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Design of optimal sampling times for pharmacokinetic trials via spline approximation

Musa Hakan ASYALI
Department of Electrical and Electronics Engineering, Zirve University
Kızılhisar 27260, Gaziantep-TURKEY
e-mail: asyali@zirve.edu.tr

Abstract

Understanding and comparison of different drug delivery formulations are based on pharmacokinetic parameters (PKP) such as area under curve, maximum concentration, and time to reach maximum concentration. Accurate estimation of PKP is of critical importance in capturing drug absorption and elimination characteristics and in reaching bioequivalence decisions. Since PKP are estimated from a limited number of samples, the timing of the samples directly influences the accuracy of estimation. Optimization of the sampling times may not only increase the accuracy of PKP estimation, but also reduce the number of samples to be drawn, which in turn lessens the inconvenience to the subjects and the cost of the study. In this study, as an alternative to conventional piece-wise linear approximation, we proposed cubic spline approximation to the time-concentration curve and also introduced a global optimality criterion that focuses on the closeness of all the pharmacokinetic parameter estimators to their true values simultaneously. By minimizing the criterion function over sampling times and watching the regulatory practice/guidance for sample collection, we designed optimal sampling times that can be used in pharmacokinetic studies. We demonstrated that using our approach it is possible to obtain more accurate estimates of PKP with fewer samples.

Key Words: *Pharmacokinetic parameters, optimal sampling time design, spline approximation, sequential quadratic programming*

1. Introduction

Drug delivery is studied by means of distinctive pharmacokinetic parameters (PKP) such as area under the curve (AUC), maximum blood or plasma concentration ($C_{\max}$), and the time to reach the maximum concentration ($t_{\max}$) [1, 2]. After drug administration, subjects are monitored for a while to draw a certain number of blood samples and construct an approximate concentration-time curve (CTC). Since only a limited number of blood samples can be drawn and the estimation of PKP relies on the corresponding approximation to CTC, a judicious selection of sampling times will increase the accuracy of PKP estimation. Furthermore, optimization of sampling times may offer a smaller set of optimal sampling times with which PKP can be estimated accurately.

The literature on the issue of optimal sampling in PK is somewhat limited. Chang and Wong [3], and Kong and Gonin [4] suggested an optimal sampling time design procedures to improve the accuracy of AUC estimation only. They used sequential quadratic programming method to minimize the area between the actual CTC and its trapezoidal approximation.

Atkinson et al. [5] introduced an *ED*-optimal design, where E refers to expectation and D refers to the determinant of the Fisher's information matrix (FIM). D'Argenio [6] considered the issue of optimal sampling in the context of estimation of the compartmental model parameters such as the absorption and elimination rate constants, k_a and k_e, and the apparent volume of distribution, V. He suggested a sequential design that used results from previous subjects to determine the optimal sampling times for the next subject. Tod and Rocchisani [7] have compared different optimization strategies that can be used to obtain optimal sampling time design to accurately estimate PK model parameters. They concluded that, compared to *ED*-optimal design, *EID* (expectation of determinant of the inverse of FIM) and *API* (expectation of logarithm of the determinant of FIM) optimal designs may increase the accuracy and precision of the PK model parameter estimates. Random and gradient-search based algorithms are used to determine the optimal sampling times, however this study mostly focused on the estimation of underlying model parameters (i.e. k_a, k_e, and V), not on the PK parameters used to reach BE decisions (i.e. AUC, $C_{\max}$, and $t_{\max}$).

In this study, we use a first order model for drug distribution and assume that either the model parameters or their distributions are known. We first introduce a cubic spline approximation based method for PK parameter estimation, as an alternative to the conventional piecewise linear approximation. Previously, properties of spline approximation were investigated in the context of AUC estimation only [8]. Secondly, using these improved estimates, we define a generalized optimality criterion that simultaneously takes the accuracy of spline approximation based estimation of all the three PK parameters into account. We then minimize this criterion function, using sequential quadratic programming, to obtain the optimal sampling times.

2. Methods

2.1. One-compartmental model

The optimization methodology developed in this study focuses on a one-compartmental model with first order absorption. In this drug distribution model, the CTC is given as

$$C(t) = \frac{Dk_a}{V(k_a - k_e)} \left(e^{-k_e t} - e^{-k_a t}\right); k_a > k_e > 0;\ D, V > 0; 0 \leq t < \infty, \tag{1}$$

where $C(t)$ is the concentration, t is the time in hours (h), D is the dose administered, V is the apparent volume of distribution, and k_a and k_e are the absorption and elimination rate constants respectively. In general, k_a, k_e, and V are random variables that vary with study subjects and D is fixed and known. Following Kong and Gonin (4), we will assume the following example values for D and model parameters: $D = 400$ mg, $V = 40$ L, $k_a = 0.2\ \mathrm{h}^{-1}$, and $k_e = 0.1\ \mathrm{h}^{-1}$. The AUC from 0 to ∞ can be obtained by integrating $C(t)$, from 0 to ∞, which gives $AUC(0-\infty) = D/(Vk_e) = 100$. Similarly, $t_{\max}$ and $C_{\max}$ can be obtained from equation

(1) as

$$t_{\max} = \frac{\ln\left(\frac{k_a}{k_e}\right)}{(k_a - k_e)} = 6.93, \tag{2}$$

and

$$C_{\max} = C\left(t_{\max}\right) = \frac{Dk_a}{V\left(k_a - k_e\right)}\left(e^{-k_e t_{\max}} - e^{-k_a t_{\max}}\right) = \frac{D}{V}\left(\frac{k_e}{k_a}\right)^{\frac{k_e}{k_a - k_e}} = 5 \tag{3}$$

According to this model, if k_a is sufficiently larger than k_e, the concentration for $t > t_{\max}$ can be approximated as $C(t) \cong C_{\max} e^{-k_e(t - t_{\max})}$. Then, the half-life or $t_{1/2}$, defined as the time after $t_{\max}$ at which the concentration drops to half $C_{\max}$, i.e. $C\left(t_{\max} + t_{1/2}\right) = \frac{C_{\max}}{2}$, is given by

$$t_{1/2} \cong \frac{\ln(2)}{k_e} = 6.93 \tag{4}$$

The $t_{1/2}$ is important in determining the extent of the sampling period. Sampling should be continued until the remaining area beyond the last sampling time, that we call as the final time T_f, is negligible. A sampling interval up to three or four half-lives after $t_{\max}$ is considered sufficient and truncated AUC values are now acceptable to regulatory agencies. For our example values, $t_{\max} + 3t_{1/2} = 27.73$ h. Due to practical considerations, we will assume that $T_f = 24$ h and focus on estimating AUC from 0 to 24 h. For the given values, this value is

$$AUC\left(0 - 24\right) = \int_0^{24} C\left(t\right) dt = 82.68 \tag{5}$$

2.2. Point estimators for PK parameters

We denote the set of parameters used in BE studies with θ, that is, $\theta = \left(AUC\ C_{\max}\ t_{\max}\right)^t$. We consider estimating θ from the measurements of concentration $C(t)$ at a given set of sampling times t_1, t_2, ..., t_N. We assume that there is a default sample at $t_0 = 0$ with $C(t_0) = 0$. We further assume that the last sample is taken at the final time, i.e. $t_N = T_f$. We will call the point estimators for AUC, $C_{\max}$, and $t_{\max}$ as $\hat{AUC}$, $\hat{C}_{\max}$, and $\hat{t}_{\max}$, respectively, so that $\hat{\theta} = \left(\hat{AUC}\ \hat{C}_{\max}\ \hat{t}_{\max}\right)^t$. Conventionally, i.e. based on a piecewise linear approximation to CTC, the AUC from 0 to t_n is calculated by using the trapezoidal approximation as $\hat{AUC}\left(0 - t_N\right) = \sum_{i=1}^{N} \frac{(C_i + C_{i-1})(t_i - t_{i-1})}{2}$. The $\hat{C}_{\max}$, and $\hat{t}_{\max}$ are simply the maximum of the observed concentrations and the time at which the maximum is attained.

We introduce the estimators for AUC, $C_{\max}$, and $t_{\max}$ based on a cubic spline approximation to CTC. The approximation is constructed using patches of cubic polynomials [9–11]:

$$\hat{C}_i\left(t\right) = \sum_{k=0}^{3} a_{ik}\left(t - t_i\right)^k, \tag{6}$$

where a_{ik} are the polynomial coefficients, on the i^{th} subinterval $t_{i-1} \leq t < t_i; i = 1, 2, ..., N$. While putting the patches together to obtain the composite approximation or spline interpolation over the entire interval,

a provision can be made to blend the pieces smoothly, so that the approximation has several continuous derivatives at the break points. Cubic splines became very popular in function approximation since cubic polynomials represent a good tradeoff between the flexibility and complexity and it is possible to obtain two continuous derivatives at the break points. We will call the cubic spline interpolation (CSI) for $C(t)$ as $S(t)$; that is, $S(t) = \hat{C}_i(t)$ for $t \in [t_{i-1}, t_i), i = 1, 2, ..., N$, where $\hat{C}_i(t)$ is given by Equation (6). A matrix equation expressing all of the continuity conditions at the break points is solved to obtain the polynomial coefficients.

To form a CSI for $C(t)$, we have added two extra points at times $2T_f = 48$ h and $3T_f = 72$ h to make sure that the CSI approximates the $C(t)$ accurately beyond 24 h as well. Since we assume that the model and its parameters are known apriori, the $C(t)$ is known at any time and therefore such a modification is feasible. We can also provide the slopes at the end points (0 and 72 h), since the derivative of the $C(t)$ is also available at any time. Figure 1 clearly demonstrates the advantages introduced by these extra conditions/provisions, where we investigate the behavior of different CSIs over the time interval [0 100] h. The left end point is always at 0. The solid lines are the actual $C(t)$, whereas the dashed and dotted lines are the CSIs obtained with and without the end-point-slopes respectively. The $C(t)$ and CSIs were evaluated at times separated by 0.5 h, from 0 to 100, i.e. $t = 0$ (0.5) 100 h. In the top panel, the right end point is at 24 h and the breaks are at 0 and 13 h. In the middle panel, the right end point is at 48 h and the breaks are at 0, 13 and 24 h. In the bottom panel, the right end point is at 72 h and the breaks are at 0, 13, 24, and 48h. As we note, the CSI obtained

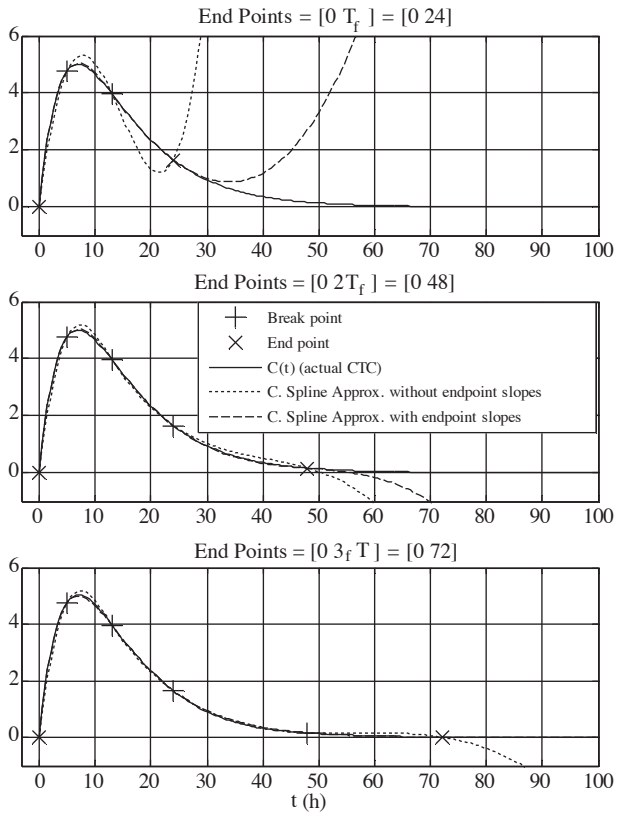

Figure 1. Demonstration of the effects of using extra break points and end point slopes in cubic spline approximation.

with the end points at 0 and 72 h and end-point-slopes provides the best approximation. The approximation corresponding to these conditions (the dashed line in the bottom panel) is almost indistinguishable from the exact curve (the solid line).

In the optimization runs that will be covered subsequently, after the CSI is constructed using the extra conditions discussed, the $A\hat{U}C$ (0-24) is obtained by integrating the CSI from 0 to 24 as

$$A\hat{U}C\,(0-24) = \int_0^{24} S\,(t)\,dt = \sum_{i=1}^{N} \int_{t_{i-1}}^{t_i} \hat{C}_i\,(t)\,dt, \tag{7}$$

Since $\hat{C}_i\,(t)$s are cubic polynomials, the integrals in Equation (7). can easily evaluated. To obtain $\hat{C}_{\max}$ and $\hat{t}_{\max}$, we have used a fine time grid where the interval $[0\ldots 24]$ h was divided into equally spaced subintervals of length 1 min each, i.e. the time grid was $t_s = 0\ (1/60)\ 24$ h. Therefore, the point estimators for the remaining two PK parameters of interest are given as

$$\hat{C}_{\max} = \max\left\{C\,(t_s)\right\}, \tag{8}$$

$$\hat{t}_{\max} = \arg\max_{t_s}\left\{C\,(t_s)\right\}. \tag{9}$$

2.3. Obtaining optimal sampling times: global optimality criterion and optimization

Given N, the number of samples to be taken, and all the parameters that describe the CTC, we would like to select the sampling times t_1, t_2, $\ldots$,t_N, such that the estimates of PK parameters will be as close to the actual PK parameters as possible. We will discuss the issue of determining an appropriate or optimal value for N later.

Optimization of sampling times should involve criterion function that indicates how accurate AUC(0-24), $C_{\max}$ and $t_{\max}$ can be estimated from a given set of sampling times. We propose the following global optimality criterion (GOC) function that employs "Euclidian norm" as a measure of closeness of the estimated values to actual values:

$$GOC\,(t_1, t_2, ..., t_N) = \left\|\hat{\theta} - \theta\right\|^2 = \left(A\hat{U}C\,(0-24) - AUC\,(0-24)\right)^2 + \left(\hat{C}_{\max} - C_{\max}\right)^2 + \left(\hat{t}_{\max} - t_{\max}\right)^2.$$

Here, the actual values of PK parameters, i.e. AUC(0-24), $C_{\max}$ and $t_{\max}$, are respectively given by equations 5, 3, and 2, and the estimators $A\hat{U}C$(0-24) , $\hat{C}_{\max}$, and $\hat{t}_{\max}$are respectively given by equations 7, 8, and 9. The optimal sampling times can be found by minimizing the GOC with respect to t_1, t_2, $\ldots$,t_N. However, this optimization problem is not a trivial one because of several reasons:

i) There are the following constraints due to the natural ordering of the sampling times:

$$0 < t_1 < t_2 < ... < t_{N-1} < t_N = T_f \tag{10}$$

ii) Optimization techniques have already been developed to deal with this kind of constrained nonlinear optimization or "nonlinear programming" problem. The underlying assumptions for these techniques are that

the function to be minimized is continuous, i.e. differentiable with respect to all of its arguments and that only a local minimum can be returned.

iii) Depending on the randomly chosen initial point in the N-dimensional space of sampling times, optimization algorithms may not converge to a solution, in which case selecting another random point or changing algorithms' tolerances may be necessary.

We note that $\hat{C}_{\max}$, and $\hat{t}_{\max}$ may introduce discontinuities in the GOC, which may adversely affect the optimization. In Figure 2, we demonstrate such a case where there are three samples: t_2 and t_3 are fixed at 12.85 and 24, respectively, so that t_1 can vary from $t_0 = 0$ up to t_2 due to the constraint given by Equation (10). The upper panel shows the CTC obtained by using our example values. $C(t)$ attains level 4 at $t \approx 3.25$ and $t \approx 12.85$. As shown in the middle panel, the $\hat{C}_{\max}(t_1, t_2 = 12.85, t_3 = 24)$ stays flat at 4 as t_1 varies from 0 to 3.25, after that it follows $C(t)$ up to t_2. Therefore $\hat{C}_{\max}$ is discontinuous around $t = 3.25$. In the lower panel, we observe the behavior of $\hat{t}_{\max}(t_1, t_2 = 12.85, t_3 = 24)$ as t_1 varies from 0 to 12.85. Till time 3.25, the $\hat{t}_{\max}$ stays at t_2 where the maximum is attained, after which it follows t_1 up to t_2. Similar to $\hat{C}_{\max}$, $\hat{t}_{\max}$ is also discontinuous around $t = 3.25$. Although there exist such discontinuities, we have not run into any convergence problems during the optimization runs. This indicates that there were no discontinuities near the solutions. However, even if convergence is achieved, the solution returned is not guaranteed to be the global minimum. To deal with this complication, we have run optimization algorithm with several different random initial points and selected the solution that offers the minimum value for the GOC as the optimal solution.

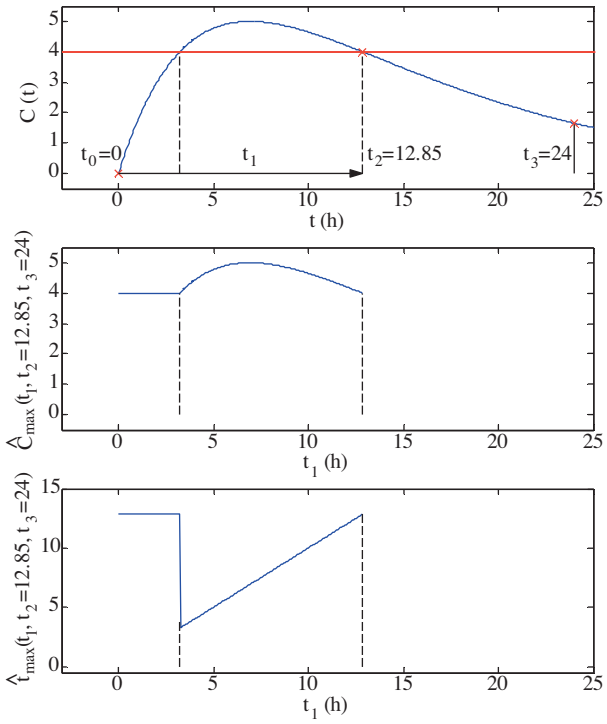

Figure 2. Demonstration of discontinuity in $\hat{C}_{\max}$, and $\hat{t}_{\max}$.

We have carried out the optimization using *fmincon* routine [12] of the MATLAB^{TM} (The MathWorks Inc., Natick, MA) Optimization Toolbox. This routine implements a sequential quadratic programming method

where a quadratic programming subproblem is solved at each iteration. An estimate of the Hessian of the Lagrangian is updated at each iteration using the well-known Broyden-Fletcher-Goldfarb-Shanno formula [13, 14]. We have chosen this gradient-based technique for the optimization of GOC over other techniques like Nedler-Meads simplex [15] or adaptive random search [16] because of its speed advantage.

We decomposed the constraints given by Equation (10) into the following "linear inequality" and "lower and upper bound" constraints as follows: $t_{i+1}-t_i > 0.1, i = 1, 2, ..., N-2; t_N > T_f$ (linear inequality constraints) and $0 < t_i < T_f$, $i = 1, 2,..., N$ (lower and upper bound constraints). Setting the constraints this way ensures that i) the t_N lands exactly on $T_f = 24$ during the optimization, as there are two competing constraints on $t_N, t_N \geq T_f$ by the inequality constraints and $t_N \leq T_f$ by the upper boundary constraints, and ii) the separation between the adjacent sampling times is more than 0.1 h (6 min) which signifies a useful constraint, i.e. practically it is difficult or inconvenient to take two consecutive samples in less than 5-6 min.

3. Results

3.1. Optimal sampling times for fixed k_e and k_a

We have let N, number of samples, vary from 3 to 9 due to practical considerations and done the optimization using our example parameters, i.e. $D = 400$, $V = 40$, $k_a = 0.2$, and $k_e = 0.1$. In each case, i.e. for different values of N, we have repeated the optimization runs 100 times with different initial conditions to increase the chances of establishing the global minimum. Slightly changing with the N value, each run takes about 5 seconds on a PC with 384 MB of memory, 1.6 GHz Pentium^{TM}-4 processor, running under Microsoft Windows 2000^{TM}. The results of optimization, i.e. the optimal sampling times and corresponding optimal values of the estimated PK parameters and the GOC, are shown in Table 1. Figure 3 shows the optimal $\hat{AUC}$(0-24) , $\hat{C}_{\max}$, $\hat{t}_{\max}$ and GOC as a function of N. We note that $N = 5$ is an optimal choice for the number samples, since there is a considerable decrease in the value of the optimal GOC as N moves from 4 to 5, whereas there is negligible decrease from 5 to 6. In Figure 4, we see the location of optimal sampling times for $N = 5$ overlaid on the actual CTC plot.

Table 1. Optimization results for $N = 3, 4, \ldots, 9$; $D = 400$, $V = 40$, $k_a = 0.2$, and $k_e = 0.1$.

	Optimal Sampling Times (h)									Optimal values of the estimated PK Parameters and the GOC			
N	t_1	t_2	t_3	t_4	t_5	t_6	t_7	t_8	t_9	$\hat{AUC}$(0-24)	$\hat{C}_{\max}$	$\hat{t}_{\max}$	GOC_{opt}
3	6.02	14.04	24.00	-	-	-	-	-	-	83.08	5.00	7.00	0.16126
4	4.18	9.22	15.98	24.00	-	-	-	-	-	82.81	5.01	6.93	0.01711
5	3.21	6.91	11.47	17.45	24.00	-	-	-	-	82.74	5.00	6.93	0.00315
6	2.58	5.82	9.00	13.15	18.37	24.00	-	-	-	82.71	5.00	6.93	0.00087
7	2.22	4.31	6.74	9.80	13.86	18.63	24.00	-	-	82.70	5.00	6.93	0.00033
8	2.09	4.20	6.68	9.60	11.78	14.86	19.08	24.00	-	82.69	5.00	6.93	0.00014
9	1.47	3.48	6.07	8.65	10.01	12.84	16.24	19.98	24.00	82.69	5.00	6.93	0.00008

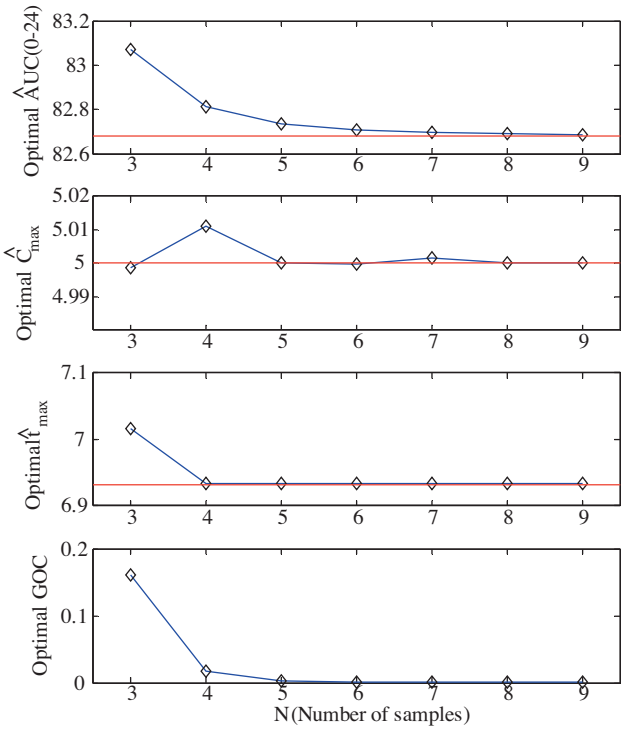

Figure 3. The optimal $A\hat{U}C$(0-24) , $\hat{C}_{\max}$, $\hat{t}_{\max}$ and GOC as a function of N (the number of samples) for the example case ($D = 400$, $V = 40$, $k_a = 0.2$, and $k_e = 0.1$). The horizontal lines in the upper 3 panels show the actual values of the PK parameters.

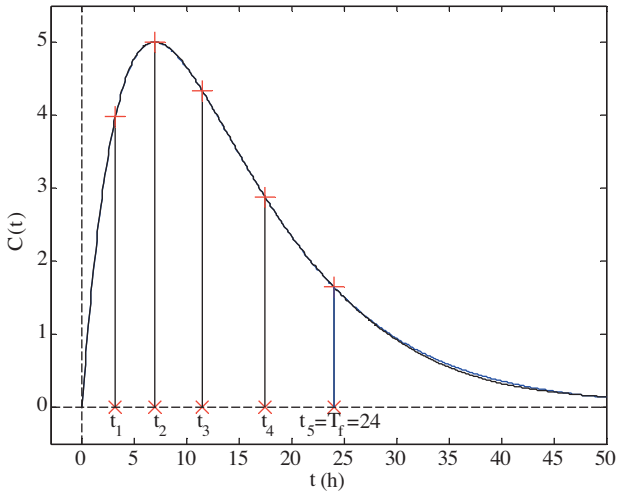

Figure 4. The arrangement/location of the optimal sampling times for the example case ($D = 400$, $V = 40$, $k_a = 0.2$, and $k_e = 0.1$) for $N = 5$, overlaid on the actual $C(t)$ plot.

3.2. Optimization for random k_e and fixed k_a

In this case, we assume that k_a is fixed as 0.2 and k_e is a random variable with a doubly truncated lognormal distribution, i.e. $\ln k_e \approx N\left(\mu_{k_e}, \sigma^2_{k_e}\right)$. Following Kong and Gonin (4), we have selected the parameters of this distribution as $\mu_{k_e} = 0.1$ and $\sigma_{k_e} = 0.05$. For the underlying compartmental model to be valid, k_a should be larger than k_e, we have therefore set an upper bound on k_e as $0.75\,k_a$. On the other hand, if k_e value is too small, meaning a long elimination time, then the value that we have assumed for the final time, i.e. 24 h, may not be appropriate. Therefore, to avoid such cases, we have also imposed a lower bound on k_e as $0.25\,k_a$.

We have generated 500 random k_e samples from this distribution to simulate a population, so that we can investigate the effect of subject variation on the optimal sampling times. For each value of k_e and N (from 3 to 9), we have carried out the optimization procedure to find the optimal sampling times as described in the previous section where both k_a and k_e were fixed. In each case, we have repeated the optimization attempts 10 times with different initial conditions to locate the global minimum. The total run time of this experiment was (5 seconds per case) $\times$ (10 runs each case) $\times$ (500 different k_e values) $\times$ (7 different N values) = 48.61 h. This way, we have obtained the distribution of optimal sampling times for a simulated population.

Figure 5 shows the mean and median of the optimal GOC values as a function of number of samples N. We again note that $N = 5$ is the optimal selection for the number of samples. Figure 6 shows the distributions of optimal sampling times for $N = 5$. Figure 7 provides a global picture for the results of the population simulation for $N = 5$, where the histograms of optimal $A\hat{U}C$, $\hat{C}_{\max}$ and $\hat{t}_{\max}$, along with the distributions of actual PK parameters are given. We note that the distributions of the estimated PK parameters are very close to those of the actual values.

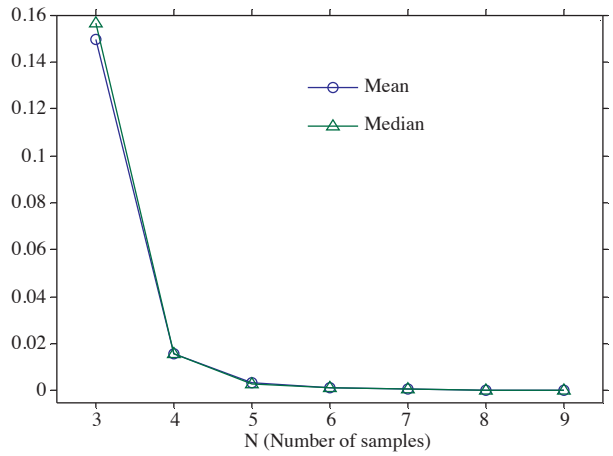

Figure 5. Mean and median of the optimal GOC values ($n = 500$) as a function of number of samples (N) for random k_e and fixed k_a.

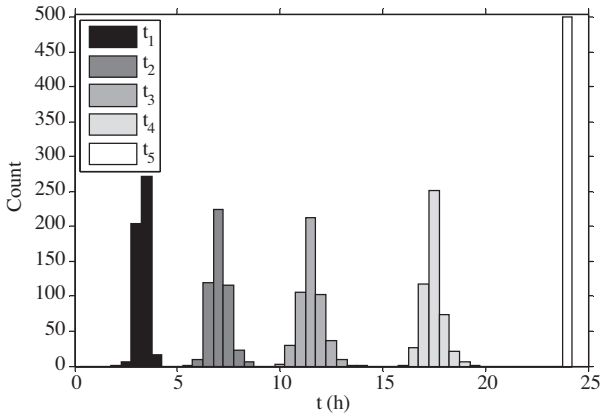

Figure 6. Histograms of the optimal sampling times for $N = 5$ (after $n = 500$ trials).

Table 2 presents summary statistics for the optimal sampling times. The means, medians, standard deviations (SD) and their standard errors (SE) were computed by using the bootstrap technique [17, 18] with 1000 bootstrap samples. We suggest the Mean $\pm$ 2SEM (SE of the Mean)for each sampling time as an "optimal

sampling interval." These optimal sampling intervals and their length in minutes are reported in the last two rows of Table 2.

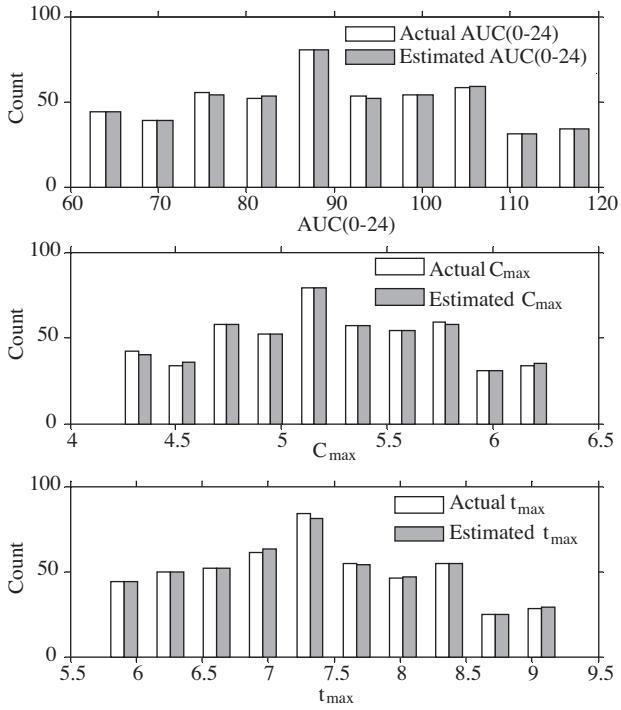

Figure 7. Histograms of optimal $\hat{AUC}$(0-24), $\hat{C}_{\max}$ and $\hat{t}_{\max}$ (n = 500) along with the distributions of the actual PK parameters for random k_e and fixed k_a.

Table 2. Summary statistics of the optimal sampling times for $N = 5$.

	Optimal sampling times (h)				
	t_1	t_2	t_3	t_4	t_5
Mean (SE)	3.273 (0.010)	7.039 (0.019)	11.552 (0.024)	17.471 (0.021)	24.000 (0.000)
Median (SE)	3.263 (0.003)	7.061 (0.012)	11.584 (0.024)	17.483 (0.014)	24.000 (0.000)
SD (SE)	0.244 (0.010)	0.443 (0.015)	0.532 (0.020)	0.455 (0.017)	0.000 (0.000)
Optimal Sampling Interval (Mean ± 2SEM)	3.252 - 3.294	7.000 - 7.077	11.505 - 11.599	17.429 - 17.512	24.000 - 24.000
Length (in minutes) of the Optimal Sampling Interval	2.503	4.595	5.652	4.962	0

4. Discussion and conclusion

Proper of estimation of PK parameters are of crucial importance in understanding drug dynamics and making bioequivalence decisions. In PK studies, the PK parameters estimated from a limited number of samples are compared, therefore the timing of the samples is very important to achieve accurate parameter estimates. In previous studies, piece-wise straight line fit approximation to the concentration-time-curve is used and the optimization of the sampling times is done based only on one of the PK parameters, which is the AUC mostly. Other optimal design strategies based on Fisher's information matrix suffer from the requirement of having

accurate prior information and large variances for the estimated optimal sampling times, especially when the number of samples is small. In this study, we have suggested a cubic spline approximation based method for PK parameter estimation and introduced a global optimality criterion (GOC) that can simultaneously consider all the PK parameters. We have considered several provisions to improve the spline approximation and obtained optimal sampling times by minimizing the GOC using sequential quadratic programming. Along with the estimation of optimal sampling times, we have also devised a technique to determine the optimal number of samples to be drawn for accurate and efficient/economical PK parameter estimation.

While forming the GOC, a time resolution of 1 min was used to find $\hat{C}_{\max}$ and $\hat{t}_{\max}$ values. By increasing this resolution, it should be possible to obtain better $C_{\max}$ and $t_{\max}$ estimates and therefore lower GOC values. However, as Figures 3 and 5 show clearly, it is possible to obtain very accurate PK parameter estimates even with this resolution.

After validating the proposed technique on a sample case where all the model parameters were fixed, we have assumed that k_e, which varies among individuals considerably, comes from a realistic density and studied the optimal sampling times for a population. We have obtained the optimal sampling times for each generated value of k_e and different N, i.e. number of samples. We have concluded that $N = 5$ is an optimal choice for the number of samples for the example and studied the distributions of optimal sampling times for this value of N. We therefore reported Mean $\pm$ 2SEM, which approximately corresponds to 95% confidence interval for the mean, for each sampling time as an optimal sampling interval. (As we forced the last sample t_N to be taken at 24 h exactly, there is not any interval associated with t_N. However, it is possible to relax this constraint and obtain an optimal sampling interval for t_N as well.) The use of optimal sampling intervals instead of optimal sampling times/instants has many convenient practical implications.

As for the future direction of our study, we will assume that all the model parameters come from a suitable realistic joint lognormal density whose parameters are determined from earlier tests. We will simulate or generate data (i.e., different k_a, k_e, and V combinations) from the assumed multivariate density and obtain the optimal sampling times for each case. Then, following the procedure we have introduced in this study, we will study the characteristics of the optimal sampling intervals that can be suggested for the whole population. Although we introduced our approach based on a one-compartmental model, the ideas involved in it are very general and therefore it can be applied to general PK models as well. We are also planning to evaluate the impact of optimal sampling time designs in bioequivalence studies in a future study.

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