Experimental Design and Efficient Parameter Estimation in Preclinical Pharmacokinetic Studies**

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Monte Carlo simulation technique used to evaluate the effect of the arrangement of concentrations on the efficiency of estimation of population pharmacokinetic parameters in the preclinical setting is described. Although the simulations were restricted to the one compartment model with intravenous bolus input, they provide the basis of discussing some structural aspects involved in designing a destructive ("quantic") preclinical population pharmacokinetic study with a fixed sample size as is usually the case in such studies. The efficiency of parameter estimation obtained with sampling strategies based on the three and four time point designs were evaluated in terms of the percent prediction error, design number, individual and joint confidence intervals coverage for parameter estimates approaches, and correlation analysis. The data sets contained random terms for both inter- and residual intra-animal variability. The results showed that the typical population parameter estimates for clearance and volume were efficiently (accurately and precisely) estimated for both designs, while interanimal variability (the only random effect parameter that could be estimated) was inefficiently (inaccurately and imprecisely) estimated with most sampling schedules of the two designs. The exact location of the third and fourth time point for the three and four time point designs, respectively, was not critical to the efficiency of overall estimation of all population parameters of the model. However, some individual population pharmacokinetic parameters were sensitive to the location of these times.

KEY WORDS: population pharmacokinetics; preclinical; destructive (quantic); simulation; experimental design; parameter estimation; design number; confidence intervals coverage.

INTRODUCTION

Most pharmacokinetic and toxicological studies are conducted according to standard guidelines, and no effort is made to optimize experimental protocols on the basis of sound pharmacokinetic knowledge (1). The number of samples to be collected may be limited to a large extent by the sample size in destructive (quantic) animal pharmacokinetic studies in which one animal supplies only one observation. In situations which allow for serial sampling the total amount of blood that can be withdrawn is limited. The balance, particularly in small animals (eg., rats and mice), between providing realistic pharmacokinetic data and increasing the sample size to unmanageable proportions is narrow. Although the number of blood samples and the spacing of sampling times can be easily controlled for efficient experimental design (2, 3), examples abound in the literature of poor sampling strategy in animal pharmacokinetic studies designed for parameter estimation (1, 4). In animal pharmacokinetic studies the sample size is usually fixed so that the arrangement of samples in time needs to be given adequate consideration.

Sampling times can be manipulated to improve the information content of the available concentration—time data. Obtaining measurements at informative times which will contain the maximum pharmacokinetic information about model parameters in acute individual studies have been discussed by various authors (3, 5, 6, 7, 8). Two sampling times are needed for the efficient estimation of model parameters, clearance (CL) and volume (V), of the one compartment model (6). However, there is no formal solution to the optimal sampling question in the population setting. Using Monte Carlo simulation in the population setting with multiple samples per subject, Al-Banna *et al* (9) examined the impact of two sampling times (an early and a late sampling time) and three sampling times (where the first and last samples were obtained at early and late times and the third time varied between the two) on parameter estimation. They concluded that efficient estimates of population mean parameters and their variances were obtained with the 3 point design.

To test the validity of this approach in the quantic sampling setting, a Monte carlo simulation study was carried out to investigate the effect of the arrangement of concentrations in time on the efficiency of estimation of population pharmacokinetic (PK) parameters and associated variability, given a fixed sample size. This was done using two different sampling strategies (the three time point design, and the four time point design), and comparing different sampling schedules within each design to determine the best sampling strategy.

METHOD

A monoexponential model with intravenous bolus dose (D) was assumed, and the concentration after drug administration at a time t was given by

$$C_j = f(p_j, D, t)(1 + \epsilon_j) \quad (1)$$

where f is the model predicting the true concentration in the jth animal and $p_j = (p_{1j}, p_{2j}, \ldots, p_{mj})$ are the m PK parameters in the jth animal. ϵ_j represents the residual departure of the model from the observed concentration available from the jth animal. σ_ϵ (the concentration measurement error or residual intra-animal variability) was set to 15% of the true

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concentration value in the simulation. The kth element of p_j is modelled as

$$p_{kj} = \theta_k + \eta_{kj} \quad (2)$$

where θ_k is the typical population value of p_{kj} and η_{kj} expresses the random difference between θ_k and p_{kj}. ϵ_j were assumed independent normally distributed with mean zero and variance σ^2. η_{kj} represents inter-animal variability, and were assumed to be independent normally distributed, with mean zero and variance σ^2_k. The inter-animal variability expressed in this form is additive to the population mean, and σ_k approximates the inter-animal standard deviation for associated parameters. No covariance was assumed between the elements of η. (Note that σ_ϵ could not be estimated since each experimental unit provided only one concentration—time point.)

Sampling Design

For the purpose of this study, sampling time ranged from as early as possible after the beginning of the experiment (t_{min} = 5 min) to some value (t_{end} = 240 min), the latest time that could be contemplated in actual experiment, taking into consideration the "average" $t_{1/2}$ (84 min) of the drug. 48 observations corresponding to 48 animals were used in each design. Two sampling designs were studied: the three, and four time point designs.

The Three Time Point Design

In this design t_{min} (first time point) and t_{end} (second time point) were fixed at 5 min and 240 (± 7.5) min, respectively, and the third sampling was varied between 30 and 210 (all ± 7.5) min after dose in steps of 30 min, yielding 7 sampling schedules shown in Table I. 16 animals were sampled at each time point.

The Four Time Point Design

In this case, t_{min} and t_{end} were fixed as in the previous cases at 5 min and 240 min, respectively. The third time point was fixed at 30 (± 7.5)min, while the fourth sampling time was varied between 60 and 210 (all ± 7.5) min in steps of 30 min (Table I). 12 animals were sampled at each time point.

Table I. Formulation of Sampling Schedules for the Three and Four Time Points Designs

Three Time Points Design				Four Time Points Design			
(t_1 (min)	t_3 (min)	t_2 (min)		t_1 (min)	t_3 (min)	t_4 (min)	t_{end}
	5	30	240	5	30	60	240
	5	60	240	5	30	90	240
	5	90	240	5	30	120	240
	5	120	240	5	30	150	240
	5	150	240	5	30	180	240
	5	180	240	5	30	210	240
	5	210	240				

Simulation and Data Structure

With the assumed structural and variance model parameters defined above, simulated data sets were generated for each sampling design using the method described by Bard (10). Briefly, individual CL values (CL_j's) were obtained by sampling from the population distribution (θ_{CL}, σ^2_{Cl}) using a random number generator. V_j's were similarly generated. Using the appropriate sampling time (t_j), the true concentration (C^*_j) was computed. A random error, proportional to C^*_j was then added to give the observed concentration (C_j). This was repeated for each animal comprising the data set. The lower limit of quantitation (LLQ) of C_j was set at 0.1 μg/ml, and C_j was not allowed below LLQ.

Population parameter values of the drug having the characteristics of avicin, a cytotoxic agent (11), were used for this simulation study. The typical population parameter values were CL = 1.3 ml/min; V = 162.5 ml. σ_{Cl}, σ_V, and σ_ϵ were set to 15%. 30 replicates of data sets were generated for each sampling schedule within a study design. Altogether, 390 data sets were generated for the two sampling designs and analyzed assuming zero covariance between parameters.

Analysis

Prediction Error

NONMEM (12) was used to estimate Cl, V, σ_{Cl}, σ_V for each design. Since this was not a study involving multiple sampling, σ_ϵ could not be estimated.

Given that the "true" parameter values were known, the efficiency with which each model parameter was estimated could be studied. Percentage prediction errors (%PE) were computed in order to express the accuracy and precision for all parameters on the same scale. Thus, for each run and for each parameter, the difference between the "true" value (θ^*_i) and the estimated value (θ_i) was expressed as a percentage of the "true" value.

$$\%PE = [(\theta_i - \theta^*_i)/\theta^*_i] \cdot 100 \quad (3)$$

The mean %PE for each of the 30 replicates was used as a measure of the accuracy with which each parameter was estimated. An estimate of the precision with which each parameter was estimated with a particular sampling schedule was obtained from the standard deviation of %PE, denoted "SD of %PE" (13). Estimates with SD of %PE ≤ 25% were accepted as precise. %PE was plotted across the designs for all parameter estimates. Statistical significance of nonzero %PE's was tested using the two-tailed t test.

Individual Confidence Interval Coverage for Parameter Estimates

A cut off rule was established as an aid to determining the impact of standard error on confidence interval (CI) coverage for a parameter estimate. For efficient estimation of CL and V, percent relative SE (%RSE) (i.e. $SE(\theta_i)/\theta_i)\cdot 100$) had to be ≤20%, while %RSE ≤ 50% was used for the variance parameters for any given run (14, 15). To determine the runs in a simulated data set which covered the "true" values, 99% confidence intervals were calculated, as a reason-

able approximation for confidence interval estimates to contain 95% of the estimates produced by NONMEM (16).

Bias in estimates production, and SE of estimates are some of the factors that affect confidence interval coverage. The confidence intervals (CIs) coverage table used for the presentation of the results of this study is divided into three sections, taking these factors into account as has been previously described (14, 15). The sections are:

Section I: "Success/Total" Ratio

This gives CIs coverage for parameter estimates when the cut off rule is not applied. The coverage here is primarily determined by bias (14, 15).

Section II: "Success—Excluded / Total—Excluded" Ratio

This shows coverage for interval estimates when the cut off rule is applied to both the numerator and denominator during confidence interval coverage computation. The estimates not used for the construction of these confidence intervals are herein referred to as "catastrophic" estimates. Thus, this section gives an indication of how good the coverage is if catastrophic estimates were deleted from the results.

Section III: "Success—Excluded / Total" Ratio

The coverage when the catastrophic estimates are excluded in the numerator for confidence interval coverage computation is provided in this section. This reveals the influence of SE on CI coverage. With this section the acceptability of an estimate can be judged in combination with the accuracy with which such an estimate is produced.

Joint Confidence Intervals Coverage for All Parameter Estimates

Since model parameters are estimated as a set, this was considered in the interpretation of the results of this study. The joint confidence interval coverage for all parameter estimates as has been previously described (14, 15) was computed as an aid to the interpretation of the efficiency with which all parameters were estimated. Briefly, the approximate 99% joint confidence interval coverage for all parameter estimates was computed from the count of the number of runs containing "true" parameter values for all parameters of the model. Thus, 99% joint confidence intervals coverage for parameter estimates were determined for each combination of variability studied.

The chi-squared test ($p < 0.01$) was used to determine whether the individual or joint confidence intervals coverage for parameter estimates was significantly different from the expected values (e.g., 0.95 and 0.81 (4 parameters only), for the individual and joint confidence intervals coverage, respectively, for the parameters of the one compartment model with IV bolus injection).

Design Number

The design number is a statistic that is used for judging the efficiency of model parameter estimation either individually or jointly (14, 15). It can be used to choose the best sampling strategy amongst a set of similar sampling schedules within a study (14, 15). The design number, Φ_{ir} for each parameter is defined:

$$\Phi_{ir} = \Phi_i / \text{Max}\ (\Phi_i) \quad (4)$$

where Max (Φ_i) is the maximum value of the unscaled design number for a given parameter from all runs across sampling schedules, and Φ_i (unscaled design number) is given by:

$$\Phi_i = \{(\theta_i - \theta^*_i)/\ \theta^*_i)^2\} \cdot SE(\theta_i)/\theta^*_i \quad (5)$$

Φ_{ir} is Φ_i rescaled to give equal weighting to all parameters.

From Eq. (5), Φ_i defines a design number for each parameter viewed independently. Since model parameters are estimated as a set and one is interested in choosing a sampling design which produces the most efficient parameter estimates, combining all the design numbers for individual parameters yields the "overall design number". Thus, the overall design number (Φ_r) is computed as follows:

$$\Phi_r = 1/n \sum_{i=1}^{n} \Phi_{ir} \quad (6)$$

where n is the number of estimated parameters.

The design number, Φ_{ir}, and the overall design number, Φ_r calculated using Eq.'s (4) and (6) were used in comparing the efficiency of parameter estimation from the different two sampling times designs using the Kruskal Wallis ANOVA ($p < 0.05$) with multiple comparisons. The most efficient parameter estimate(s) is (are) obtained with the design yielding the lowest average rank of Φ_{ir} (Φ_r).

Correlation Plots (17)

Since the simulated data were generated assuming zero covariance, a high degree of parameter estimation efficiency would be associated with low correlation coefficients between parameter estimates. Star plots of correlation between estimates were generated as aids for judging the efficiency of parameter estimation. The plots were generated for each sampling schedule within a given sampling design. The star points represent a correlation of +1, and the center of the polygon corresponds to a correlation of −1. Zero correlation is midway between +1 and −1 on the points of the polygon (in this case a hexagon, corresponding to the number of off-diagonal elements of the NONMEM correlation matrix). An efficient sampling schedule would be expected to yield correlation coefficients as close to zero as possible. A pair-wise correlation coefficient of ± 0.75 is termed high, otherwise, it is termed low.

RESULTS

The Three Time Point Design

The estimates of CL and V were precise and minimally negatively biased for most sampling schedules (Fig. 1a & b). The most precise estimate of V was obtained when the third sampling time was located at 60 min, while a location of the third sampling time at 210 min yielded the least precise estimates. Although some of the estimates of CL and V were statistically significantly biased, the biases did not exceed 5%. Except for the location of the third sampling time at 30 or 60 min there was a general trend for the bias in the esti-

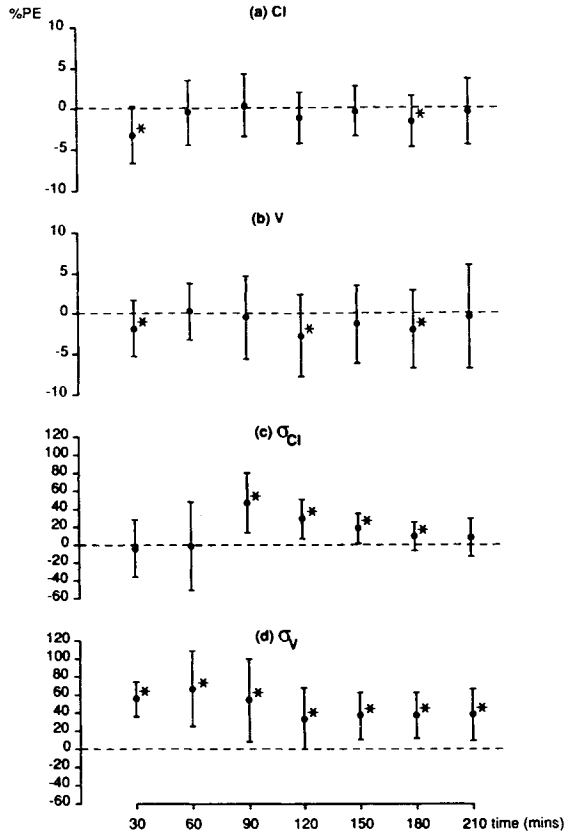

Fig. 1. Bias and precision expressed as %PE (mean ± standard deviation, respectively) for estimated parameters. The horizontal axis represents the different time points for the three time point design. Each vertical bar expresses the bias and precision of the population parameter estimate. Significant ($p < 0.05$) biases are indicated by asterisks. CL = clearance, V = Volume, σ_{CL} = interanimal variability in CL, σ_V = interanimal variability in volume.

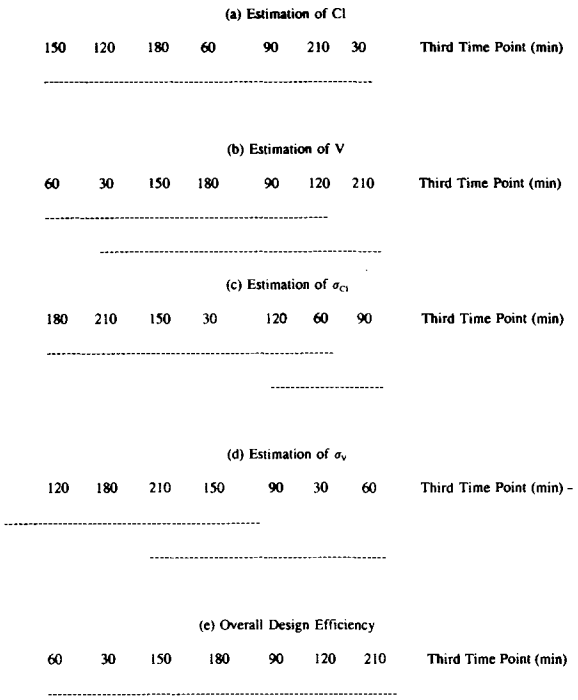

Fig. 2. Summary of significant differences in the efficiency (measured with design number) with which parameters were estimated using the three time point design. Rank order of design numbers increased from left to right. See **Methods** for the computation of the design number. The lines beneath the sampling time values indicate a lack of significant difference (using Kruskal Wallis ANOVA) between those sampling schedules. CL = clearance, V = Volume, σ_{CL} = interanimal variability in CL, σ_V = interanimal variability in volume.

mation of σ_{CL} to decrease as the third sampling time was located at later times (Fig. 1c). σ_{CL} estimates were acceptably precise when the third sampling time was located at ≥ 120 min, while the most imprecise estimates were obtained with the third sampling time at 60 min. σ_V estimates were significantly positively biased and imprecise (Fig. 1d). An acceptably precise estimate of σ_V was obtained with the third sample at 30 min.

The results of the multiple comparisons of the design numbers obtained for the various sampling schedules designs were summarized in Fig. 2. The design numbers for the sampling designs are ranked in increasing order from left to right with the design yielding the least efficient parameter estimate having the highest average rank order. Where two designs are connected with a line, this indicates that there was no significant difference in the efficiency with which a parameter is estimated with the designs considered. When two designs are unconnected with each other by a line, this is an indication that there was a significant difference in the efficiencies with which the parameters were estimated. Using the design number approach to determine the efficiency of parameter estimation showed that there was no significant difference in the efficiency of estimation of CL (Fig. 2a). V was best estimated when the third sampling time was at 60 min (Fig. 2b), but this was only significantly better than the estimates of V when the third sampling time was set at 210 min. The best and worst estimates of σ_{CL} were obtained with the third time located at 180 and 90 min, respectively. σ_V was poorly estimated (Fig. 2d). Overall, the exact location of the third sampling time was not critical for efficient estimation of model parameters since there was no significant difference in the efficiency with which parameters were estimated with the different sampling schedules of the three time point design (Fig. 2e).

Without excluding catastrophic NONMEM runs, the confidence interval coverage was good for all sampling schedules (Table II, Section I). Excluding catastrophic estimates in the numerator during CI coverage computation, the coverage for σ_{CL} was reduced for the design with the third sampling time at 60 min and significantly so at 30 min (Table II, section III). A similarly reduced coverage was obtained for the joint confidence intervals for parameter estimates with these two sampling schedules compared to other sampling schedules. The coverage for σ_{CL} and joint confidence intervals obtained for the design in which the third sample was at 30 min was significantly different from the expected values of 0.95 and 0.81, respectively (Table III, Section III).

The sampling schedules with the third time point at 60 and 90 min yielded two NONMEM runs (6.7%) with high correlation coefficients (i.e. $r > 0.75$) (Fig. 3). η_1 and θ_1, η_2 and θ_2 were the parameters highly correlated for the sam-

Table II. 99% Confidence Interval Coverage for Individual Parameter Estimates Obtained with the Three Time Point Design

Sampling Times (min)	Fraction of Coverage Including True											
	Section I Success / Total				Section II (Success—Excluded) / (Total—Excluded)				Section III (Success—Excluded) / Total			
	CL	V	σ_{Cl}	σ_v	CL	V	σ_{Cl}	σ_v	CL	V	σ_{Cl}	σ_v
30	30/30	29/30	29/30	19/30	30/30	29/30	10/11	19/30	30/30	29/30	10/30*	19/30
60	30/30	30/30	29/30	27/30	30/30	30/30	20/20	23/26	30/30	30/30	20/30	23/30
90	30/30	30/30	30/30	30/30	30/30	29/29	26/26	23/23	30/30	29/30	26/30	23/30
120	30/30	28/30	29/30	30/30	30/30	28/30	29/30	30/30	30/30	28/30	29/30	30/30
150	29/30	28/30	29/30	29/30	29/30	28/30	29/30	29/30	29/30	28/30	29/30	29/30
180	30/30	29/30	30/30	29/30	30/30	29/30	24/24	27/28	30/30	29/30	24/30	27/30
210	30/30	28/30	30/30	30/30	30/30	28/30	23/23	28/29	30/30	28/30	23/30	28/30

* $p < 0.01$ (from X^2).
Success = number of NONMEM runs in which CI contain "true" parameter estimates.
Excluded = NONMEM runs with catastrophic estimates (see methods).

pling schedule with the third time point at 60 min, while η_2 and $\theta 2$, and η_1 and η_2 were the ones highly correlated for the design with the third time at 90 min. In general, there were no marked differences between the correlation plots of the different sampling schedules (Fig. 3).

The Four Time Point Design

CL estimates were least biased and most precise when the fourth sampling time was set at 210 min (Fig. 4a). On the other hand, the least biased and most precise estimates of V were obtained with a design in which the fourth time was set at 60 min (Fig. 4b). The biases in CL and V did not exceed 5%. The estimates of σ_{CL} were almost unbiased when the fourth time point was at 60 or 210 min (Fig. 4c). As was the case with the three time point design, there was a general trend towards decrease in bias in the estimation of σ_{CL} as the fourth sampling time was set at late times. The estimates of σ_{CL} were imprecise, except for the design with the fourth sampling time set at 180 min. The most imprecise estimates were obtained when the fourth time point was set at 60 min.

Table III. 99% Joint Confidence Intervals Coverage for All Parameter Estimates Obtained with the Three Time Point Design

Sampling Times (min)	Fraction of Coverage Including True		
	Section I Success / Total	Section II (Success—Excluded) / (Total—Excluded)	Section III (Success—Excluded) / Total
	JCIC	JCIC	JCIC
30	29/30	8/11	8/30*
60	29/30	18/20	18/30
90	30/30	21/21	21/30
120	29/30	28/30	28/30
150	29/29	25/30	25/30
180	30/30	25/30	21/30
210	30/30	21/22	20/30

* $p < 0.01$ (from X^2).
JCIC = Joint confidence interval coverage for all parameter estimates.

σ_V estimates were significantly positively bias and imprecise.

Using the design number approach to examine the efficiency of parameter estimation, it was found that CL was most efficiently estimated when the fourth time point was set at 210 min (Fig. 5a). V was estimated with similar efficiency with all designs (Fig. 5b). However, the best (least biased and most precise) estimate of V was obtained with the fourth time at 60 min. σ_{CL} was best estimated with the fourth time set at 180 min, and least efficiently estimated with the fourth set at 90 min (Fig. 5c). The efficiency of estimation of σ_V obtained with different sampling schedules was indistinguishable (Fig. 5d). Equally, there was no significant difference in the overall efficiency with which all the parameters were estimated (Fig. 5e).

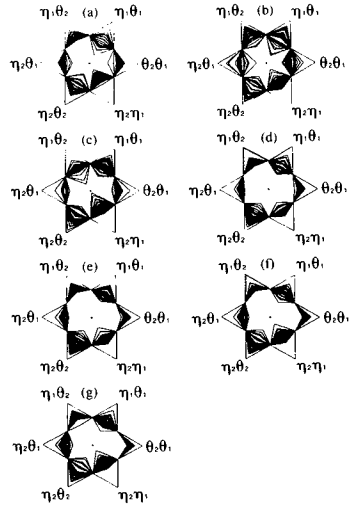

Fig. 3. Star plots for visualizing correlation matrices from the three time point design. The third time points are: (a) 30 min, (b) 60 min, (c) 90 min, (d) 120 min, (e)150 min, (f) 180 min, and (g) 210 min. Each of the plots of the signed correlations were generated from 30 simulations. The center of the hexagon corresponds to a correlation of -1, while the star points represent a correlation of $+1$. Zero correlation is midway between $+1$ and -1 on the points of the hexagon, and this corresponds to the number of off-diagonal elements of the NONMEM correlation matrix.

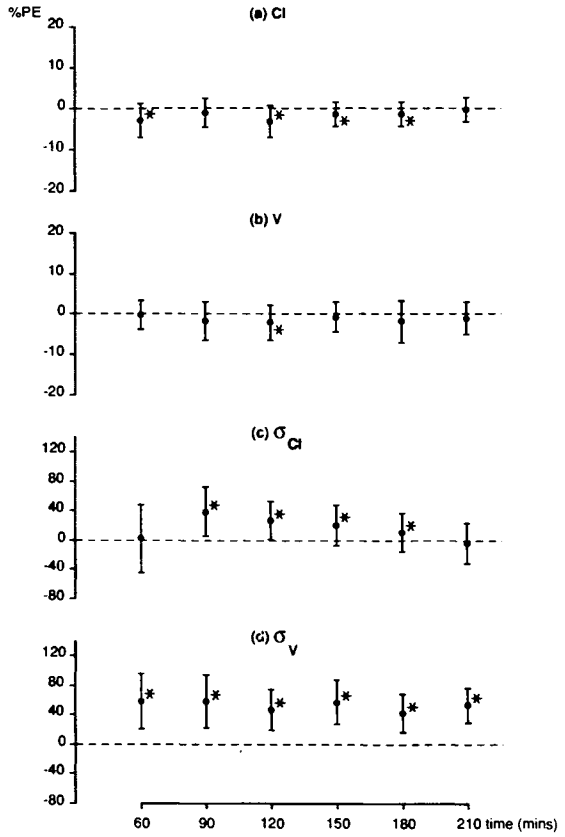

Fig. 4. Bias and precision expressed as %PE (mean ± standard deviation, respectively) for estimated parameters. The horizontal axis represents the different time points for the four time point design. Each vertical bar expresses the bias and precision of the population parameter estimate. Significant ($p < 0.05$) biases are indicated by asterisks. CL = clearance, V = Volume, σ_{CL} = interanimal variability in CL, σ_V = interanimal variability in volume.

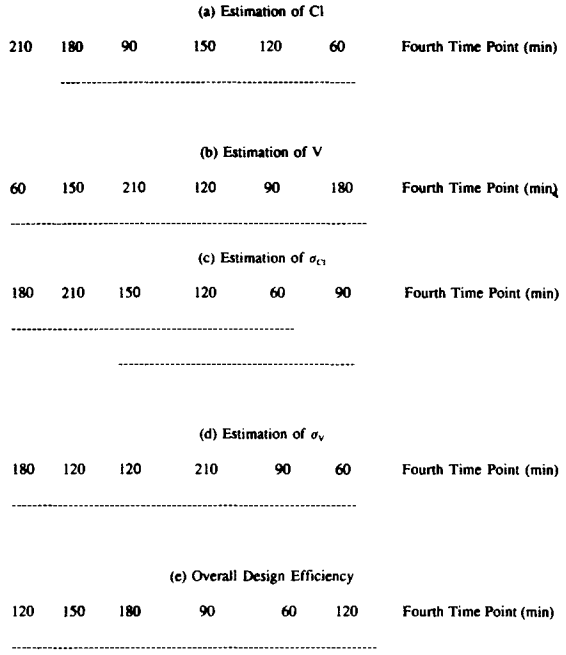

Fig. 5. Summary of significant differences in the efficiency (measured with design number) with which parameters were estimated using the four time point design. Rank order of design numbers increased from left to right. See **Methods** for the computation of the design number. The lines beneath the sampling time values indicate a lack of significant difference (using Kruskal Wallis ANOVA) between those sampling schedules. CL = clearance, V = Volume, σ_{CL} = interanimal variability in CL, σ_V = interanimal variability in volume.

Good coverage was obtained for individual and joint confidence intervals for parameter estimates with all sampling schedules (Table IV, Section I & II). When runs with catastrophic estimates were excluded in the numerator to examine the influence of standard errors on confidence intervals coverage, the design with the fourth time at 60 min was found to yield estimates of σ_{CL} with a confidence interval coverage significantly less than the expected value of 0.95 (Table IV, Section III). This sampling schedule yielded 23 runs with catastrophic estimates of σ_{CL}. On the other hand, the design with the fourth sample at 210 min also produced estimates with reduced coverage, but this was not significantly different from the expected value of 0.95. In this case, 11 NONMEM runs had catastrophic estimates of this parameter. Apart from the design with the fourth time at 60 min, the other sampling schedules produced estimates of parameters whose joint coverage was not significantly different from the expected value of 0.81 (Table V).

Only the sampling schedule with the fourth time point at 60 min yielded three NONMEM runs (10%) with high correlation coefficients (i.e. $r > 0.75$) between η_1 and θ_1, η_2 and θ_2, and η_1 and η_2 (Fig. 6). This not withstanding, the correlation plots for all sampling schedules for the four time point design were similar.

DISCUSSION

The Three Time Point Design

This sampling design with its distribution of samples produced the most precise estimates of CL and V when the third time was at late and early times, respectively, where more information was available for the estimation of these parameters. The biases in CL and V did not exceed 5%, and are not detrimental to the estimates. It is well known and often shown that nonlinear estimates are biased (9, 13). However, in this case and in many situations the biases are small relative to the standard deviation. That some estimates of CL and V were statistically significantly different from zero are an indication that the sample size was large enough to detect bias. Also, the negative bias associated with the estimation of CL and V may either be due to estimation error or the nature of the NONMEM program because the fixed effect (structural model) parameters enter the regression model nonlinearly and the random effect parameters linearly by first order approximation (14). Although estimates of σ_{CL} obtained with the third sample at 30 and 60 min, respectively, were relatively unbiased, these estimates were associated with large percent relative standard errors. The improvement in precision when the third time was set at late times was due to the increased amount of information (data points) available for σ_{CL} (CL) estimation. More precise estimates of σ_V obtained with the third time at 30 min was a result of having more data points in the early times. The positive bias

Table IV. 99% Confidence Interval Coverage for Individual Parameter Estimates Obtained with the Four Time Point Design

Sampling Times (min)	Fraction of Coverage Including True											
	Section I Success / Total				Section II (Success—Excluded) / (Total—Excluded)				Section III (Success—Excluded) / Total			
	CL	V	σ_{Cl}	σ_v	CL	V	σ_{Cl}	σ_v	CL	V	σ_{Cl}	σ_v
60	29/30	30/30	30/30	25/30	29/30	30/30	7/7	22/26	29/30	30/30	7/30*	22/30
90	30/30	30/30	30/30	29/30	30/30	29/30	22/23	24/30	30/30	29/30	22/30	24/30
120	29/30	29/30	29/30	27/30	29/30	29/30	29/30	27/30	29/30	29/30	29/30	27/30
150	30/30	30/30	28/30	28/30	30/30	30/30	26/28	28/29	30/30	30/30	26/30	28/30
180	30/30	29/30	30/30	27/30	30/30	29/30	24/24	27/30	30/30	29/30	24/30	27/30
210	30/30	30/30	26/30	26/30	30/30	30/30	15/19	26/30	30/30	30/30	15/30	26/30

* $p < 0.01$ (from X^2).
Success = number of NONMEM runs in which CI contain "true" parameter estimates.
Excluded = NONMEM runs with catastrophic estimates (see methods).

associated with the estimation of σ_V with all designs and σ_{Cl} for some designs, was due to due to the lack of information in the data sets about concentration measurement error (σ_ϵ), since NONMEM was estimating composite inter-animal variability and concentration measurement error.

The exact location of the third sampling time was inconsequential to the estimation of CL. On the contrary, the location of the third time at early times (≤ two thirds the $t_{1/2}$, e.g. 30 and 60 min) led to more efficient estimation of V. Location of the third sampling time at any time greater than 1.4 times the $t_{1/2}$ of the drug led to efficient estimation of CL. The poor estimates of σ_V obtained with all designs seems to be a characteristic of quantic pharmacokinetic studies (14, 15). The similar efficiency of estimation of all population PK parameters obtained with all sampling schedules of the three time point design investigated indicated that the exact location of the third time was not critical.

Bias and precision are some of the factors which determine the properties of interval estimates. The interplay of these factors produced CIs for fixed effect parameter estimates which had coverage near the expected value of 0.95. The reduced confidence interval coverage obtained for the estimation of σ_V with the design having the third time at 30 min was due to the associated bias. On the other hand, the significantly reduced coverage obtained for σ_{CL} estimates with designs having the third sampling time at 30 or 60 min, when NONMEM runs with catastrophic estimates were excluded in the numerator during confidence intervals coverage computation (to reveal the influence of standard error on confidence intervals coverage), indicated that the estimates obtained for this parameter were not very reliable. The good coverage obtained for σ_{CL} irrespective of the manner in which confidence intervals coverage were computed using other sampling schedules indicated that those estimates were reliable. Apart from the design with the third time at 30 min, the joint confidence intervals coverage for parameter estimates were good.

With the sampling schedules considered here, the exact location of the third time was not critical to the efficiency of population pharmacokinetic parameters estimation, and the

Table V. 99% Joint Confidence Intervals Coverage for All Parameter Estimates Obtained with the Four Time Point Design

Sampling Times (min)	Fraction of Coverage Including True		
	Section I Success / Total	Section II (Success—Excluded) / (Total—Excluded)	Section III (Success—Excluded) / Total
	JCIC	JCIC	JCIC
60	24/30	7/7	7/30*
90	29/30	17/23	17/30
120	24/30	24/30	24/30
150	26/29	23/30	23/30
180	26/30	21/24	21/30
210	22/30	14/19	14/30

* $p < 0.01$ (from X^2).
JCIC = Joint confidence interval coverage for all parameter estimates.

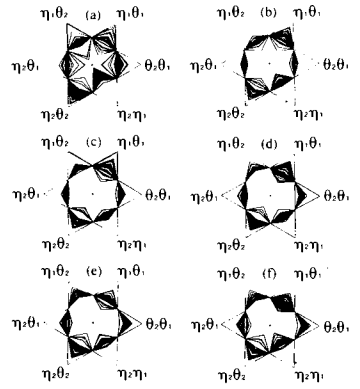

Fig. 6. Star plots for visualizing correlation matrices from the four time point design. The fourth time points are: (a) 60 min, (b) 90 min, (c) 120 min, (d)150 min, (e) 180 min, and (f) 210 min. Each of the plots of the signed correlations were generated from 30 simulations. The center of the hexagon corresponds to a correlation of −1, while the star points represent a correlation of +1. Zero correlation is midway between +1 and −1 on the points of the hexagon, and this corresponds to the number of off-diagonal elements of the NONMEM correlation matrix.

lack of any notable high correlation between parameter estimates contributed to this. This is evident in the similarity of the correlation plots (Fig. 3). The results obtained in the present study are similar to the results of the simulation study which involved multiple sampling of subjects reported by Al-Banna et al (9). The study design was that of a one compartment model with I.V. bolus administration in which subjects were sampled thrice (i.e. the first and second time points fixed, while the third time point was varied) and the exact location of the third time point was not critical to parameter estimation efficiency.

The Four Time Point Design

This design was studied to examine the impact of less intensive sacrifice of animals at a given time point on parameter estimation efficiency. The most efficient estimate of CL was obtained with the fourth time at 210 min where more information was contained in the data sets for this parameter. V was estimated with similar efficiency (similar bias and precision) with all sampling schedules. The first two time points supplied all the needed information about V such that the addition of an extra time point did not provide any significant improvement in the estimation of this parameter. As with the three time point design, the negative bias associated with the estimation of CL and V may either be due to estimation error or the nature of the NONMEM program because the fixed effect (structural model) parameters enter the regression model nonlinearly and the random effect parameters linearly by first order approximation (14). σ_{CL} was more efficiently estimated with the fourth sample at ≥ 1.4 times the $t_{1/2}$ of the drug, since this provided more information on this parameter. The efficiency of estimation of this parameter with the fourth sample at 60 min, although not significantly different from the results with the fourth time at 120, 150, 180, and 210 min, was not acceptable. This was due to this sampling schedule having 23 NONMEM runs with percent relative standard error > 50%. The similar poor efficiency with which all designs estimated σ_V is a consequence of the quantic study design. The exact location of the fourth sample was not critical in the overall estimation of parameters. The specification of two samples at not greater than one third the elimination $t_{1/2}$ of the drug, with the last sample at approximately three times the $t_{1/2}$ of the drug contributed to this observation.

The predominant factor governing confidence intervals coverage for the variance parameters was standard errors. Large standard error of estimates was responsible for the significantly reduced coverage of σ_{CL} estimates and the joint confidence intervals for parameter estimates with the design having the fourth time at 60 min.

The lack of notable high correlation between parameter estimates seen in the similarity of the correlation plots (Fig. 6) contributed to the lack of significant difference in the overall efficiency with which model parameters were estimated. Although the exact location of the fourth sample was not critical, the specification of the fourth sample at ≥ 2.5 times the $t_{1/2}$ of the drug would result in more efficient parameter estimation.

In quantic studies involving the use of the one compartment model with IV bolus administration in which a two time point design was used, it was reported that the location of the first time point at less than $0.06\ t_{1/2}$ and the second time point at $\geq 1.4\ t_{1/2}$ of the drug resulted in efficient estimation of population pharmacokinetic parameters (15, 16). While the appropriate arrangement of concentrations in time in quantic pharmacokinetic studies is crucial for efficient parameter estimation with the two time point design (15, 16), the results of this study show that for the one compartment pharmacokinetic model with IV bolus administration model considered here, once the first and last sampling times have been located at < 0.06 and ≈ 3 times the $t_{1/2}$ of the drug, the addition of the third time point improved parameter estimation to the extent that the location of the third time point is not important in the overall estimation of model parameters. The four time point design is not markedly better than the three time point design in overall efficiency. For efficient use of time and resources, therefore, the three time point design would provide good estimates of parameters.

In summary, the sampling designs considered in this study can be contrasted with the traditional sampling design in which 3 to 6 animals are sacrificed per time point over 10 to 12 time points. Using the traditional sampling design with 10 time points, 15% level of inter-animal variability and the same population parameters as the ones used in this study it was found that at least 60 animals (6 animals / time) was needed for efficient estimation of population parameters (15) compared to 48 in this study (16 animals/ time at 3 time points). The sampling designs considered in this study do not only yield efficient designs for quantic animal pharmacokinetic studies but would also lead to fewer number of animals (in this case 48) being used when compared with numbers used in traditional designs. Savings in time and labor cost are added benefits.

The three and four time point sampling as well as the two time point design (14) yielded parameter estimates which for the most part were not highly correlated with each other in contrast with the traditional design which yielded some parameter estimates with high pair-wise correlations (15).

In contrast to the work of Al-Banna *et al* (9) in which multiple sampling of subjects (assuming intersubject variability of 15%) was employed permitting the estimation of intrasubject variability, intra-animal variability could not be estimated in this destructive sampling study because each animal was sampled once. The similarity between this study and that of Al-Banna *et al* (9) is the fact that the existence rather than the timing of the third time point was crucial for efficient parameter estimation.

Although the results are depicted for a drug with a short $t_{1/2}$, the findings are applicable to any drug that exhibits a one compartment model kinetics with quantic sampling and administered by IV bolus.

The 15% level of inter-animal variability was chosen to provide a basis for examining the structural aspects involved in designing a quantic sampling animal pharmacokinetics study with a fixed sample size. The results of this study may not be extrapolated to situations with higher levels of inter-animal variability. However, a 15% level of inter-animal variability is not unrealistic in homogenous population of rats.

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