METHODS FOR ESTIMATING THE AUC AND ITS VARIABILITY IN SPARSE SAMPLING DESIGNS.

Navarro-Fontestad MC[1], González-Álvarez I[1], Fernández-Teruel C[2], Bermejo M[1] Casabó VG[1].

[1]Department of Pharmaceutics and Pharmaceutical Technology. College of Pharmacy. University of Valencia.
Av. Vicente Andrés Estellés s/n Burjassot 46100 Valencia, España.
[2]Intervalence Biokinetics S.L.
Benjamin Franklin, 12 (CEEI Parc Tecnològic) Paterna 46980 Valencia, España.

Introduction and objectives

The area under the curve (AUC) is a pharmacokinetic parameter widely used in toxicokinetics and bioequivalence studies as a measure of drug exposure. In order to perform statistical comparisons, the parameter values and their variances in the different groups/treatments are necessaries.

Estimation of AUC variance in serial sacrifice or sparse sampling designs (where each animal is sampled once or more than once but not at all time points) is not straightforward. The aim of this study is to compare different methods for estimating the AUC and its variability in batch sampling designs. (1-3)

Materials and Methods

Data were simulated using NONMEM 5.0. The parameters used for simulation are shown in Table 1.

The data were simulated as 100 groups with 20 subjects each one. The selected sampling design was a "batch" design. In each group of 20 subjects, each subject is sampled at 3 time point. A batch of subjects is a group of five subjects who are sampled at the same 3 time points. This way, there are 4 batches covering a total of 12 time points.

Estimation of the AUC value was done with the rectangular method (equation 1-3).

$$AUC_o^t = \sum_{i=1}^{T} w_i \cdot C_i \qquad \text{Eq. (1)}$$

$$w_i = \frac{t_{i+1} - t_{i-1}}{2} \qquad \text{Eq. (2)}$$

$$w_T = \frac{t_T - t_{T-1}}{2} \qquad \text{Eq. (3)}$$

The statistical comparisons were made using SPSS v. 12.0.

Parameter	Value	Variability
Kp (h^{-1}) loss rate constant in lumen	1.22	0
Ka (h^{-1}) absorption rate constant	1.73	0.198
K (h^{-1}) elimination rate constant	0.488	0.053
Vc (L)	0.944	0
K23 (h^{-1}) distribution rate constant	1.18	0
K32 (h^{-1}) return rate constant	1.56	0.022
σ^2	-	0.0213
Dose (mg)	4	

Table 1: Parameters used in data simulation.

The variability estimation was made by four different methods:

1. Partial AUC Method (Method 1): the individual AUC is created for each individual at all the times it has been sampled at. The individual AUC are combined to create a

2. partial AUC for each batch (or time) of the design and then the partial AUC are added to estimate the complete AUC.

$$A\bar{U}C_b = \sum_{i=1}^{N_b} \frac{AUC_{bi}}{N_b} \qquad \text{Eq. (4)}$$

$$A\bar{U}C_{complete} = \sum_{b=1}^{B_k} A\bar{U}C_b \qquad \text{Eq. (5)}$$

Using this method is relatively straightforward to calculate the AUC variance:

$$S^2_{A\bar{U}C_b} = \frac{\sum_{i=1}^{N_{kb}} \left(AUC_{bi} - A\bar{U}C_b\right)^2}{N_b} \qquad \text{Eq. (6)}$$

$$S^2_{A\bar{U}C_{total}} = \sum_{b=1}^{B} \frac{S^2_{A\bar{U}C_b}}{N_b} \qquad \text{Eq. (7)}$$

3. Jackknife method: it calculates the AUC without the i-th data, after that the AUC variance was calculated as:

$$V_i = n \cdot A\hat{U}C - (n-1) \cdot AUC_i \qquad \text{Eq. (8)}$$

$$S^2_{AUC} = \frac{\sum_{i=1}^{n} \left(V_i - \bar{V}\right)^2}{n \cdot (n-1)} \qquad \text{Eq. (9)}$$

$$\bar{V} = \frac{\sum_{i=1}^{n} V_i}{n} \qquad \text{Eq. (10)}$$

Where AUC_i is the AUC calculated without the 'i' data. We consider that the 'i' data could be the 'i' plasmatic concentration (Method 3) or the 'i' subject with all its plasmatic concentrations (Method 2).

4. Satterthwaithe's approximation method (Method 4): this method tries to calculate the AUC variance bearing in mind the covariance terms between data that belong to the same subject. The area under the curve is calculated by rectangular method, as in previous methods. When each subject is sampled at two or more different times, the variances are correlated in these data. By means of a complex matrix calculation, this expression is obtained:

$$S^2_{A\bar{U}C} = \sum_{i=0}^{m} \frac{w_i^2 \cdot s_i^2}{r_i} + 2 \cdot \sum_{i<j} \frac{w_i \cdot w_j \cdot r_{ij} \cdot s_{ij}}{r_i \cdot r_j}$$

Eq. (11)

Where: w_i and w_j are the weights used in the AUC estimation, corresponding to the i and j times respectively. ri and r_j are the number of subjects sampled at i and j times, and r_{ij} is the number of subjects sampled at both i and j times. Finally, s_{ij} is the covariance value between the datas that belong to the same subject, which have been taken at i and j times.

Results and discussion

From the 100 subject groups, an AUC estimated value was obtained (as the mean value of those 100 estimations) and its standard error. The result (mean ± standard error of the mean) 4.2374 (± $2.40 \cdot 10^{-2}$) mg·h/L, was compared to the true AUC value, considering the true AUC value the population estimate given by NONMEN. There were not statistical differences.

The variability values were statistically evaluated and the analysis of variance showed statistical differences among methods (P<0.05). Table 2 shows the mean variance estimated values and their standard errors of the mean, for each method:

	Mean standard variance estimated (standard error of the mean)
Method 1	0.0769 (0.0028)
Method 2	0.0769 (0.0026)
Method 3	0.0388 (0.0013)
Method 4	0.0608 (0.0022)

Table 2: Mean values of variability estimated by three different methods.

The posthoc ANOVA analysis (Scheffe test) showed significant statistical differences among every methods except Partial AUC and Jackknife (using subjects as 'i' data) methods (Methods 1 and 2 respectively).

On the other hand, the confidence intervals (CI) coverage are summarized in table 4. CI coverages were obtained by checking if the true AUC value was included or not in the confidence interval.

	CI coverage
Method 1	99%
Method 2	99%
Method 3	90%
Method 4	97%

Table 4: Confidence intervals coverage (%) for each method.

The confidence interval calculated using the variance obtained with method 3 did not achieve the nominal coverage, whereas the variances obtained with the other methods lead to confidence interval coverages larger than the nominal one (95%).

References

1. Yuan J. Estimation of variance for AUC in animal studies. *J.Pharm.Sci.* 1993; **82:** 761-763.
2. Nedelman JR, Gibiansky E. The variance of a better AUC estimator for sparse, destructive sampling in toxicokinetics. *J.Pharm.Sci.* 1996; **85:** 884-886.
3. Bonate PL. Coverage and precision of confidence intervals for area under the curve using parametric and non-parametric methods in a toxicokinetic experimental design. *Pharm.Res.* 1998; **15:** 405-410.
4. Holder DJ, Hsuan F, Dixit R, Soper K. A method for estimating and testing area under the curve in serial sacrifice, batch, and complete data designs. *J.Biopharm.Stat.* 1999; **9:** 451-464.
5. Nedelman JR, Jia X. An extension of Satterthwaite's approximation applied to pharmacokinetics. *J.Biopharm.Stat.* 1998; **8:** 317-328.