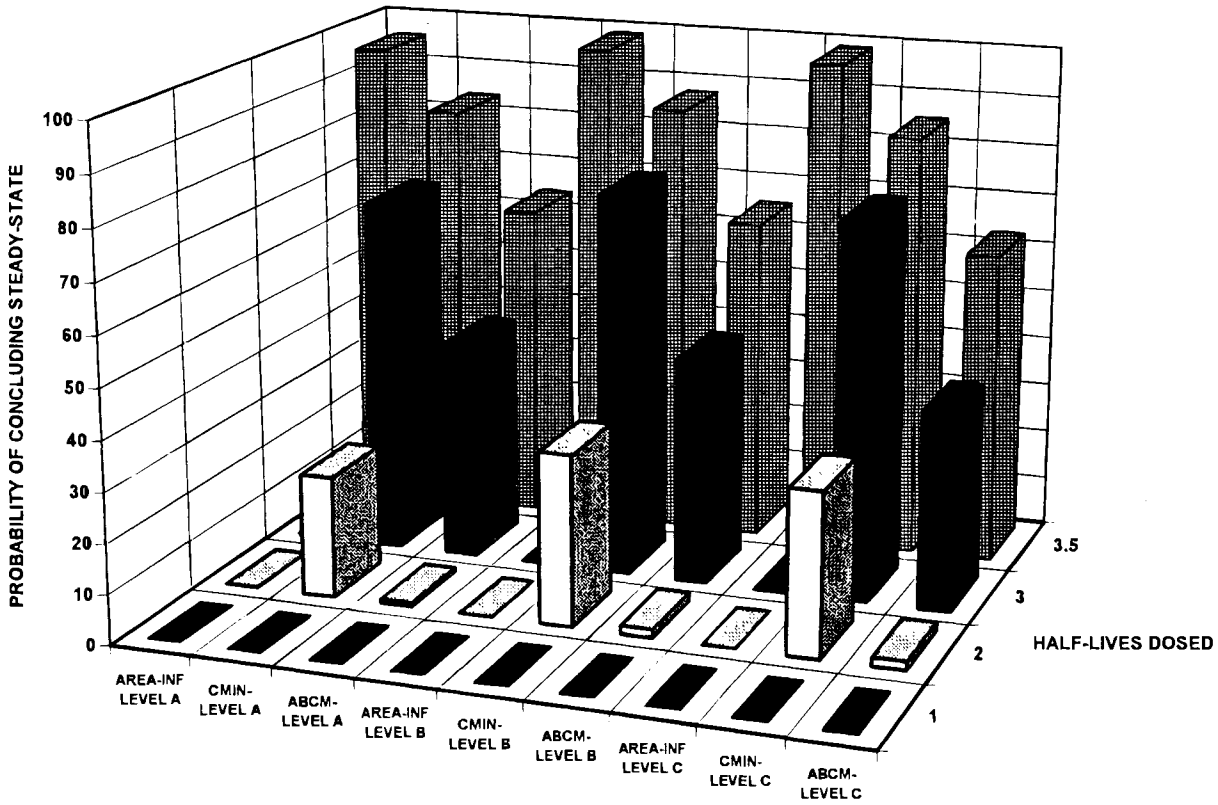

Fig. 2. Data from Monte Carlo simulations comparing the probability of calculating the true per cent of steady-state achieved after dosing for up to 3.5 half-lives in the one-compartment model for the test formulation (immediate-release) and the effect of three levels of intrasubject variability in Ka, clearance and volume of distribution. Level A is; Ka = 40%, CL = 40%, V = 15%; Level B is Ka = 20%, CL = 20%, V = 7%; Level C is Ka = 10%, CL = 10% and V = 3%.

Data Analysis

The Cmin values were observed directly from the data for Scenarios I–IV just prior to dosing. The observed Cmin values were used to calculate the triangular area under the curve following the first dose (i.e., ABCM (t)-triangular) and then to calculate ABCM(n) following 1, 2, 3 and 3.5 half-lives of dosing for the IR simulations and doses 2–7 for the CR simulations using the trapezoidal rule. The probabilities of obtaining the true per cent of steady-state were estimated for both the Cmin and ABCM methods. A one-sided 90% confidence bound:

$$CI = \overline{LnD} + t_{\cdot 95} * S_{L_{n_D}} * 1/\sqrt{k}$$

where

-k is the number of subjects

-LnD is the difference in the natural logs of the mean values between either $Cmin_{(n-1)}$ and $Cmin_{(n)}$ [($Cmin_{(n)}$ and $Cmin_{(n+m)}$ for $m\tau$)], or between ABCM(n) [($ABCM_{(m)}$ for $m\tau$)], and ABCM(t), normalized by the appropriate accumulation ratio.

-$\overline{LnD}$ is the arithmetic mean of the LnD's for the k subjects in the study

-S_{lnD} is the sample standard deviation of the k LnD's

-$t_{.95}$ is the 95th percentile of student's t-distribution with (k−1) degrees of freedom, as calculated for each simulation.

For Cmin, antilogs of the CI for the ln Cmin ratios less than 1.0 (i.e., ratio that indicates ss for Cmin) were assumed **not to have reached steady-state**. For ABCM, antilogs of the CI for the ln normalized ABCM ratios less than 2m (i.e., ratio that indicates ss for ABCM) were **considered not to be at steady-state**. For the 1000 simulations run for a 24-subject bioequivalency study, the number of times that the CI's for Cmin and ABCM indicated steady-state was recorded.

Estimation of Bias in Calculation of ABCM-IR Formulations

The assumption for the use of Equation 10 is that Ka >> K which normally assumes a Ka/K ratio of approximately 10 (5). Whenever this assumption is violated the $Rn_{(IR)}$ values resulting from the ABCM method contains per cent positive bias ranging from 4.4 to 50 % as a function of (Ka/K).

Bioequivalence Studies

Subjects in the studies were males between 18 and 30 years for the chlorpheniramine study and 20–44 years of age for the quinidine study. Each subject received a physical examination before beginning the study and was randomly assigned to a sequence of treatment. Informed consent was obtained from each subject.

Multiple Dose Studies—Immediate-Release Chlorpheniramine

A three-treatment, three-period crossover MD study was done in 12 subjects. The drug treatments were 24 mg chlorpheniramine in an Oros tablet every 24 hrs (Treatment A), 4 mg immediate-release chlorpheniramine every 4 hours (Treatment B), and 12 mg chlorpheniramine controlled-release tablet every 12 hours (Treatment C). All treatments were for seven days. Blood samples were collected just prior to dosing on days 1–6. On day 7, the samples for the formulation of interest, immediate-release Treatment B, were taken at 0, 1, 1.5, 2, 4, 5, 5.5, 6, 8, 9.5, 10, 12, 13, 13.5, 14, 16, 17, 17.5, 18, 20, 21, 21.5, 22, 24, 36, 48, and 72 hours post-dose. Since the dosing interval was less than 24 hours, samples at 8:00 AM each day were used as Cmin values. There was a 7-day washout period between study phases.

Analytical Procedure

A specific high performance liquid chromatography (HPLC) assay was used to determine chlorpheniramine concentrations in plasma. The assay was linear over the

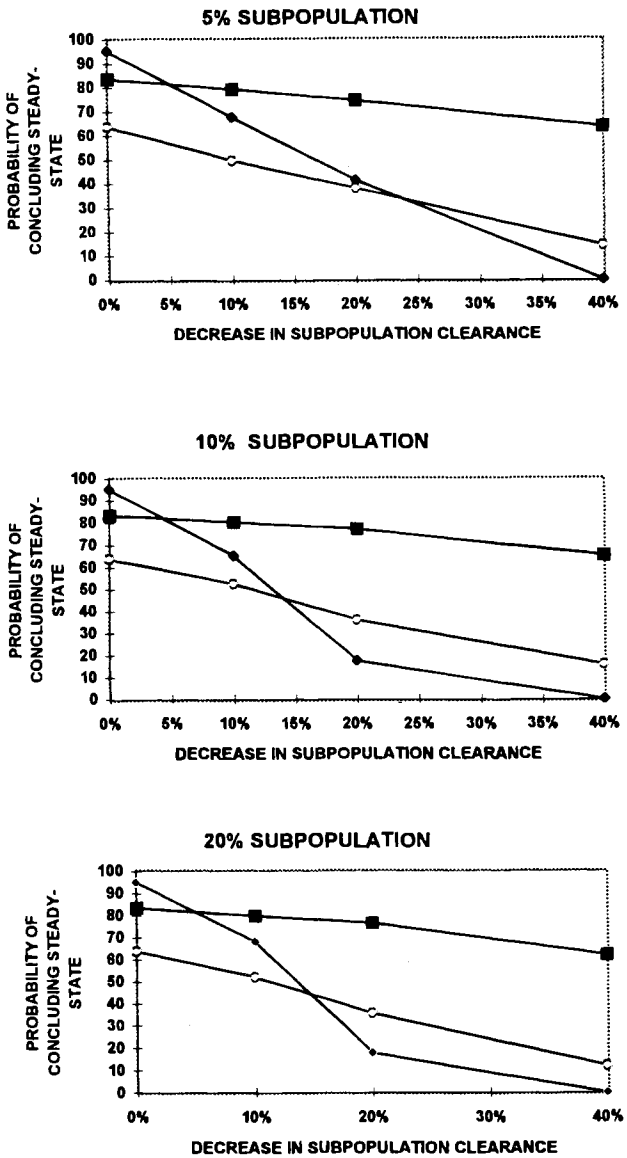

Fig. 3. Test subpopulation immediate-release simulations based upon baseline intrasubject variation Levels A. Decreased clearances of 6.75(1/hr)−10% decrease in baseline; 6.0(1/hr)−20% decrease in baseline and 3.0 1/hr−40% decrease in baseline were simulated in subpopulations of 5%, 10% and 20% of the 24 subject study population for the 3 methods F_{ss} (◆), Cmin (■) and ABCM (○).

range of 4 to 30 ng/ml with a coefficient of variation ranging from 1.1 to 8.1%. Accuracy, estimated from control samples at 30, 17, 10 and 4 ng/ml, ranged from 100% at 30 ng/ml to 91.7% at 4 ng/ml.

Single and Multiple Dose Studies—Controlled-Release Quinidine

A two-treatment, two-period, crossover, single/multiple dose study was done in 24 subjects. The drug treatments were Quinaglute^{R} 324 mg (Berlex Laboratories) and a generic quinidine gluconate 324 mg sustained release tablet. The initial 324 mg dose was used for the single dose study and was followed after 24 hours by 7 additional 324 mg oral doses administered at 12 hour intervals for the MD study. During the single dose study, blood samples were collected at 0.5, 1, 2, 3, 4, 5, 6, 8, 12 and 24 hours post-dose. For the MD study, blood samples were taken on day 4 just prior to the morning and evening doses (doses 5 and 6) and on day 5 just before dose 7 to obtain Cmin values. After the 7th dose, plasma samples were collected at 0.5, 1, 2, 3, 4, 6, 8, 10 and 12 hours. There was a 10-day washout period between study phases. Subjects were fasted for 12 hours before the single dose study but did not fast before the MD study. Cmin samples after doses 5 and 7 (morning doses) were used for the steady-state analysis.

Analytical Procedure

A specific HPLC assay was used to determine quinidine concentrations in plasma. Details of the assay have been previously descibed (7).

RESULTS

Immediate-Release

The results from the Monte Carlo simulations are presented in Figure 2 for the test. Similar results were observed for the reference and are not presented. Simulations for Cmin were evaluated by the one-sided t-test criterion. The CI for the ABCM method concluded with 90% assurance that the studies were at steady-state less frequently at 3.0 and 3.5 dosing half-lives (i.e., 86.0% and 90.0% of "true" steady-state, respectively) than did the Cmin method for all tested levels of intrasubject error. The CI for the Cmin method concluded with 90% assurance that the studies were at steady-state more often at 3 and 3.5 half-lives of dosing than did the ABCM method even when the ABCM value for test had an approximately 6% positive bias.

The simulations for the test product (Figure 3) for high intrasubject variability investigated how including subpopulations with decreased clearances would affect baseline probabilities of concluding steady-state in a 24-subject study. In all cases

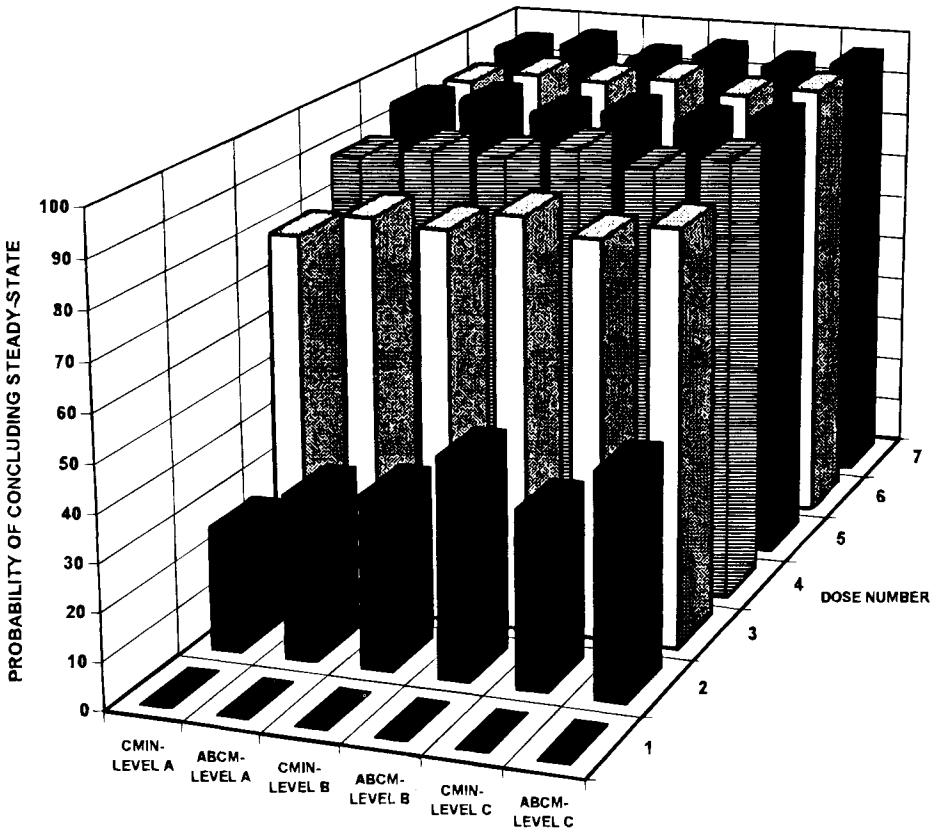

Fig. 4. Data from Monte Carlo simulations comparing the probability of calculating the true per cent of steady-state achieved after 7 doses in the one-compartment model for the test formulation for a controlled release formulation and the effect of three levels of intrasubject variability in Ka, clearance and volume of distribution. The levels of variability are the same as in Figure 2.

Table I. Results from Error-Free Calculations Estimating the Effect of K/Ka Ratio and Dosing Interval τ on the Fraction of Steady-State (F_{ss})[a] Attained and the Error[b] in the Accumulation Ratio R2 as a Function of Dose Number for the Controlled Release Simulations A and B

	K/Ka ratio	Tau	Dose number 1	2	3	4	5	6
	1.49	12						
F_{ss}			0.49	0.84	0.95	0.98	0.99	0.99
R2 Error			48%	28%	22%	20%	20%	20%
	1.87	12						
F_{ss}			0.43	0.78	0.92	0.97	0.99	0.99
R2 Error			52%	30%	21%	19%	18%	17%
	4.00	24						
F_{ss}			0.53	0.83	0.94	0.97	0.99	0.99
R2 Error			37%	14%	6%	3%	2%	2%
	5.00	24						
F_{ss}			0.46	0.76	0.89	0.95	0.98	0.99
R2 Error			46%	21%	10%	5%	3%	2%

[a] $F_{ss} = (1 + K*e^{-nKa\tau}/Ka\text{-}K) - \{Ka*e^{-nK\tau} / (Ka\text{-}K)\}$.
[b] R2 error estimated as 1-(Experimental R2/True R2)*100 where true R2 value is that at steady-state using Cminss and experimental R2 is calculated for each N^{th} dose using the calculated $Cmin_{(N)}$ for that dose (i.e., N = 1 – 6). Cmin(1) is the same for both true and experimental R2.

studied, as subpopulation clearances decreased from 10–40% below that of the average baseline (7.5 l/min), and the proportion of the subjects exhibiting these decreased values increased from 5–20% of the population, the Cmin method more consistently concluded steady-state than did the ABCM method.

Controlled Release

Results from the Monte Carlo simulations for the test (Figure 4) and reference (not presented), show that the ABCM method correctly indicated steady-state conditions more often than did the Cmin procedure at all error levels studied.

The data in Table I comparing the effect of dose number and K/Ka ratio on R2, using error-free data, indicates that R2 is underestimated more for Simulation A than for Simulation B following each dose. Increased error in R2 resulted in a 3–10% decreased probability of confirming steady-state after Dose 2 for Simulation A compared to Simulation B (Table II). On the other hand, following Dose 3, the increased error in R2 (Simulation A) resulted in a small increase in the probability of concluding steady-state over that estimated for Simulation B.

Table II. Percent Probability of Concluding Steady-State Following Doses 2 and 3 for Controlled Release Simulations A (Ka_T= 0.1155 hr-1, Ka_R = 0.0924 hr-1, K_e = 0.173 hr-1, τ = 12 hr) and B (Ka_T = 0.0577 hr-1, Ka_R = 0.0433 hr-1, K_e = 0.173 hr-1, τ = 24 hr) at High (40%), Medium (20%), and Low (10%) Error Levels on Clearance

Clearance error level (%)	Dose 2		Dose 3	
	Test	Reference	Test	Reference
40 A	24.9	8.4	86.2	74.6
40 B	34.8	15.2	84.4	70.4
20 A	42.3	16.5	88.8	82.9
20 B	46.2	20.8	87.4	77.6
10 A	44.3	20.2	89.1	80.1
10 B	47.0	24.0	87.6	77.2

Note: Data were analyzed using ABCM method.

When subpopulations with low clearance levels were included in the study, the ABCM method had a higher probability of concluding steady-state than did the Cmin method when the true level of steady-state was 98% after Dose 4, although the overall effect of subpopulations on baseline values was small. Results for the simulations for the test product are presented in Table III.

Analysis of the experimental data for chlorpheniramine (Table IV) indicated the attainment of steady-state by day 6 using both methods. For the quinidine data (Table IV steady-state was confirmed by Dose 7 for the ABCM method (Rn $\geq$ 4.0), but not by the consecutive Cmin procedure (R2 $<$ 1.0).

DISCUSSION

Both the Cmin and ABCM methods may be used to verify the attainment of steady-state in MD bioequivalence studies. The Cmin and ABCM methods, both at steady-state and after a single dose, are dependent upon absorption and distribution, in addition to elimination. However, an important advantage to the ABCM method is that it allows for normalization of intra-subject clearance differences, unlike those methods currently used. However, the ABCM method may not be the method of choice to determine ss conditions for immediate-release formulations due to the bias in the method if the assumption of Ka $>$ K is violated. For IR products, it appears prudent to prefer the Cmin method, which provides a better probability of correctly determining steady-state, even when low-clearing subpopulations are present.

The normalization of Rn was done using R2 (Cminss/Cmin), since the model employed in the simulations has continuous absorption. Therefore, the rate of elimination is controlled by the rate of release (e.g., absorption) which makes the accurate estimation of K for R3 difficult. The error involved in using R2 (Table I depends upon whether each dose is being administered in the postabsorptive phase of the preceding dose (5). Controlled-release simulation studies A and B demonstrated that error present in R2 led to an underestimation of the true minimum concentration at steady-state, $(Cinf)_{min}$, resulting in

Table III. The Effect of Subpopulations (5%, 10%, and 20%) on the Probability of Determining Steady-State for the Simulated Test Controlled Release Product, at High (40%) and Low (10%) Intrasubject Clearances After Dose 4 for the Cmin (A) and ABCM (B) Methods

		Decrease in low intrasubject clearance						Decrease in high intrasubject clearance					
		10%		20%		40%		10%		20%		40%	
Method		A	B	A	B	A	B	A	B	A	B	A	B
Percent of population	5%	91.5	94.6	94.0	96.3	90.6	93.5	90.0	92.9	89.5	92.4	89.7	96.6
	10%	92.6	95.4	93.0	95.2	91.1	94.1	88.1	91.5	88.3	91.2	89.5	92.5
	20%	93.2	95.0	91.9	94.0	91.1	94.2	90.9	93.5	89.7	92.9	91.6	94.4

Note: The mean half-lives for elimination and absorption are 4 hours and 16 hours, respectively. The clearance (1.73 l/hr) was decreased by 10%, 20%, and 40% in each of the subpopulations. Baseline mean values were ABCM (94.3%-low; 91.3%-high) and Cmin (94.3%-low; 91.4%-high).

Table IV. 1-Sided Confidence Intervals for Cmin and ABCM Methods (Normalized by R3) for Immediate-Release (IR) Chlopheniramine and for Controlled Release (CR) Quinidine (Normalized by R2)

	Chlorpheniramine-IR			Quinidine-CR	
Data Analysis Method	Day 4	Day 5	Day 6	Test Treatment	Reference Treatment
Cmin	0.98	0.99	1.06	0.94	0.99
ABCM	11.64	12.00	12.18	4.63	4.87

Note: The Cmin values used in the calculations were at the same clock time (ie 8 AM every 24 hours) since the dosing interval was less than 24 hours. Therefore, values for ABCM are based upon mtau.

a small (1–4%) underestimation of the probability of Simulation A concluding steady-state at all error levels investigated compared to Simulation B by Dose 5 or 6. For the controlled-release Simulation B, the ABCM method also gave a higher probability of predicting steady-state than did the Cmin method, as the true level of steady-state increased with successive doses, either with or without the inclusion of subpopulations. However, the inclusion of subpopulations had less impact than for the immediate-release simulations. In fact, there was a slight random increase in the probability of the Cmin and ABCM methods compared to baseline, probably due to slightly higher $Cmin_{(n)}$ values resulting from the decreased clearance. The methods in this paper assumed the simplest absorption scenario for the application of the ABCM method to controlled-release simulations. Therefore, further testing will be required to assess how well the method applies to more complex absorption cases (i.e. non first-order or discontinuous input). Nonetheless, the non-normalized Cmin would still be applicable.

The ABCM method also provides the framework for testing individual study subjects that "appear" not to be at steady-state and may be causing the CI to be less than 2. This can be accomplished by using the subject's trough "steady-state" values and the population variance to construct an $ABCM(i^{th\ subject})$ CI. If the resulting value is less than 2, one has reason to exclude that subject from the analysis.

Application of the Cmin and ABCM methods requires that a theoretical value (such as 86.5% or 90% of steady state) be established to represent "true steady-state." Successful application of the ABCM method requires that: (1) studies specify that a Cmin sample be collected after the first dose and (2) for IR formulations, sufficient samples be collected after the final dose to determine K. The current practice of dosing for approximately 3 times the drug half-life of the subjects with lowest reported clearances could be continued if 86.5% of steady-state is deemed an acceptable standard of performance.

The Cmin and ABCM procedures can both verify attainment of SS conditions consistent with kinetic theory without necessitating a major change in the current experimental design used for multiple-dose bioequivalence studies. In summary, the methods:

1. define a criterion based upon the Cmin values for the final dosing interval.
2. provide a statistic consistent with current regulatory criteria to define steady-state (i.e., 10% consumer risk and Ln differences in means).

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