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# A Comparison of the Standard Approach and the NONMEM Approach in the Estimation of Bioavailability in Man

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## Abstract

There has recently been concern about confidence intervals calculated using the standard error of parameter estimates from NONMEM, a computer program that uses a non-linear mixed-effects model to calculate relative bioavailability (F), because of possible downward bias of these estimates. In this study an alternate approach, the log-likelihood procedure, was used to calculate the confidence intervals for F from NONMEM. These were then compared with those calculated using the standard error of the parameter estimates, the traditional NONMEM approach, and the standard model-independent method, to determine whether bias exists.

By use of data from a single dose, open cross-over study of ibuprofen using 14 healthy male volunteers, NONMEM was shown to give results consistent with those obtained using the standard model-independent method of analysis and could be a useful tool in the determination of F where conditions for using the standard method of analysis are not optimum. The width of the confidence interval for F using the log-likelihood procedure was narrower and non-symmetrical when compared with that obtained using the traditional NONMEM approach. The width of the confidence interval obtained using the traditional NONMEM method was similar to that from the standard approach, however the parameter estimate for F was higher than that obtained from the standard method. This could have been because of an outlier in the data set to which the standard approach is more sensitive.

No downward bias was found in the confidence intervals from NONMEM. The bioavailability data set was of relatively low variability and more research with highly variable data is necessary before it can be concluded that the confidence intervals calculated from NONMEM can be used for hypothesis testing.

A standard model-independent method is routinely used to determine the relative bioavailability of drug products. A method of population pharmacokinetics that uses a non-linear mixed-effects model (NONMEM) was introduced by Sheiner & Beal (1980). For experimental data, tests have shown the assessment of bioavailability furnished by NONMEM to be consistent with results obtained using the standard model-independent method (Graves & Chang 1990; Kaniwa et al 1990). NONMEM is often used for assessment of pharmacokinetic parameters from routine clinical data or from incomplete data. This would make NONMEM particularly useful for comparison of bioavailability in situations where conditions are not optimum for the standard method of analysis.

There is a potential problem with proving bioequivalence using the confidence intervals calculated from NONMEM. The maximum-likelihood procedure (extended least-squares) used by NONMEM has been shown to lead to downward bias of the standard error of the parameter estimates in the non-linear model, resulting in over-optimistic confidence intervals (Sheiner & Beal 1987; White et al 1991). Two studies have also found that the parameter estimates produced by NONMEM have a relatively small bias if the variability of the data is relatively small (Vozeh et al 1990; White et al 1991). This is an issue of concern, because the accuracy and validity of both the parameter and interval estimates obtained from NONMEM are in question. It has even been suggested (White et al 1991) that the confidence intervals computed from NONMEM should not be routinely employed as a method of hypothesis testing.

An alternative method, the log-likelihood procedure, is available for obtaining the confidence intervals from NONMEM. This is less reliant on approximations than that which uses confidence intervals which are based on the standard error of the parameter estimates, and will also enable estimation of confidence intervals which are not symmetrical.

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The purpose of this study was to determine the relative bioavailability of a product using the computer program NONMEM, and to find the confidence intervals of this parameter using two different NONMEM methods: the traditional NONMEM method, using standard error of the parameter estimates to calculate confidence intervals, and the log-likelihood procedure. These values were compared with those obtained from the standard model-independent method of analysis to investigate the possibility of bias in the parameter estimates of F and the confidence intervals.

## Methods

This study involved the analysis of data from a bioequivalence study of two products containing ibuprofen (as conducted at the Drug Studies Unit at the University of Durban-Westville). The bioequivalence study was a single-dose, randomized, open cross-over design involving 14 healthy male volunteers. Eighteen blood samples were obtained from each volunteer per phase of the study.

The parameter relative bioavailability (F) and the 90 and 95% confidence intervals of this parameter were calculated using the standard model-independent method and two NONMEM methods.

### *The standard model-independent method of analysis*

This was previously conducted at the Drug Studies Unit at the

University of Durban Westville. The pharmacokinetic parameters calculated were: peak drug concentration ($C_{max}$), time to peak plasma concentration ($T_{max}$) and area under the curve (AUC). These values were then subjected to analysis of variance to obtain the population parameter-estimates. The mean squared error from analysis of variance was used to construct confidence intervals. The 90 and 95% conventional *t*-confidence intervals were calculated for the ratio of the test product to the reference product, using log-transformed data.

*NONMEM analysis*

Data analysis was performed using NONMEM version IV, level 1·0, double precision. The population pharmacokinetic parameters were estimated by fitting a one-compartment pharmacokinetic model, with first-order absorption and elimination, to the data set.

The confidence intervals for F were calculated using both the standard error of the estimates and the log-likelihood procedure.

*Calculation using the standard error of the estimates*

Percentiles of the normal distribution of the standard error of the estimates were used to calculate the bounds of the confidence intervals for the parameter estimate from the equation (Boeckmann et al 1991):

$$\theta \pm Z_{1-\alpha/2}\text{SEE} \quad (1)$$

where $\theta$ is the parameter, $Z_{1-\alpha/2}$ denotes the $1 - \alpha/2$ percentile of the normal distribution, and SEE is the standard error of the estimate.

*Log-likelihood procedure*

For a given model, the sample likelihood can be used to determine the confidence intervals for any parameter estimate by evaluating the minimum objective function associated with a change in parameter value (Sheiner et al 1974).

The minimum objective function for the final regression model was first computed. The value of the parameter for which the confidence intervals were to be calculated (F in this example) was then fixed at various values. The model was then re-optimized under this restricted condition, computing the values of all the other parameters, and the minimum objective function of this new model was found. The difference between the minimum objective function for the models yields the statistic $c^2$ (Boeckmann et al 1991).

$$c^2 = I_r - I_f \quad (2)$$

where $I_f$ is the lowest minimum objective function with the parameter fixed at a specific value and $I_r$ is the corresponding quantity with the parameter fixed at a different value.

This statistic is approximately a chi-square ($\chi^2$) distribution. The number of degrees of freedom of this distribution is equal to the number of parameters that are fixed. In this example there was only one such parameter (F), all the other parameters were free to vary. The $c^2$ statistic therefore had a $\chi^2$ distribution with one degree of freedom. The confidence intervals were found by comparing $c^2$ with ${\chi_{1-\alpha}}^2$ (q), which represents the $100(1 - \alpha)$ percentile of the $\chi^2$ distribution (critical value).

In this example the 95% and 90% confidence intervals were that value of F where $c^2$ was equal to the critical values of 3·84 and 2·71, respectively. These were associated with *P* values less than 0·05 and 0·1, respectively.

## Results

The results obtained from the different methods of analysis are listed in Table 1.

Fig. 1 illustrates the plot of the fixed value of the parameter F against the minimum objective function. The trend of the curve was followed to obtain the value for F and the confidence intervals. The lowest minimum objective function gave the point estimate for F (110%). The points at which the minimum objective function exceeded the critical values of 2·71 and 3·84 from the minimum gave the upper and lower limits of the 90 and 95% confidence intervals of 106·7–112·9 and 106·1–113·5%, respectively.

Three points did not fall on the curve, possibly because of local minima. Perturbation of initial estimates did not solve this. This might be the result of a suboptimum model relating to absorption rate.

## Discussion

The estimate of relative bioavailability obtained using both NONMEM methods (110%) was greater than that obtained from the standard model-independent method (105·1%). This could possibly be because of a downward bias of the standard approach caused by outliers in the data set, to which the standard approach is more sensitive.

In the standard method the value of the parameter is found by calculating the mean of all the individual parameters. It is, therefore, possible that a single outlying value could influence the mean, or the final parameter calculated. The median calculated for the log-normal AUC distribution of the reference product was found to be 109·1 compared with the mean of 116·8 for the same product, suggesting the presence of an outlier in the data set.

Table 1. Relative bioavailability and confidence intervals using three different methods of analysis.

| | Standard model Independent method | NONMEM Traditional method | NONMEM Log-likelihood procedure |
|---|---|---|---|
| Relative bioavailability | 105·1 | 110·0 | 110·0 |
| 90% Confidence interval | 99·6–110·9 | 104·2–115·8 | 106·7–112·9 |
| Width | 11·3 | 11·6 | 6·2 |
| 95% Confidence interval | 98·4–112·2 | 103·0–116·9 | 106·1–113·5 |
| Width | 13·8 | 13·9 | 7·4 |

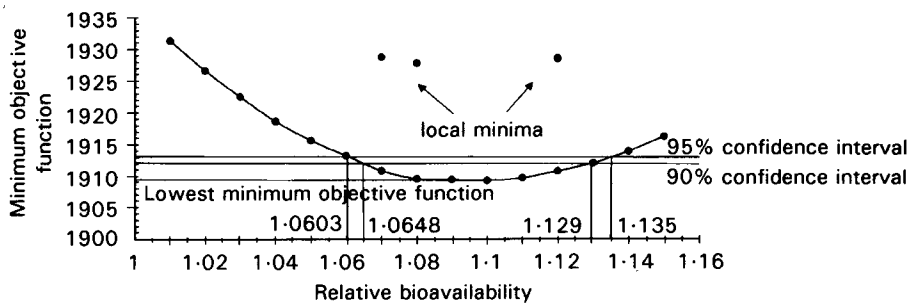


FIG. 1. Plot showing the minimum objective function obtained when fixing relative bioavailability (F) at different values using the log-likelihood procedure. The lowest minimum objective function gives the point estimate for F. The horizontal lines marked 90 and 95% are drawn 2·71 and 3·84 units above the minimum of the curve and represent the 90 and 95% confidence intervals for F.

One such outlier was found in the reference product AUC data set by use of both the Dixon Test for extreme values and the T procedure (Bolton 1990). When this value was removed, the new calculated parameter for relative bioavailability was found to be 109·1%, which is very similar to the value of 110% found by NONMEM. To check for bias with NONMEM first-order estimation, a run using first-order conditional estimation was done. Like the first-order estimation method the first-order conditional estimation uses first-order expansions about values of the $\eta_i$ (the difference between the ith individual parameter values and the population prediction for these values), but these values are conditional estimates of the $\eta_i$ rather than 0 (Beal 1995). The fit of the data did not improve and rendered an estimate for F (109%) which was similar to that obtained from the first-order estimation method. In this case, it is possible that the difference between the values obtained for relative bioavailability arose because of bias in the standard method, and not in the NONMEM approach.

If a downward bias in the standard errors did exist, as proposed by White et al (1991), the width of the confidence intervals calculated using the standard errors of estimates from NONMEM would be expected to be narrower than those from the alternative approach. When looking at the results (Table 1), it can be seen that the confidence intervals are actually wider than those of the log-likelihood approach.

In the assessment of bioequivalence, both the rate and extent of absorption of the active ingredient are determined for each product. For this data set NONMEM was unable to produce a reliable estimate of the absorption rate constant ($k_a$) for either of the products. The first-order model describing the absorption kinetics is a likely source of this problem. Erratic absorption was frequently observed, but was not modelled in this study; this could, therefore, result in inaccuracies when estimating the rate of absorption. Apart from the difficulties in the estimation of $k_a$, NONMEM appeared to perform well in the estimation of bioavailability from experimental data. It gave parameter estimates which were less influenced by outliers than the standard model-independent method. No bias was found in the parameter estimate of F or in the confidence intervals from NONMEM. All values were within the acceptable range of $\pm 20\%$. This was sufficient to prove bioequivalence.

From this study we conclude that the confidence intervals calculated using NONMEM could be employed in hypothesis testing. A necessary caution to be noted is that our bioavailability data set had relatively low variability. It has been shown by White et al (1991) that the standard errors deteriorate as the variability in the data increases. Thus, it cannot be concluded that the confidence intervals calculated from NONMEM standard error of the parameter estimates for highly variable data sets can be used for hypothesis testing. This study addresses a problem with a real data set. However, it might be worth while simulating data to investigate these issues further under different conditions.

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