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Featured Article

Performance of an Iterative Two-Stage Bayesian Technique for Population Pharmacokinetic Analysis of Rich Data Sets

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Purpose. To test the suitability of an Iterative Two-Stage Bayesian (ITSB) technique for population pharmacokinetic analysis of rich data sets, and to compare ITSB with Standard Two-Stage (STS) analysis and nonlinear Mixed Effect Modeling (MEM).
Materials and Methods. Data from a clinical study with rapacuronium and data generated by Monte Carlo simulation were analyzed by an ITSB technique described in literature, with some modifications, by STS, and by MEM (using NONMEM). The results were evaluated by comparing the mean error (accuracy) and root mean squared error (precision) of the estimated parameter values, their interindividual standard deviation, correlation coefficients, and residual standard deviation. In addition, the influence of initial estimates, number of subjects, number of measurements, and level of residual error on the performance of ITSB were investigated.
Results. ITSB yielded best results, and provided precise and virtually unbiased estimates of the population parameter means, interindividual variability, and residual standard deviation. The accuracy and precision of STS was poor, whereas ITSB performed better than MEM.
Conclusions. ITSB is a suitable technique for population pharmacokinetic analysis of rich data sets, and in the presented data set it is superior to STS and MEM.

KEY WORDS: Bayesian analysis; data analysis; mixed effect modeling; Monte Carlo simulation; population pharmacokinetics.

INTRODUCTION

Over the last two decades population pharmacokinetics has become a major topic in the field of pharmacokinetics (1). The reasons for the shift from pharmacokinetic analysis in the individual to the description of pharmacokinetics in a population are closely related to the relatively large variability in pharmacokinetics between individuals. For adequate drug treatment in individual patients, therapeutic drug monitoring techniques by Bayesian feedback have been developed (2–4). These procedures require reliable population pharmacokinetic data. In addition, analysis of the influence of covariates (e.g., body weight, body surface area, age, gender, creatinine clearance, concomitant diseases and medication) may reduce the uncertainty in the predicted individual pharmacokinetic behavior, and thus improving the precision of drug therapy.

Population pharmacokinetics has focused mainly on the analysis of sparse data from a large group of subjects, i.e., where only a few blood samples (typically 1 to 4) are taken from each individual, allowing assessment of the influence of covariates. On the other hand, in studies with a limited number of patients, a study design with rich data sampling (typically, 10 to 20 measurements in each subject, or three to five measurements for each parameter) is often more appropriate, e.g., to develop a pharmacokinetic model. The population analysis of rich data from a relatively small group of subjects (typically, 6 to 20) has received much less attention in literature. The Standard Two-Stage (STS) analysis is the traditional approach for rich data, probably due to the simplicity and the similarity to the usual procedure in descriptive statistics, i.e., calculation of means and standard deviations of parameters from a set of subjects. However, the application of STS has some obvious disadvantages, and even for rich data sets, STS overestimates the interindividual variability of model parameters.

Over the last decades several techniques have been described to allow population pharmacokinetic analysis, including nonlinear Mixed Effect Modeling (MEM; implemented in the program NONMEM) (1), nonparametric methods (NPML, NPEM, NPAG) (4), parametric expecta-

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ABBREVIATIONS: AIC, Akaike information criterion; ITSB, iterative two-stage Bayesian; ME, mean error; MEM, (nonlinear) Mixed effect modeling; RMSE, root mean squared error; STS, standard two-stage.

tion-maximization methods (MCPEM, PEM) (5,6), Iterative Two-Stage Bayesian (ITSB) methods (7–10), and Bayesian analysis using Markov chain Monte Carlo techniques (11). Recently a comparison between various methods was performed simulated using sparse data sets (12).

In the present paper a procedure for ITSB population pharmacokinetic analysis is described, and evaluated by comparison with STS and MEM analysis, using a rich data set from a clinical study with rapacuronium and data generated by Monte Carlo simulation. For the ITSB method some aspects of experiment design and data characteristics, i.e., number of subjects, number of measurements, degree of measurement error, and covariance between parameters, are also investigated.

MATERIALS AND METHODS

Data

Clinical Data Set

Data were taken from a clinical PK-PD modeling study of rapacuronium performed in our department (13). In short, ten patients (eight male, two female; aged 26–64 years; body weight 62–88 kg; ASA class I–III), undergoing elective surgery, received thiopental-fentanyl-isoflurane-O_2-N_2O anesthesia. Rapacuronium was administered as a short-term infusion, median (range) duration 4.8 (2.5–5.7) min and dose 1.01 (0.58–1.22) mg kg^{-1}. Arterial blood samples were obtained, 15 to 19 from each patient, and plasma rapacuronium concentrations were determined by high-performance liquid chromatography. The plasma concentration data are shown in Fig. 1.

Data Generation by Monte Carlo Simulation

One hundred synthetic data sets were generated by Monte Carlo simulation. The study design and population model were close to that of the clinical study (13). In the simulation study, a dose of 1 mg kg^{-1} was administered to a panel of ten subjects (body weight 70 kg) by an intravenous infusion over 4 min. Plasma concentration was measured at 18 time points (at 2, 3, 4, 6, 8, 10, 12, 15, 20, 30, 45, 60, 90, 120, 180, 240, 300, and 360 min after the start of the infusion). The population pharmacokinetic parameters of a mammillary three-compartment model with elimination from the central compartment are listed in Table I. Pharmacokinetic parameters were log-normally distributed, and covariance between the parameters was assumed to be absent. Measurement errors were log-normally distributed with a standard deviation of 0.1 (corresponding to a coefficient of variation of 10%).

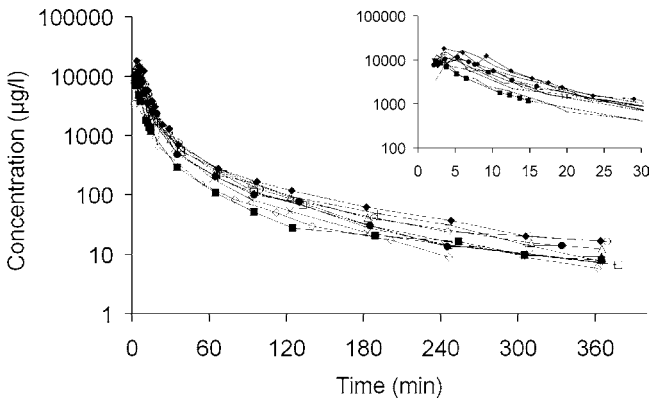

Fig. 1. Plasma concentration profiles of ten subjects receiving a short-lasting infusion of rapacuronium. The inset expands the first 30 min.

Population Analysis

Iterative Two-Stage Bayesian (ITSB) Analysis

The procedure for ITSB analysis is similar to the method described by Mentré and Gomeni (9) and by Bennett and Wakefield (10), with a few modifications. A modification of the method with a fixed value for the residual standard deviation has been described earlier by Steimer *et al.* (7), and a similar procedure is applied in the program IT2B (USC*-PACK, Laboratory of Applied Pharmacokinetics, Los Angeles, CA, USA) (4).

In short, the ITSB procedure works as follows. Rough estimates of each population parameter (mean and standard deviation) and residual standard deviation (σ_{res}) are assumed to be known. Estimates from STS analysis are usually suitable. In the first stage, the individual pharmacokinetic parameters of each patient are obtained from a Bayesian nonlinear fitting procedure, using the measurements in that patient as well as the estimated population parameters and σ_{res} as Bayesian priors. In the second stage, the population mean and standard deviation of each parameter are calculated from the individual parameters, and σ_{res} is estimated. The first stage is then repeated using the new population parameters as Bayesian priors. Both stages are repeated until the new population parameters and σ_{res} converge, i.e., are similar to the values of the previous cycle.

Stage 1. For each subject the individual pharmacokinetic parameters are estimated from the observed concentration data (measurements) by weighted nonlinear Bayesian analysis using the Marquardt algorithm (14) for the minimization of the objective function O_k, i.e., minus two

Table I. Population Parameters for Monte Carlo Simulations

Variable	Unit	Mean[a]	σ^b
CL	ml min^{-1} kg^{-1}	7.29	35.3
V_1	ml kg^{-1}	52	23.1
CL_{12}	ml min^{-1} kg^{-1}	2.43	47.1
V_2	ml kg^{-1}	43	34.9
CL_{13}	ml min^{-1} kg^{-1}	0.73	25.5
V_3	ml kg^{-1}	92	32.6
σ_{res}[b]	%	10	–

CL Clearance from central compartment, V_1 volume of central compartment, CL_{12} clearance between central and shallow peripheral compartments, V_2 volume of shallow peripheral compartment, CL_{13} clearance between central and deep peripheral compartments, V_3 volume of deep peripheral compartment, σ_{res} residual standard deviation.

[a] Geometric mean (mean of log-normal distribution).

[b] Expressed as coefficient of variation, in percent (standard deviation of log-normal distribution corresponds to value / 100).

log likelihood of the individual parameter set k, given the observed concentration data (Y-term) and the population parameter set (P-term):

$$O_k = \sum_{i=1}^{n_k} \left\{ \frac{(Y_{obs,\,k,\,i} - Y_{calc,\,k,\,i})^2}{\sigma^2_{res,\,k,\,i}} + \ln\left(\sigma^2_{res,\,k,\,i}\right) + \ln\,(2\pi) \right\} \qquad (Y-\text{term})$$
$$+[P_k - P_{pop}]^T \left[COV_{pop}\right]^{-1} [P_k - P_{pop}]$$
$$+ \ln\left(\det\left[COV_{pop}\right]\right) + m \times \ln(2\pi) \qquad (P-\text{term}) \qquad (1)$$

where

n_k	Number of measurements (observed concentrations) for subject k ($k = 1, 2, ..., n_{subj}$)
$Y_{obs,\,k,\,i}$	Observed concentration at time point i ($i = 1, 2, ...,$ n) for subject k
$Y_{calc,\,k,\,i}$	Calculated concentration at time point i for subject k
$\sigma_{res,\,k,\,i}$	Residual standard deviation (standard deviation of difference between observed and calculated concentration) at time point i for subject k
$[P_k - P_{pop}]$	Vector of differences between individual parameters of subject k (P_k) and population parameters (P_{pop})
$[COV_{pop}]$	Covariance matrix of population parameters
m	Number of pharmacokinetic model parameters

'ln' denotes natural logarithm, 'det' denotes determinant of the matrix, T denotes transpose of the matrix, and '-1' denotes the inverse of the matrix. For an assumed log-normal distribution of residual errors in the measurements, Y refers to the natural logarithm of the observed and calculated concentrations. For an assumed log-normal distribution of parameters within the population, P refers to the natural logarithm of the individual and population parameters.

If the covariance between each pair of population parameters is assumed to be zero, the P-term in Eq. (1) can be simplified:

$$\sum_{p=1}^{m} \left\{ \frac{\left(P_{k,\,p} - P_{pop,\,p}\right)^2}{\sigma^2_{pop,\,p}} + \ln\left(\sigma^2_{pop,\,p}\right) + \ln\,(2\pi) \right\} \qquad (2)$$

where

$P_{k,\,p}$	Parameter p (p = 1, 2, ..., m) for subject k
$P_{pop,\,p}$	Population parameter p
$\sigma_{pop,\,p}$	Standard deviation of population parameter p (i.e., square root of diagonal element p, p of matrix COV_{pop})

The residual standard deviation ($\sigma_{res,\,k,\,i}$) may be a function of the subject (k) and a function of the observed or calculated concentration (i). In the present paper the residual errors are assumed to be log-normally distributed with a common value σ_{res} for each subject and each measurement.

Stage 2. For each model parameter, the population mean and standard deviation are calculated assuming a normal or log-normal distribution (in the latter case the logarithms of the parameters are used for the calculation of mean and standard deviation, and transformed back to the normal scale). The population means are calculated from:

$$P_{pop,\,p} = \frac{\sum_{k=1}^{n_{subj}} P_{k,\,p}}{n_{subj}} \qquad (3)$$

where n_{subj} is the number of subjects.

The population standard deviations and the correlation matrix between the population parameters are calculated from the covariance matrix of the population parameters, which is obtained from:

$$COV_{pop,\,p,\,q} = \frac{\sum_{k=1}^{n_{subj}} \left\{ \left(P_{k,\,p} - P_{pop,\,p}\right)\left(P_{k,\,q} - P_{pop,\,q}\right) + COV_{k,\,p,\,q} \right\}}{n_{subj} - 1} \qquad (4)$$

where

$COV_{pop,p,q}$	Covariance between population parameters p and q
$COV_{k,\,p,\,q}$	Covariance between individual parameters p and q for subject k

The covariance matrix of the standard errors in the individual parameters $[COV_k]$ is obtained from (14):

$$[COV_k] = [\alpha_k]^{-1} \qquad (5)$$

where the elements of matrix $[\alpha_k]$ are calculated from:

$$\alpha_{k,\,p,\,q} = \sum_{i=1}^{n_k} \left\{ \frac{1}{\sigma^2_{res,\,k,\,i}} \frac{\partial Y_{calc,\,k,\,i}}{\partial P_p} \frac{\partial Y_{calc,\,k,\,i}}{\partial P_q} \right\} \qquad (6)$$

Note that in the denominator of Eq. (4) a value of $n_{subj} - 1$ is used, i.e., the degrees of freedom (analogous to the calculation of a standard deviation), where earlier papers (9,10) used n_{subj}. The latter method was tested also.

Assuming a common value σ_{res} for each subject and each measurement, the residual variance is calculated from the following equation, obtained upon rearrangement of Eq. (17) from Mentré and Gomeni (9):

$$\sigma^2_{res} = \frac{\sum_{k=1}^{n_{subj}} \sum_{i=1}^{n_k} \left\{ \left(Y_{obs,\,k,\,i} - Y_{calc,\,k,\,i}\right)^2 + \sum_{p=1}^{m} \sum_{q=1}^{m} \left(COV_{k,\,p,\,q} \frac{\partial Y_{calc,\,k,\,i}}{\partial P_p} \frac{\partial Y_{calc,\,k,\,i}}{\partial P_q} \right) \right\}}{\sum_{k=1}^{n_{subj}} n_k} \qquad (7)$$

Iterative Procedure. Stage 1 is performed for each subject using initial estimates for P_{pop}, COV_{pop}, σ_{res} (details

described in the next section), and P_k (details described below), followed by the calculation of P_{pop}, COV_{pop}, and σ_{res} in stage 2. Then stage 1 is repeated, using P_{pop}, COV_{pop}, and σ_{res} obtained in stage 2. This process is repeated until the relative difference of the population values obtained from Eqs. (3), (4), (5) and (6) (i.e., the mean and standard deviation of each parameter and the residual standard deviation σ_{res}) and the corresponding prior value (used in Eq. 1) is smaller than some predefined value (0.01%). For this investigation, the initial parameter values P_k in stage 1 are set equal to P_{pop} during the first 10 cycles to reduce the risk of convergence of individual k to a local minimum. Thereafter the initial parameter values P_k in stage 1 are set to the values P_k obtained in the previous stage 1 to accelerate convergence.

Measures for Goodness-of-fit. As a measure of goodness-of-fit of the final population parameter set, the sum of the objective function O_k (Eq. 1) for all subjects, representing minus two log likelihood of the total parameter set, was calculated

$$\Sigma O = \sum_{k=1}^{n_{subj}} O_k \tag{8}$$

Akaike Information Criterion (AIC) equals minus two log likelihood (ΣO) with a 'penalty' of two for each parameter to be estimated, i.e., the individual parameters for each patient, population mean and standard deviation of each parameter, estimated correlation coefficients between population parameters (if applicable), and residual standard deviation, respectively:

$$AIC = \Sigma O + 2mn_{subj} + 4m + cm(m-1) + 2 \tag{9}$$

where $c = 0$ if correlation coefficients between parameters are assumed to be absent, and $c = 1$ if correlation coefficients between parameters are estimated during the analysis.

The following equation was calculated, i.e., the sum of the weighted residuals of concentrations and parameters divided by the degrees of freedom, i.e., the total number of measurements minus the number of population parameters:

$$\Sigma WSS = \frac{\sum_{k=1}^{n_{subj}} \left\{ \sum_{i=1}^{n_k} \left(\frac{\left(Y_{obs,k,i} - Y_{calc,k,i}\right)^2}{\sigma^2_{res,k,i}} \right) + \left[P_k - P_{pop}\right]^T \left[COV_{pop}\right]^{-1} \left[P_k - P_{pop}\right] \right\}}{\left(\sum_{k=1}^{n_{subj}} n_k \right) - m} \tag{10}$$

In preliminary tests it was observed that the value of ΣWSS converges to unity in the case of a successful convergence of the population parameters and residual standard deviation.

Improving Robustness. To minimize the risk of convergence to a local minimum of ΣO (Eq. 8), an extension of the ITSB procedure was developed. The ITSB procedure was started using the initial estimates as described above. After the first ten cycles (or less, in the case that the convergence criterion was reached), the current values of ΣO, population parameter set, covariance matrix and σ_{res} were stored. Then a new set of population prior values was randomly chosen from a log-normal distribution with a standard deviation of one (corresponding to a coefficient of variation of 100%) around the initial estimates of the population means. A new value for σ_{res} was also randomly chosen in a similar manner. After ten more cycles (or less, in the case that the convergence criterion was reached), the result was compared to the stored results. If ΣO was smaller than the stored value, the stored data were replaced by the current values. Then a new set of population priors was drawn and the ITSB procedure was started again. This process of starting the ITSB procedure with a randomly drawn population prior was performed 20 times. Finally, the normal ITSB procedure was started with the 'best parameter set.'

Standard Two-Stage (STS) Analysis

In stage 1 the individual pharmacokinetic parameters of each subject were estimated as described for the ITSB analysis, leaving out the (Bayesian) P-term in Eq. (1). In stage 2 the population mean and standard deviation of each model parameter were calculated as described for the ITSB analysis, leaving out the term $COV_{k,p,q}$ in Eq. (4). STS can be performed in a single cycle of stage 1 + 2, since the parameter set minimizing the first term of Eq. (1) is independent of $\sigma_{res,k,i}$ if a common value $\sigma_{res,k}$ is assumed for each measurement. The residual standard deviation $\sigma_{res,k}$ was calculated from:

$$\sigma^2_{res,k} = \frac{\sum_{i=1}^{n_k} \left\{ \left(Y_{obs,k,i} - Y_{calc,k,i}\right)^2 \right\}}{n_k - m} \tag{11}$$

and the population residual standard deviation σ_{res} was obtained from:

$$\sigma^2_{res} = \frac{\sum_{k=1}^{n_{subj}} \sigma^2_{res,k}}{n_{subj}} \tag{12}$$

Nonlinear Mixed Effect Modeling (MEM) using NONMEM

For comparison the same data sets were also analyzed by nonlinear mixed effect modeling using NONMEM (NONMEM V Version 1.1; Globomax, Hanover, MD). Settings were similar to those used for ITSB, i.e., the same model and parameters, a log-normal distribution of interindividual variability of the population parameters and a log-normal distribution of the residual error. The conditional method (FOCE) was used, and concentration measurement data were transformed logarithmically. To avoid an interchange of the parameters CL_{12} and V_2 with CL_{13} and V_3, possibly disturbing the population estimates, one parameter of the population model was reparametrized to ensure that CL_{12} and V_2 correspond to the rapidly equilibrating compartment, i.e., that $k_{21} > k_{31}$ where $k_{21} = CL_{12} / V_2$ and $k_{31} = CL_{13} / V_3$. Initial estimates were equal to the exact values. If this did not result in successful convergence, the initial value of the residual error variance was increased, and the process was repeated for that particular data set. If convergence still did not occur, various initial values were tested until convergence was achieved. The NONMEM control file is listed in the Appendix.

Evaluation

Analysis of Data Sets

The clinical data set and the 100 simulated data sets were analyzed by the ITSB, STS and MEM methods as described above. Unless stated otherwise, the ITSB analysis was performed assuming that correlation coefficients between parameters were absent, i.e., off-diagonal values of COV_{pop} were calculated from Eq. (4) for evaluation of accuracy and precision, but Eq. (2) was used in stage 1 (equivalent to setting off-diagonal values of COV_{pop} to zero in Eq. 1).

Unless stated otherwise, initial estimates of the individual parameters were set to the exact parameter values, to test the accuracy and precision of the methods under optimal conditions. The procedure for improving robustness was not applied in these cases.

In addition, the robustness of the ITSB analysis was investigated using a set of perturbed initial estimates: all population means for clearance parameters were multiplied by 4, and their corresponding standard deviations by 0.5; all population means for volume parameters were multiplied by 0.25, and their corresponding standard deviations by 2; the residual standard deviation was multiplied by 4. These initial estimates were tested both without and with the procedure for improving robustness.

Accuracy and Precision of Estimated Parameter Values

The accuracy and precision of each parameter (population means, interindividual standard deviations, correlation coefficients, and residual standard deviation for population results, and individual parameter values for individual results) were evaluated from 100 sets for each modification of the data set and for each method of analysis, and were calculated as mean error (ME, or bias) and root mean squared error (RMSE), respectively (15). Both ME and RMSE were expressed as a percentage of the exact value (except for correlation coefficients):

$$\mathrm{ME} = \frac{\left(\dfrac{\sum_{j=1}^{n_{\mathrm{set}}} \left(P_{\mathrm{j}} - P_{true,\,j}\right)}{n_{\mathrm{set}}}\right)}{P_{\mathrm{exact}}} \times 100\% \tag{13}$$

$$\mathrm{RMSE} = \frac{\sqrt{\dfrac{\sum_{j=1}^{n_{set}} \left(P_{\mathrm{j}} - P_{\mathrm{true},\,j}\right)^2}{n_{\mathrm{set}}}}}{P_{\mathrm{exact}}} \times 100\% \tag{14}$$

where n_{set} is the number of data sets (100 data sets for the population results, 1,000 data sets for the individual results), P_{j} is the estimated parameter of set j, $P_{\mathrm{true},j}$ is the corresponding true parameter value, and P_{exact} is the exact parameter value (i.e., the parameter value without random error). For the population results, $P_{\mathrm{true},j}$ is the actual geometric mean, standard deviation, correlation coefficients, or residual standard deviation, as obtained from the 'true' individual parameters and 'true' concentrations from set *j*. For the individual results, $P_{\mathrm{true},j}$ is the 'true' individual parameter of the same subject. For the correlation coefficients, ME and RMSE were not expressed as a percentage of their true value (zero) by omitting the factors P_{exact} and 100%. ME and RMSE of all 15 correlation coefficients between the 6 parameters were reported as a single mean value.

Standard Errors of Population Parameters

ITSB does not provide standard errors of population parameters, in contrast to MEM. Therefore standard errors were obtained using the results from the Monte Carlo analysis. We assumed that the standard error of each parameter (means, standard deviations, correlation coefficients, and residual standard deviation) for each set could be approximated from the corresponding RMSE (in percent).

Evaluation of Standard Errors

Assuming a Student *t*-distribution of the estimated population and individual parameters around their true value, the 95% confidence interval of the estimated parameter was obtained from:

$$\mathrm{CI}(95\%) = P \pm t_{0.025,\,df} \times \mathrm{SE} \tag{15}$$

where P is the estimated parameter value, SE its standard error (in the same unit as P), and t the tabulated value of the student *t*-distribution with $\alpha = 0.025$ and df is the degrees of freedom.

For population parameters the degrees of freedom was $n_{\mathrm{set}} - 1$ ($df = 99$), and for individual parameters $n_{\mathrm{subj}} - 1$ ($df = 9$). The calculated standard errors of the estimated population and individual parameters were evaluated by counting the number of sets (population parameters) or subjects (individual parameters) for which the true parameter value was within the calculated 95% confidence interval.

Influence of Covariance

To investigate the performance of the ITSB procedure in the case of significant covariance between population parameters, several modifications of the data set with known covariance between each pair of population parameters (true value of correlation coefficients varying between −0.9 and 0.9) were generated (100 sets for each modification) and analyzed by ITSB, setting initial estimates to zero for each correlation coefficient.

Influence of Study Design

To investigate the influence of the study design on the performance of the ITSB procedure, the following modifications of the data set were generated and analyzed by ITSB (100 sets for each modification):

Number of subjects. Data sets with 4,6,10 (= original data set), 20, and 40 subjects.

Number of measurements per subject. Data sets with 6, 12, 18 (= original data set), 24 and 36 measurements per

subject. The time points were selected between 1 and 480 min after the start of the infusion, with gradually increasing intervals similar to the original data set, and were equal for each subject.

Number of subjects and measurements per subject. Data sets with the same total number of measurement (144 measurements), but with a different number of subjects and different number of measurements per subjects: 24 subjects with 6 measurements each, 12 subjects with 12 measurements each, 8 subjects with 18 measurements each, 6 subjects with 24 measurements each, and 4 subjects with 36 measurements each.

Measurement error level. Data sets with measurement error standard deviation of 0.02, 0.05, 0.1 (= original data set), 0.2, and 0.4 (i.e., 2 to 40% error).

Computer Implementation

The Monte Carlo simulations, nonlinear Bayesian fitting, and ITSB and STS procedures were performed using the program MW\Pharm version 3.61 (written by the author, and available from MediWare BV, Zuidhorn, the Netherlands) (16). To validate the computer program code, the ITSB and STS procedures were also performed using MultiFit (written by the author), a program written in a different computer language (Borland Pascal instead of Visual Basic), not sharing any part of the source code. MEM was performed using NONMEM V Version 1.1.

RESULTS

Clinical Data Set

The three methods STS, ITSB, and MEM were applied to a real data set (Fig. 1) from a clinical study with rapacuronium, and the results are summarized in Table II. Depending on the initial estimates for the population parameters and σ_{res}, ITSB gave two different solutions, both complying with the convergence criteria, but with different AIC values (−56.46 and −61.64). The solution with the lowest AIC (−61.64), i.e., with the highest likelihood, is considered the best global solution, and that with the higher AIC (−56.46) as a local minimum. The procedure for improving robustness of ITSB described in "Materials and Methods" resulted in the solution with a lowest AIC value (AIC −61.64).

Population means agreed reasonably well between STS, MEM, and the global solution of ITSB. However, population standard deviations varied widely between the methods, and were higher for STS, and lower for MEM compared to ITSB. Using MEM the standard deviations of the parameters CL_{12} and CL_{13} were close to zero.

ITSB Analysis of Monte Carlo Data Sets

The ITSB analysis performed satisfactorily, as can be concluded from the ME (Table III) and RMSE (Table IV) values. Bias (ME) of population means and standard deviations obtained from ITSB was small, and negligible compared to RMSE. The precision of the individual parameters (Table V) is in agreement with the precision of the population parameters: the RMSE of the individual values is about three time higher (square root of the number of subject per data set (ten subjects)) than the RMSE of the population means. It was confirmed that ΣWSS (Eq. 10) converged to a value close to unity (1 ± 0.0001) in all data sets.

Using poor initial estimates, deviating markedly from the true population values, the performance of ITSB without the procedure for improving robustness was not satisfactory. Although the calculation converged to the same final population parameters in 81 out of 100 sets, the results in the remaining data sets deviated markedly from the true values, resulting in larger values for ME and RMSE. The procedure for improving robustness resulted in convergence to values close to that obtained with the exact values as initial estimates ('best case scenario'; data not shown).

In addition, ITSB analysis was performed without the assumption of absence of covariance between the parameters, i.e., using the complete covariance matrix obtained from Eq. (4) in Eq. (1). In this case the precision of the population estimates was slightly less, but the estimates of the correlation coefficients between the parameters were close to their true value zero (Table III).

To check to correctness of our modification in Eq. (4), we tested the original version of Eq. (4) with denominator n_{subj} replacing $n_{subj}-1$ (Table III). This modification had no

Table II. Population Parameters [Geometric Mean (Coefficient of Variation, in percent)] for the Clinical Rapacuronium Data Set, Obtained by STS, ITSB, and NONMEM

Variable	Unit	STS	ITSB[a]	ITSB[b]	NONMEM
CL	ml min^{-1} kg^{-1}	6.55 (28)	6.55 (25)	6.57 (24)	6.57 (21)
V_1	ml kg^{-1}	51.8 (23)	53.0 (18)	52.8 (17)	53.1 (14)
CL_{12}	ml min^{-1} kg^{-1}	2.15 (53)	2.13 (33)	2.13 (24)	2.11 (0)
V_2	ml kg^{-1}	39.6 (40)	46.2 (15)	42.9 (18)	42.1 (5)
CL_{13}	ml min^{-1} kg^{-1}	0.740 (24)	0.638 (32)	0.692 (17)	0.700 (0)
V_3	ml kg^{-1}	93.4 (30)	97.9 (24)	92.9 (23)	92.6 (18)
σ_{res}	%	16.1 (21)[c, d]	16.5[d]	17.1[d]	18.2[d]

[a] Local minimum (AIC −56.46).
[b] Global minimum (AIC −61.64).
[c] Mean value of residual standard deviation in ten patients.
[d] Expressed as coefficient of variation, in percent (standard deviation of log-normal distribution corresponds to value / 100).

Table III. Accuracy of population mean, standard deviation (σ), correlation coefficient (*r*), and residual standard deviation (σ_{res}) of a panel of ten subjects

Variable	STS	ITSB	ITSB Correlation coefficients estimated	ITSB Using n_{subj} in Eq. (4)	NONMEM
CL	−0.9	0.1	0.1	0.1	0.2
V_1	−0.9	−0.5	−0.6	−0.5	0.0
CL_{12}	−1.0	1.5	1.5	1.4	1.2
V_2	−6.4	−0.1	−1.3	0.0	−0.2
CL_{13}	0.3	0.9	0.5	0.7	1.0
V_3	11	−0.3	0.5	−0.7	0.0
σ(CL)	5.5	−0.2	−0.0	−5.1	−5.0
σ(V_1)	4.3	−0.4	0.1	−6.5	−6.4
σ(CL_{12})	38	4.3	8.5	−2.7	−2.0
σ(V_2)	50	−5.6	3.7	−13	−12
σ(CL_{13})	71	−0.0	18	−12	−10
σ(V_3)	68	0.2	4.2	−6.6	−6.3
r^a	0.01	0.01[b]	0.00	0.00[b]	–[c]
σ_{res}	−0.6	−0.8	−2.5	−0.3	−0.5

The presented values are ME (in percent of the exact values) of 100 panels, obtained by Standard Two-Stage (STS), Iterative Two-Stage Bayesian (ITSB), ITSB with correlation coefficients estimated, ITSB using n_{subj} instead of $n_{subj} - 1$ in Eq. (4), and nonlinear Mixed Effect Modeling (NONMEM).
[a] Mean value of 15 correlation coefficients between 6 model parameters; untransformed values (not expressed in percent).
[b] Correlation coefficient calculated from Eq. (4), but not used in stage 1.
[c] Correlation coefficients not provided by NONMEM.

visible effect on parameter means, correlation coefficients and σ_{res}, but interindividual standard deviations were underestimated by about 7% on average.

STS Analysis

The STS analysis performed poorly compared to ITSB, as can be concluded from the ME (Table III) and RMSE (Table IV) values. Only for V_1, STS performs almost as good as ITSB. STS overestimated the population standard deviations of each parameter considerably, except for CL and V_1 (Table III). The individual parameter estimates were much less precise than that obtained from ITSB (Table V).

NONMEM Analysis

The NONMEM analysis resulted in good accuracy (Table III) and precision (Table IV) of the population estimates. The same holds for the precision of the individual values (Table V). However, the performance of MEM was

Table IV. Precision of population mean, standard deviation (σ), correlation coefficient (*r*), and residual standard deviation (σ_{res}) of a panel of ten subjects

Variable	STS	ITSB	ITSB Correlation coefficients estimated	ITSB Using n_{subj} in Eq. (4)	NONMEM
CL	3.5	0.9	0.9	0.9	1.0
V_1	2.8	2.6	2.6	2.5	2.7
CL_{12}	13	7.5	8.8	7.5	8.0
V_2	16	5.5	9.9	5.7	5.8
CL_{13}	12	5.7	9.3	5.8	6.2
V_3	22	4.6	6.7	4.7	4.5
σ(CL)	25	2.6	2.7	5.8	5.7
σ(V_1)	13	12	12	13	13
σ(CL_{12})	87	17	27	16	17
σ(V_2)	95	17	24	21	20
σ(CL_{13})	99	21	40	26	27
σ(V_3)	126	18	21	20	20
r^a	0.36	0.18[b]	0.27	0.18[b]	–[c]
σ_{res}	3.3	3.8	4.4	3.8	3.8

The presented values are RMSE (in percent of the exact values) of 100 panels, obtained by Standard Two-Stage (STS), Iterative Two-Stage Bayesian (ITSB), ITSB with correlation coefficients estimated, ITSB using n_{subj} instead of $n_{subj} - 1$ in Eq. (4), and nonlinear Mixed Effect Modeling (NONMEM).
[a] Mean value of 15 correlation coefficients between 6 model parameters; untransformed values (not expressed in percent).
[b] Correlation coefficient calculated from Eq. (4), but not used in stage 1.
[c] Correlation coefficients not provided by NONMEM.

Table V. Precision of Individual Parameters

Variable	STS	ITSB	NONMEM
CL	11	2.8	2.8
V_1	7.8	7.0	7.1
CL_{12}	53	19	20
V_2	59	16	17
CL_{13}	40	15	15
V_3	52	13	13

The presented values are RMSE (in percent of the exact values) of 1,000 subjects, analyzed as 100 panels of 10 subjects, by Standard Two-Stage (STS), Iterative Two-Stage Bayesian (ITSB), and nonlinear Mixed Effect Modeling (NONMEM).

less good than for ITSB, because population standard deviations were underestimated by MEM (Table III).

Influence of Covariance on Accuracy and Precision of ITSB Analysis

Accuracy and precision of the population means, standard deviations, correlation coefficients, and residual standard deviation obtained by ITSB analysis were not markedly affected by the presence of significant covariance between each pair (true value of correlation coefficients varying between −0.9 and 0.9; data not shown). In general the RMSE values were slightly smaller than that listed in Table IV for ITSB with correlations estimated.

Influence of Study Design on Accuracy and Precision of ITSB Analysis

Number of Subjects

Bias (ME) in the population means was relatively independent of the number of subjects (Fig. 2, first row). Only for four subjects, bias in V_2 and CL_{13} exceeded 2%. Bias in the population standard deviations was more pronounced, but decreased with the number of subjects, except for V_3, and was less than 6% for six subjects or more. Bias in σ_{res} decreased with the number of subjects and was less than 2% in all cases. RMSE of all parameters decreased with the number of subjects. RMSE reduced by about 50% by increasing the number of subjects by a factor 4 (Fig. 3, first row).

Number of Measurements Per Subject

A similar pattern was found for the number of measurements. Bias was pronounced for six measurements per subjects, but was hardly affected if the number of measurements increased from 12 to 36 (Fig. 2, second row). As for the number of subjects, RMSE of all parameters reduced by about 50% by increasing the number of measurements by a factor 4 (Fig. 3, second row).

Number of Subjects and Measurements Per Subject

The influence of the number of subjects and measurements was also investigated while the total number of measurements was kept constant (144 measurements) by varying the number of measurements and subjects simultaneously. Only for six measurements per subjects (24 subjects) bias in the population parameters exceeded 2%, and in almost all cases bias in the standard deviations was less than 4% (Fig. 2, third row). RMSE was relatively independent of the number of measurements per subject. For the population means RMSE was minimal for 12 measurements (12 subjects; Fig. 3, third row). In contrast, RMSE of the standard deviations was lowest for six measurements, whereas RMSE of σ_{res} decreased with the number of measurements. The results show that the number of measurements is not a critical factor: bias and RMSE for six measurements (24 subjects) were not essentially different for 36 measurements (four subjects).

Measurement Error

Both bias (Fig. 2, last row) and RMSE (Fig. 3, last row) increased with increasing measurement error in a broadly linear manner, indicating numerical stability with up to 40% measurement error.

Evaluation of Standard Errors

The standard errors of population and individual parameters obtained from ITSB were evaluated by counting the number of subjects for which the true parameter value was within the calculated 95% confidence interval. Of the true population means, 94.7% was within the calculated confidence interval, ranging from 93 to 96% for the six parameters; for the standard deviations this value was 94.0% (range 91–97%), for the correlation coefficients 95.1% (range 93–98%) and for the residual standard deviation 95%. Of the individual parameters, 94.9% of the true individual parameters was within the calculated confidence interval (range 93.4–96.5%).

Model Selection

The ITSB analysis was also performed with a two-compartment model (with parameters CL, CL_{12}, V_1, V_2). In each of the 100 sets the two-compartment model resulted in a higher AIC value than the three-compartment model. The mean difference in AIC was 291 (range 175–374). Therefore it can be concluded that the ITSB algorithm is able to identify the three-compartment model as the better fitting model compared to the two-compartment model in all sets.

DISCUSSION

The results demonstrate that ITSB is a suitable technique for population pharmacokinetic analysis of rich data sets, and is superior to STS and MEM in the analysis of the presented data set. ITSB performed well under a wide variety of conditions with respect to the number of subjects, number of measurements, degree of measurement error, and covariance between parameters.

The method described to improve the robustness of ITSB by testing multiple initial estimates for the population parameters and residual standard deviation (σ_{res}) produced reliable results independent of the initial estimates for the data

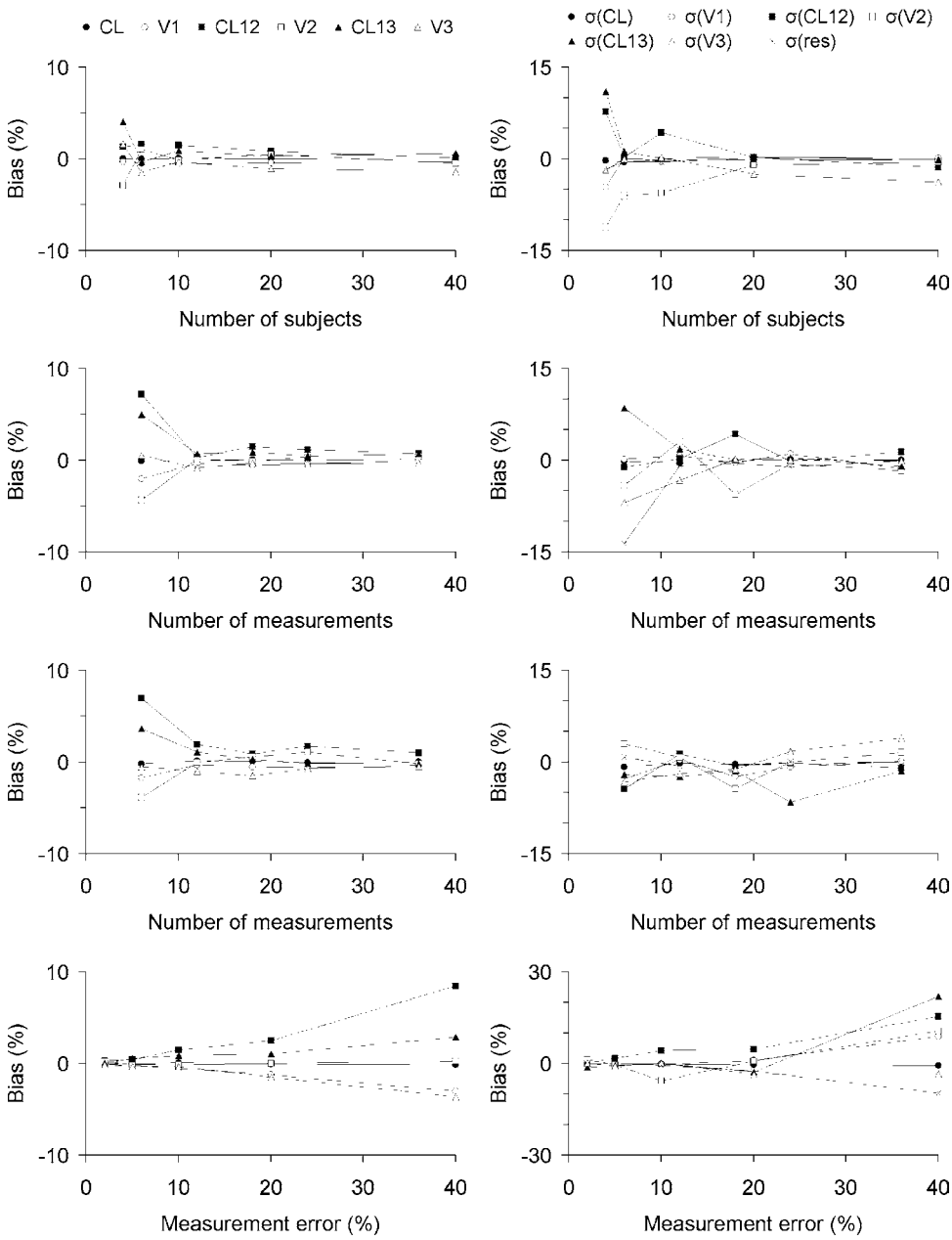

Fig. 2. Influence of number of subjects, number of measurements, number of measurement and subjects (total number of measurements 144), and degree of measurement error on accuracy (expressed as percent of exact value) of population means (*left column*), population standard deviations and residual standard deviation (*right column*) obtained by ITSB.

sets tested. Without this method ITSB was found to be moderately sensitive to the initial estimates of the population parameters and σ_{res}, sometimes converging to a local minimum instead of the global minimum, as observed both with the clinical data set and with the Monte Carlo simulated data sets. Therefore we recommend using this procedure routinely for the analysis of real data.

The results obtained with the clinical data set (Table II) showed some characteristic properties of the three methods for population analysis. Population means agreed reasonably well between the three methods. In contrast, population standard deviations (coefficient of variation) for STS were markedly higher than for ITSB, in agreement with the overestimation of the standard deviation for STS in the simulation study (Table III). NONMEM analysis results in lower standard deviations than ITSB, and the standard deviation of the parameters CL_{12} and CL_{13} are close to zero. The latter occurred also in four sets of the simulation study.

From Tables III and IV it can be seen that the accuracy and precision of the population means are much better than that of the population standard deviation, as expected from statistical theory. The estimation of the correlation coeffi-

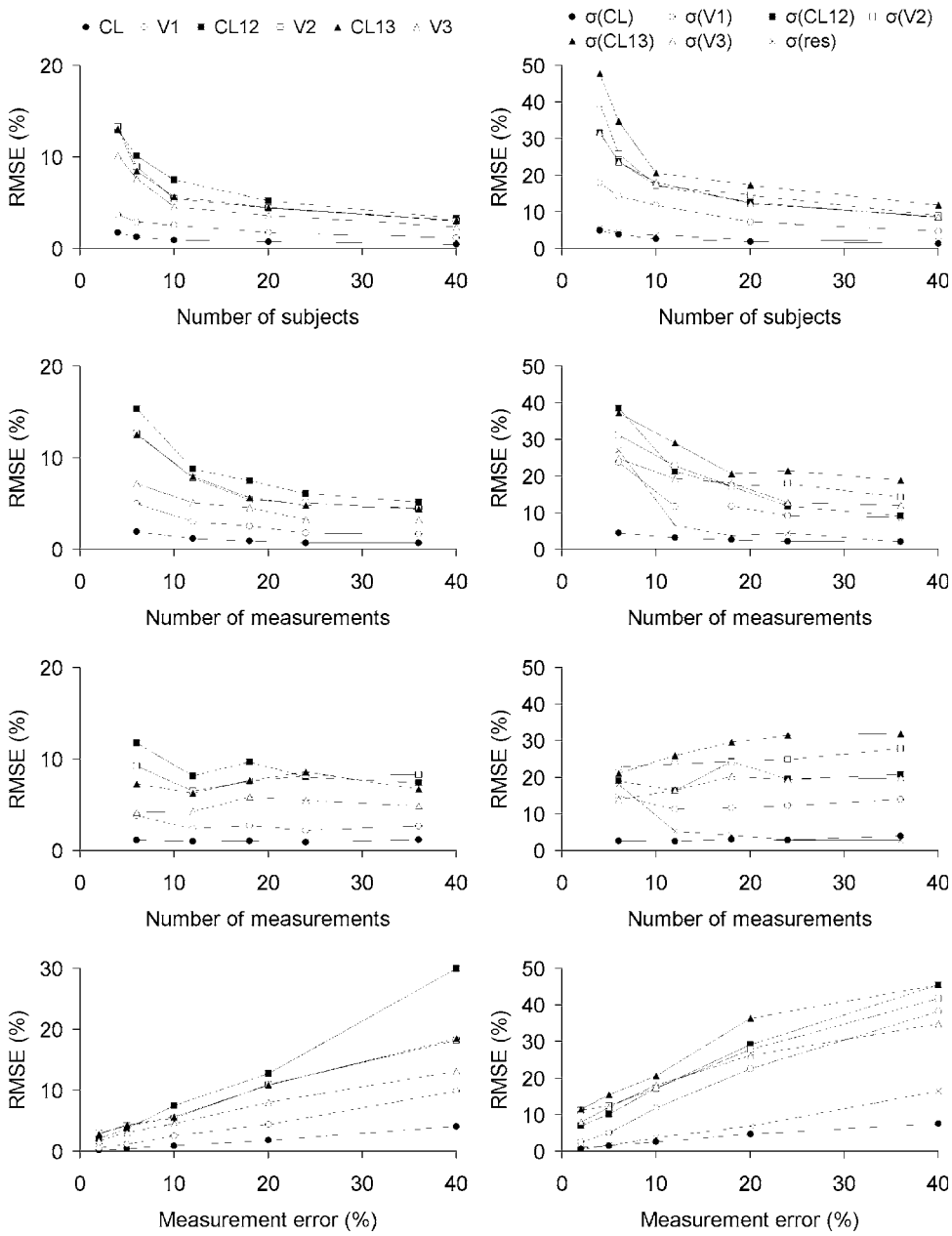

Fig. 3. Influence of number of subjects, number of measurements, number of measurement and subjects (total number of measurements 144), and degree of measurement error on precision (expressed as percent of exact value) of population means (*left column*), population standard deviations and residual standard deviation (*right column*) obtained by ITSB.

cients between the population parameters results in a decreased precision of parameter estimates, but the estimated correlation coefficients were unbiased.

RMSE of all values reduces by about 50% by increasing the number of subjects or the number of measurements by a factor 4, i.e., RMSE is inversely proportional to the square root of the total number of measurements, as expected from statistical theory. This does not apply to bias: increasing the number of subjects from 10 to 40, or increasing the number of measurement from 12 to 36 hardly affects ME. However, the level of bias is small compared to RMSE, even for 40 subjects or 36 measurements, and thus it does not play a significant role.

In the calculation of RMSE values, the actual mean value and sample standard deviation of each panel of subjects were considered as the 'true' values for that particular panel. Therefore the RMSE values reflect the suitability of the population analysis to reconstruct the actual population parameters (mean and standard deviation) for that particular panel of subjects, rather than the 'exact' parameters of the underlying statistical distribution from which the subjects were drawn. If the latter 'exact' values would be used as the

'true' values, RMSE values would be somewhat larger, and less discriminating between methods.

The applied procedure for ITSB analysis was similar to a method described in literature (9,10), with a few modifications. In the denominator of Eq. (4) a value of $n_{subj} - 1$ was used, where the cited papers used n_{subj}. Our equation resulted in unbiased estimates of the population standard deviation (ME on average 0.1%), whereas the original equation using n_{subj} resulted in an underestimation of the standard deviations by on average 7.2%. This finding is in agreement with statistical theory, which predicts an error of $\sqrt{(n-1/n)}$ for $n = 10$ patients, resulting in an underestimation of 5.1%.

We performed the ITSB analysis with two different sets of initial estimates. Initially we used the exact parameter values (mean, standard deviation and residual error standard deviation) as initial estimates. These initial estimates were also used for STS and MEM analysis. This allows a comparison of the methods under the most favorable circumstances, avoiding poor performance due to problems with convergence to the optimal solution as a result of poor initial estimates. However, in practice these 'ideal' initial estimates are not known, and the robustness of the method to poor initial estimates may become a major issue. We indeed observed that ITSB performed less well when started with poor initial estimates. In a number of data sets ITSB converged to a solution with a markedly higher objective function value than that obtained starting with the exact values as initial estimates. The proposed procedure for improving robustness, exploring the parameter space by testing lower and higher values of each parameter, was found to be adequate and resulted in virtually identical results as when starting with the exact values. It should be noticed that in practical situations the problem of convergence to a local minimum may be minimized by the user, e.g., by carefully selecting the initial estimates based on prior information, and by repeating the analysis with different sets of initial estimates. Our procedure for exploring the parameter space is an automated version of the latter approach.

In contrast to MEM, ITSB does not provide standard errors of population parameters. Therefore we estimated the standard errors from the results obtained in the Monte Carlo analysis, assuming that the standard error of each parameter (means, standard deviations, correlation coefficients, and residual standard deviation) for each set could be approximated from the corresponding RMSE. The evaluation of the standard errors showed that this procedure results in reliable estimates for the standard error of each population parameter.

The application of STS has some obvious disadvantages, mainly due to the nature of the individual parameters. Individual pharmacokinetic parameters (e.g., clearance and volume of distribution in a particular subject) are not obtained by a direct and accurate measurement (in contrast to, e.g., the subject's weight), but by PK modeling of a set of measurements. Each of these measurements is perturbed by a certain level of measurement error, which may be relatively large (typically, 5 to 25%). Moreover, the modeling procedure will introduce some degree of bias, since there will be always some degree of model misspecification. Finally, the identification of model parameters from a series of measurements may be cumbersome. Even in the absence of model misspecification, relative small measurement errors may have a profound influence on parameter estimates. As a result, parameter estimates have a limited degree of precision, as reflected in their standard error estimated during the fitting procedure. The problem in STS analysis becomes apparent if the standard error in the parameters is of the same order of magnitude as, or even larger than, the interindividual variability of the parameter, as reflected in the interindividual standard deviation. The significant overestimation of the interindividual standard deviations by STS is due to the large standard errors in the estimates of parameters CL_{12}, CL_{13}, V_2, and V_3. These parameters are not well defined during the fitting procedure, and their value may deviate significantly from the true value to obtain the minimum of the objective function. This problem is avoided by ITSB, since such parameters are influenced in the direction of the population mean due to the Bayesian 'penalty.' The results of this study demonstrate that the standard deviations obtained by ITSB are unbiased.

Using STS with multi-compartment models, it is a well-known problem that the data cannot be described by the same model for each subject. For example, in a majority of the subjects a three-compartment model fits significantly better to the data, whereas in some subjects no acceptable parameters for this model can be obtained, and/or the two-compartment model cannot be rejected on statistical grounds, e.g., by an F-test or AIC value. As a result, a two-compartment model must be accepted in these subjects. In this case the second stage is hampered by the application of two different models, and a consistent analysis is not possible. This problem may be, at least partly, responsible for the poor performance of STS in our simulations. This problem does not arise during the application of ITSB. If the data of a particular subject contain insufficient information about one or more parameters, the Bayesian procedure warrants that these particular parameters do not deviate too far from the population average, and that it has a relative large standard error. As a result this particular estimate hardly affects the new population mean, and therefore does not perturb the performance of the population analysis. Parameter constraints or special intervention in the procedure are not necessary.

The poor performance of STS implies that STS should not be considered as a reliable method for population analysis of rich data in more complex models. Although STS may still be useful as a simple and fast tool for a preliminary analysis, it should not be used to provide final results.

The performance of MEM was less good than for ITSB, because population standard deviations were underestimated by MEM (Table III). In 4 out of 100 sets the NONMEM analysis resulted in very small values for $\sigma(CL_{13})$, despite the known interindividual variability in that parameter. This problem was not observed in ITSB.

The NONMEM analysis took much more time to execute compared to ITSB: a single data set took about 110 s (with exact initial estimates), compared to about 2 s (without procedure for improving robustness, using exact initial estimates) to 10 s (with procedure for improving robustness, using poor initial estimates) for ITSB (execution times on a PC with processor operating at 900 MHz).

We suggest that Monte Carlo simulation and analysis should be routinely performed for each real data set, regardless of the population analysis technique used. This allows an evaluation of the precision of the results obtained by population analysis, including standard errors and confidence intervals of each population and individual parameter.

In conclusion, ITSB is a suitable technique for population pharmacokinetic analysis of rich data sets, and in the presented data set it is superior to STS and MEM.

APPENDIX

The following control file was used in the NONMEM analysis. For practical reasons parameters were expressed in liters and liters per minute for volume and clearance parameters, respectively.

```
$PROB  MCRA
$DATA mcra.dat
      IGNORE = C
$INPUT ID TIME WGT AMT RATE DV
$SUBROUTINES ADVAN11 TRANS4
$PK
      CALLFL = 1
      V1=THETA(1)*EXP(ETA(1))
      V2=THETA(2)*EXP(ETA(2))
      V3=THETA(3)*EXP(ETA(3))
      CL=THETA(4)*EXP(ETA(4))
      Q3=THETA(6)*EXP(ETA(6))
      Q2=(THETA(2)*(THETA(6)/THETA(3) + THETA
        (5)))*EXP(ETA(5))
      S1=V1
$ERROR
      CALLFL = 0
      Y=LOG(F)+ERR(1)

; Starting at the exact values
$THETA (0, 3.64)(0, 3.01)(0, 6.44)(0, 0.51)(0, 0.048)(0, 0.051)
$OMEGA 0.0533 0.1217 0.1063 0.1245 0.2215 0.0650
$SIGMA 0.01

;Without POSTHOC only typical values are in the table
$ESTIMATION MAX = 9999 SIG = 6 METHOD = COND
  NOABORT POSTHOC
$TABLE TIME V1 V2 V3 CL Q2 Q3 DV
```

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