Evaluation of Pharmacokinetic Studies: Is It Useful to Take into Account Concentrations Below the Limit of Quantification?

Henri Humbert,[1,2] Marie Danièle Cabiac,[1] José Barradas,[1] and Christophe Gerbeau[1]

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Purpose. Based on real data, to evaluate the usefulness of taking into account samples with values below the limit of quantification (LOQ) for the evaluation of pharmacokinetic studies.

Methods. To compare for two drugs, after single dose administration the pharmacokinetic parameters obtained by using a poorly sensitive assay (PSA) and a highly sensitive assay (HSA), acting as reference; To evaluate the results of pharmacokinetic studies in the light of different values for the LOQ.

Results. Under certain conditions, such as homogeneous population, sufficient subject number, sufficient sampling times and acceptable accuracy (CV < 20%) for the concentrations, it is possible to get valuable and more reliable kinetic information by using concentrations obtained with a poor precision (CV > 20%). This is especially true for the parameters associated with the terminal phase, such as $t1/2\beta$ and AUC, but also for parameters depending to a lesser extent on the terminal phase, such as $t1/2\alpha$ and AUC_{tn}. Moreover, the mean concentration time curve is by far best defined by using all the concentrations.

Conclusions. In some situations, it is preferable to use concentrations with values below the LOQ to evaluate the results of pharmacokinetic studies. However, this should not be the rule, especially when this does not bring any additional information, or when it is possible to increase the sensitivity of the bioanalytical assay.

KEY WORDS: pharmacokinetics; precision; accuracy; limit of quantification.

INTRODUCTION

Results from pharmacokinetic studies depend to a high extent on the sensitivity of the bioanalytical method. Few years ago, the calculation of the limit of quantification (LOQ) of a bioanalytical method fully relied on the analyst, since few guidelines and numerous theories were available (1). As a result, for a same method, with comparable data, the LOQ defined in different laboratories could have significantly different values. It was up to the Conference Report on analytical method validation (2) to define guidelines on this topic, allowing an objective approach to calculate only one value of LOQ from a given set of data. For instance LOQ is obtained from the sample concentration presenting an accuracy and precision ≤20%. In fact, accuracy is not really a problem, since in case of a large inaccuracy, the data can and should be corrected taking into account the bias (3).

For numerous drugs, the bioanalytical assay is sensitive enough to clearly characterize the kinetic parameters. However, for some drugs at therapeutic dosage, despite a lot of efforts to improve the sensitivity of the assay, numerous samples from the elimination phase exhibit concentrations below the LOQ. Nevertheless, these samples certainly contain information which is not available by other ways, and the question is: Could discarding samples with concentration below the LOQ bias the conclusion of a study?

The analyst involved in pharmacokinetic calculation knows that in such a situation, the LOQ has an important impact on the calculation of the area under the concentration time curves (AUC_t), and the value of the terminal half-life. This terminal half-life could play a crucial role to explain a longer pharmacological (clinical) effect (5,6) or to predict the steady state concentrations (7) or more simply to calculate the extrapolated AUC (4). Additionally, the calculation of the mean concentration time curve, useful tool to summarize and present the data, is difficult, since numerous individual concentrations are below the LOQ.

Based on real data, the present paper tries to answer to the following questions: is it useful under certain circumstances, to take into account concentrations below the LOQ? And what information can be expected from such data?

METHOD

In the following, the formal names of the drugs are not mentioned, as the results of this analysis are not especially related to a specific drug but more generally to any drug where the same kind of problem is encountered. For each study, the protocol was approved by the local Ethics Committee. All the participants gave their written informed consent.

Single Dose Kinetics

A first study was performed after a single oral dose administration of drug A to 20 healthy subjects. Blood samples were collected just before drug administration (blank) and 0.17, 0.33, 0.50, 0.75, 1.00, 1.33, 1.67, 2, 3, 4, 6, 9, 12, 18, 24, 28, 36, 40, 48, 54, 60, and 72 hours after.

A second study was performed after a single oral dose administration of drug B to 16 healthy subjects. Blood samples were collected just before drug administration (blank) and 1, 2, 3, 4, 5, 6, 8, 10, 12, 24, 32, and 48 hours after drug administration.

For each drug, the clinical samples were firstly analysed with a poorly sensitive assay (PSA). In a second step, after improvement of the assay, all the clinical samples near or below the recommended LOQ of the PSA were reanalysed with a highly sensitive assay (HSA). For both assays, the quality control samples (QCS) were choosen to be in the range of concentrations with good precision (<20%) and accuracy (<15%). However, with the PSA additional QCS were also assayed, with very low concentrations corresponding to good accuracy (<15%) but poor precision (>20%). For each drug, with the PSA, an overall coefficient of variation (CV) was obtained for each value of QCS (8). A relationship was then established between CV and concentration. The best fit among different possibilities (9) was obtained with the simple equation CVc =

[1] Pharmaceutical Research Centre, Sandoz Laboratories, 92506 Rueil-Malmaison, France.

[2] To whom correspondence should be addressed.

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(a/C) + b, where C is the concentration corresponding to the CVc; a and b are constants. From this equation, it was thus possible to define a so-called "limit of evaluation" (LOE) of the data, associated to a given CV, for the PSA method.

Comparison of the Mean Concentration Time Curves

For each drug, a reference mean concentration time curve was established from the results of the HSA method. The sample concentrations from the PSA were then validated according to different values of the LOE, corresponding to a CV of 20, 30, 50, 70, 100 and 200% respectively. For each value of the LOE, two mean curves were calculated with the data of the PSA, either by setting all the values below the LOE to zero, or by keeping only the values above the LOE. These two ways of calculation will be summarized in the following as: setting or not to zero for individual values below the LOE.

Comparison of Pharmacokinetic Parameters

Each individual concentration time curve was fitted according to a two-compartment open model with zero-order input and elimination from the central compartment (10), using ELSFIT (11). The evaluations were performed for each individual data set obtained from the HSA, and also, for those obtained from the PSA, assuming two values for the LOE, corresponding to a CV of 30 and 200%. The use of a CV of 20%, according to the conference report on analytical method validation (2), instead of 30% would have been too discriminatory for the results of the PSA. Compartmental parameters were obtained by iterative weighted non-linear regression analysis using weights that were inversely proportional to the observed concentrations (12). The half-lives of the distribution and elimination phases were calculated as $t1/2\ \alpha = (Ln2)/\alpha$ and $t1/2\beta = (Ln2)/\beta$ respectively, where α and β are the relevant rate constants. Calculation was performed in each case only with the values above the corresponding LOE. The individual area under the plasma concentration time curves (AUC_{tn}) was then determined by the linear trapezoïdal rule to the last concentration $Ctn \geq LOE$. The AUC was calculated from AUC_{tn} by extrapolation to infinity by the addition of the term Ctn/β. The truncated $AUC[12 - tn]$ and $AUC[12 - \infty]$ were also calculated by substraction of the AUC12h to AUC_{tn} and AUC. Statistical analysis including paired t test and search for linear correlation (13) were performed between the PSA and HSA results. In tables, the results of paired t test analysis were reported as: NS (non significant), * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$).

Single Dose Linearity Study

A single dose linearity study was performed, according to a cross-over design in 18 healthy subjects, with a drug C at three different dosages 2.5, 5 and 10 mg. Blood samples were collected just before drug administration (blank) and 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 12, 16, 20, 24, 30, 36, 48, 60, and 72 hours after drug administration. The samples were assayed only with a PSA. For each individual concentration time curve the AUC_{tn} was calculated as described above, and assuming two values of LOE; defined by a CV of 30%, or 200%. As no reliable half-life values could be produced for the lower dose as compared to the higher dose neither terminal half-lives, nor AUC were calculated. The linearity of absorption was then tested by comparison of the AUC_{tn} increase versus the dose increase.

Single Dose Bioequivalence Study

A single dose bioequivalence study was performed, according to a cross-over design in 18 healthy subjects, with two formulations (standard and slow released) of drug D at the same dosage. Blood samples were collected just before drug administration (blank) and 0.17, 0.33, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 9, 12, 16, 20, 24, 30, and 36 hours after drug administration. The samples were assayed only with a PSA. For each individual concentration time curve the AUC_{tn} was calculated as described above, and assuming two values of LOE; defined by a CV of 30%, or 200%. The terminal half-lives and consequently AUC were not calculated, because no reliable half-life values could be produced for the slow release formulation. The relative bioavailability of the slow release formulation was then calculated from the AUC_{tn} ratio.

RESULTS

Single Dose Kinetics

Comparison of the Mean Concentration Time Curves

For drug A the synoptic view of the individual plasma concentration time curves obtained with the PSA and with the HSA is shown in Figure 1. The mean curves obtained from the PSA, assuming different values for the LOE and setting or not to zero the values below the corresponding LOE, are shown in Figure 2, in comparison to the reference mean curve obtained from the HSA. This figure shows that, as compared to the curve

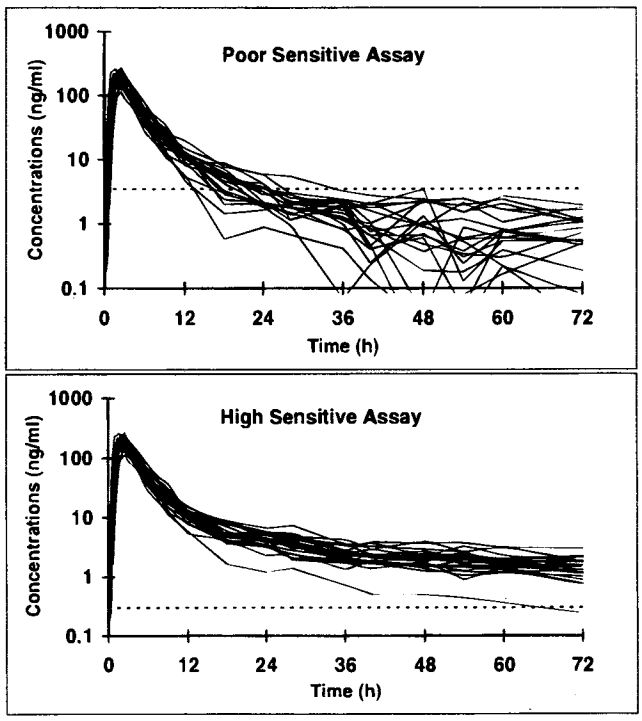

Fig. 1. Individual plasma concentration time curves (N = 20) for drug A after single oral dose administration in healthy volunteers (....) LOQ for each assay.

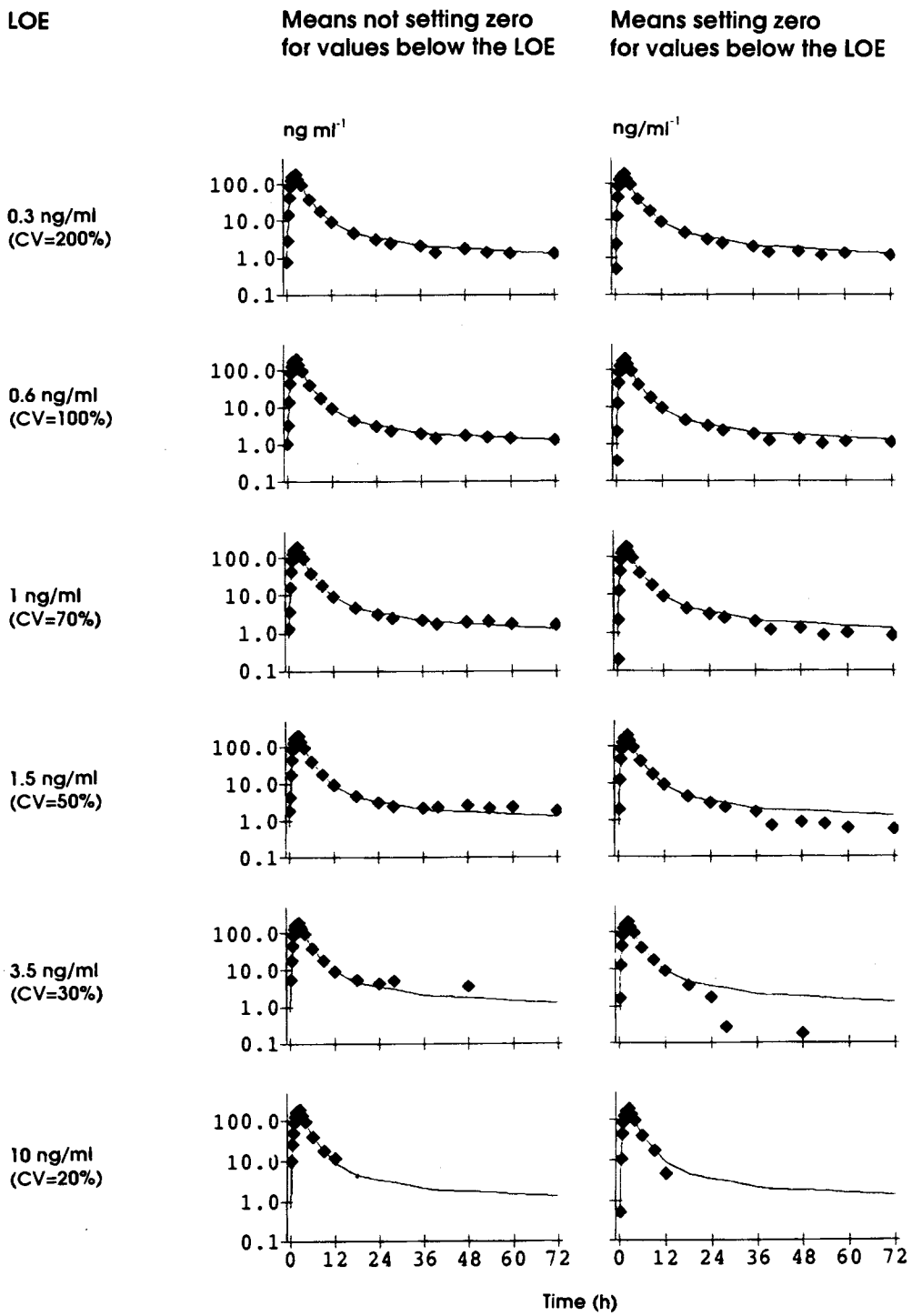

Fig. 2. Mean (N = 20) plasma concentration time curves for drug A after single oral dose administration in healthy volunteers, with a HSA (——) and a PSA (♦) according different values of LOE.

obtained with the HSA, the best results deduced from the PSA are obtained for a LOE corresponding to a CV $\geq$ 100%. In this case, the two mean curves from the PSA are pratically superimposable to that of the HSA. From the PSA results, assuming a LOE with a CV $\leq$ 30%, there is a dramatic impact on the mean curve after the 24th hours when setting or not to zero for the individual values below the LOE. Moreover, in that situation, none of the two mean curves deduced from the PSA, fit with the mean reference curve (HSA) after the 24 h. The worst result is observed with the PSA data assuming a LOE with a CV $\leq$ 20% (recommended LOQ), with essentially no available pharmacokinetic informations after the 12th hour.

For drug B, the same observation can be done with regard to the different mean curves deduced from the PSA results

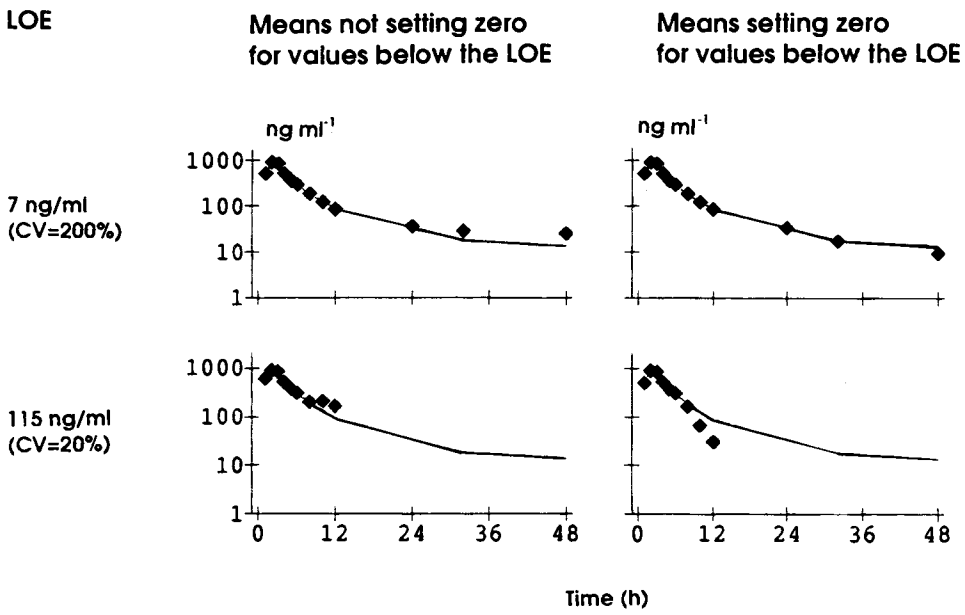

Fig. 3. Mean (N = 16) plasma concentration time curves for drug B after single oral dose administration in healthy volunteers, with a HSA (——) and a PSA (♦) according different values of LOE.

when compared to the mean reference curve (HSA). This is clearly shown in Figure 3 where the two extreme cases corresponding to a LOE associated with a CV of 200 and 20%, are represented for drug B.

Comparison of Kinetic Parameters

The mean pharmacokinetic parameters obtained for drug A, are displayed in Table I. From the PSA results and assuming a LOE of 0.3 ng/ml (CV = 200%) the means of AUC_{tn} and AUC, differ by less than 3% as compared to the HSA results. Assuming a LOE of 3.5 ng/ml (CV = 30%) a difference of about 10% is observed for these two parameters between the HSA and PSA results. This difference is obviously due to the last part of the kinetics and appears more clearly for the comparison of the truncated AUC[12 − tn] (Tables I and II). The best fit, closed to the identity line, and with the highest coefficient of correlation (r = 0.794), is obtained with the PSA results corresponding to the LOE defined from a CV of 200%. In the other case, the coefficient of correlation is worse (r = 0.464) and the straight line of the correlation is clearly different from the identity line. Similar conclusions (Tables I and II) can be drawn from the analysis of the truncated AUC[12 − ∞]. Concerning the mean half-lives t1/2α and t1/2β, the best agreement with the HSA is observed for the PSA results assuming a LOE corresponding to a CV of 200% (Table I). As expected, this is especially the case for t1/2β where a large under estimation of the half-life value is noticed, with the PSA results assuming a LOE corresponding to a CV of 30% (Table I). From the kinetic profile after 6 h, for drug A (Figure 1), it is clear that, taking into account the samples with concentrations below 3.5 ng ml^{-1} (CV 30%) until 0.3 ng ml^{-1} (CV 200%), dramatically increases the individual number of sampling times allowing the evaluation of t1/2β. The statistical analysis shows that the best correlation (Table II) between the HSA and the PSA results for t1/2α is observed with the lowest LOE (CV = 200%). For t1/2β, despite comparable mean values (Table I) between the HSA and the PSA results assuming the lowest LOE (CV = 200%), the correlation on individual values is

Table I. Pharmacokinetic Parameters of Drugs A and B, After Single Oral Dose Administration to Healthy Subjects and Different Assay Conditions

	Drug A (N = 20)			Drug B (N = 16)		
Parameters	HSA LOQ: 0.3 ng/ml (CV 20%)	PSA LOE: 0.3 ng/ml (CV 200%)	PSA LOE: 3.5 ng/ml (CV 30%)	HSA LOQ: 10 ng/ml (CV 20%)	PSA LOE: 7 ng/ml (CV 200%)	PSA LOE: 65 ng/ml (CV 30%)
AUC_{tn} (ng $ml^{-1}h$)	873 ± 173	859 ± 172 NS	783 ± 159***	5409 ± 2196	5406 ± 2063 NS	4838 ± 2245***
AUC[12 − tn] (ng $ml^{-1}h$)	157 ± 44	142 ± 47*	64 ± 42***	1155 ± 625	1144 ± 625 NS	511 ± 613***
t1/2 α (h)	1.63 ± 0.26	1.67 ± 0.34 NS	1.20 ± 0.42***	1.34 ± 0.46	1.33 ± 0.42 NS	0.83 ± 0.54***
t1/2 β (h)	27.13 ± 7.48	22.88 ± 10.82 NS	10.22 ± 8.65***	11.12 ± 5.77	14.80 ± 13.34 NS	5.57 ± 5.03***
AUC (ng $ml^{-1}h$)	925 ± 187	900 ± 176*	849 ± 162***	5680 ± 2363	6008 ± 2482 NS	5161 ± 2694*
AUC [12 − ∞] (ng $ml^{-1}h$)	210 ± 61	183 ± 61**	124 ± 85***	1425 ± 905	1746 ± 1353 NS	834 ± 1127**

Value are mean ± SD.

Table II. Results of the Linear Correlation Between Pharmacokinetic Parameters Obtained with HSA and PSA Assuming Two Different Values of the LOE, for the PSA

	Drug A						Drug B					
	LOE = 0.3 ng/ml (CV 200%)			LOE = 3.5 ng/ml (CV 30%)			LOE = 7 ng/ml (CV 200%)			LOE = 65 ng/ml (CV 30%)		
Parameters	Slope	Intercept	r	Slope	Intercept	r	Slope	Intercept	r	Slope	Intercept	r
AUC_{tn}	0.982	5.651	0.985	0.942	−38.44	0.966	0.898	621.8	0.956	0.992	−265.7	0.971
AUC[12 − tn]	1.115	−27.88	0.794	0.771	−65.12	0.464	0.918	29.53	0.514	0.834	−269.8	0.691
t1/2 α	1.291	−0.424	0.832	1.392	−0.950	0.324	0.888	0.416	0.956	1.112	−0.602	0.587
t1/2 β	0.974	−4.512	0.257	0.917	−14.48	0.308	1.723	−6.986	0.072	0.130	2.544	0.187
AUC	0.930	41.59	0.976	0.891	14.04	0.873	1.082	−299.2	0.836	0.843	278.6	0.935
AUC [12 − ∞]	0.967	−11.48	0.770	1.348	−184.2	0.261	1.480	−635.9	0.310	0.631	80.75	0.597

poor (Table II). This is related to the high variability of the concentrations obtained with the PSA and also to the fact that the range of individual half-life is not so wide. However, the same comparison with the data of the PSA obtained with the highest LOE (CV = 30%) leads also to a poor correlation for t1/2β; moreover the intercept is very high as compared to the value of this parameter (Table II).

For drug B, the mean kinetic parameters are displayed in Table I. The same observations as for drug A can be drawn from the comparison of the mean results of the two methods. Whatever the parameters, the mean value closest to that of the HSA is obtained with the PSA results using the lowest LOE (CV = 200%). The statistical analysis based on linear correlation with individual data for drug B exhibits a quite contrasted image (Table II). Whilst the slopes tend to be generally nearer to unity with the lower LOE (CV = 200%), the coefficient of correlation tends to be worse than with the higher LOE (CV = 30%). Again, the individual estimates of the parameters (except t1/2α) are badly affected by the high variability of the concentrations below the LOQ. Moreover, the number of subjects in study with drug B is smaller than for drug A (N = 16 vs 20), which certainly affects this type of statistical analysis. However, whatever the parameter, with regard to the slope, the intercept and the coefficient of correlation, the PSA results assuming a LOE with a CV = 200%, are not worst than the PSA results assuming a LOE with a CV = 30%.

Single Dose Linearity Study

For drug C, the mean AUC_{tn} (±SD) for the three dosages (2.5–5–10 mg) are: 1357 (±674), 2538 (±1100), 6441 (±2676) pg ml^{-1} h and 625 (±487), 1792 (±1058), 5786 (±2627) pg ml^{-1} h for the lowest (4.3 pg ml^{-1}, CV = 200%) and the highest (46.2 pg ml^{-1}, CV = 30%) LOE, respectively. Depending on the value of the LOE, the dose linearity is either not, or nearly satisfied. Indeed, the ratio of dose increases from 1 to 4 while, at the same time, the ratio of the AUC_{tn} increases from 1 to 8.7, with the highest LOE (CV = 30%) and from 1 to 4.3 with the lowest LOE (CV = 200%). This bias, between these two results is quite obvious when the mean concentration time curves for the 3 doses are displayed comparatively with the two values of the LOE (Figure 4). On this figure the mean concentration time curves have been calculated with all the concentrations ≥4 pg ml^{-1}. With the highest LOE (CV = 30%), a large part of the AUC_{tn} is not taken into account, mainly for the lowest dose.

Single Dose Bioequivalence Study

For drug D, the mean AUC_{tn} (±SD) for the reference and slow release formulation are: 1377 (±674), 1106 (±611) pg $ml^{-1}h$ and 1040 (±658), 577 (±717) pg $ml^{-1}h$ for the lowest (1 pg ml^{-1}, CV = 200%) and the highest (41 pg ml^{-1}, CV = 30%) LOE respectively. Depending on the value of the LOE

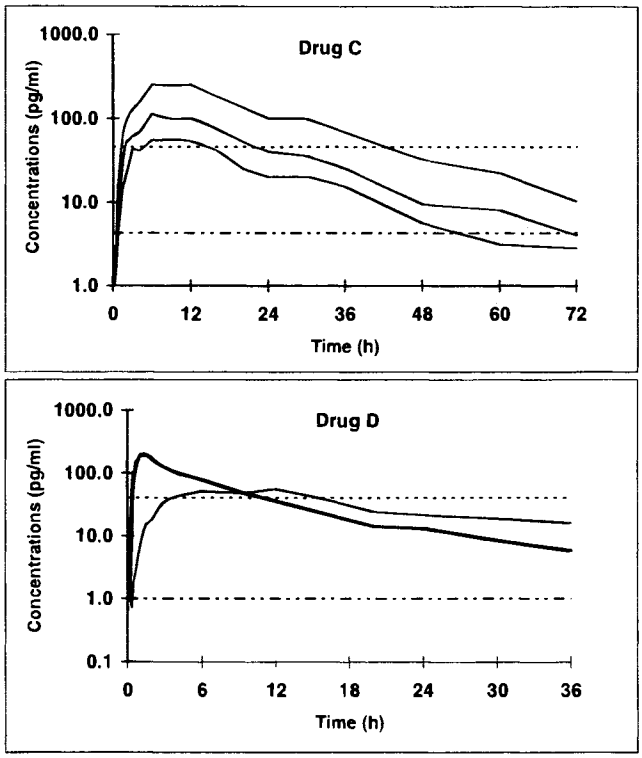

Fig. 4. Mean plasma concentration time curves as compared to a LOE defined from a CV = 30% (- - - -) or a LOE defined from a CV = 200% (-- · · --). UPPER: for drug C after single oral dose administration at three dosage in 18 healthy volunteers LOWER : for drug D after single oral dose administration of a standard (——) and a slow release formulation (——) in 18 healthy volunteers.

(assuming CV = 30 or 200%) the mean (±SD) relative bioavailability of the slow release formulation increases from 53.5% (±42.9) to 84.7% (±30.3). This discrepency between these two results is clearly illustrated in Figure 4 where the mean concentration time curves for the two formulations are displayed comparatively with the two values of the LOE. On this figure, the mean concentration time curves have been calculated with all the concentration >1 pg ml^{-1} (CV = 200%). With the highest LOE (41 pg ml^{-1}; CV = 30%) a large part of the AUC_{tn} for the slow release formulation is not taken into account due to the shape of the plasma concentration time curve.

DISCUSSION

It is obvious that all the pharmacokinetic studies analysed in this paper have been choosen consecutively to encountering difficulties to obtain a bioanalytical assay sensitive enough, at the time of the studies, to fully describe the terminal phase of the kinetics of these drugs in good conditions. Moreover, it is noteworthy that all the above examples, refer to an homogeneous population, with a quite large number of subjects, a standardized administration and a sufficient number of sampling time above the LOQ in order to define properly the last part of the kinetics. It is also important to notice that, for the PSA, information have been obtained from QCS in the low range of concentrations. These QCS, despite a poor precision must have statistically a low bias (<20%).

Under all these assumptions, if we consider the calculation of the mean concentration time curve during studies with drugs A and B, the analysis clearly shows that the best result from the PSA data, is obtained by keeping all the values of concentrations, whatever their precision (Figures 2 and 3). If only values above the LOQ are considered, none of the two ways of calculation (setting or not to zero for values below the LOQ) are satisfactory for the mean curve. A divergence rapidly occurs for the sampling time where the percentage of individual concentrations below the LOQ exceed 10 to 15% of the overall concentration at this time. So if only concentrations above the LOQ are used to calculate the mean concentration time curve it appears reasonable to stop the calculation at the sampling time where 10 to 15% of the individual values of concentration are below the LOQ.

For the pharmacokinetic parameters AUC_{tn}, AUC, $t1/2\ \alpha$, and $t1/2\ \beta$, it is also clear that the mean values are more reliable (Table I) if concentrations below the LOQ are used instead of not using them. However, at the individual level, if these results are quite reliable for AUC_{tn}, AUC and $t1/2\alpha$ this is not the case for $t1/2\beta$. Nevertheless for this parameter the individual values are at least as correct as those not using concentrations below the LOQ (Table II). So in such a situation, if information on the terminal phase of the kinetics is needed it is highly recommended to use concentrations below the LOQ.

The results of the two pharmacokinetic studies with drugs C and D are quite interesting in the light of the observations done in the studies with drugs A and B. For each of them, two different conclusions can be drawn, depending on the way the data are analysed.

For instance, for drug C, the dose linearity is not, or is satisfied depending of the value of the LOE. Since the time of this study, more recent studies at steady state and single dose, combined with a HSA have confirmed the linearity of the kinetics of drug C. The result of such a study is very important for a drug, since non linearity of the kinetics may have a dramatic impact on the clinical development. So in that situation, using concentrations below LOQ appears essential to state on the results. A possibility to get round this problem is to calculate the AUC_{tn}, for the three dosages, up to the sampling time for which the concentrations of the lowest dose were no more reliable: i.e. for study with drug C at the 16th hour. However, this is not a general acceptable way of doing, since for some drugs a non-linearity of the kinetics could appear in the terminal elimination phase. Moreover, when a study is done to compare two formulations at the same dose this way of doing seems to give worse results than using all the concentrations above the LOQ whatever the sampling time (14). Another solution would have been to perform the dose linarity study at steady state, but this is more expensive and time consuming, and moreover this is not always acceptable with certain drug in healthy volunteers. This problem of the LOQ, in dose linearity studies, has already been identified by other authors (15).

The study with drug D is also a typical situation, where the decision to further develop a slow release formulation depends on the first pharmacokinetic evaluation at single dose. For drug D, this first evaluation shows a good kinetic profile (Figure 4), however depending on the way of analysing the data, the relative bioavailability versus reference is 54% (LOE CV = 30%) or 85% (LOE CV = 200%). In the first situation, generally the decision is to stop the development of this slow release formulation due to the poor bioavailability. Nevertheless, further studies performed at steady state have shown that the relative bioavailability of this slow release form at steady state was in the range of 85%. So, this is another situation where the systematic use of concentrations below the LOQ is highly recommended. It is well known that to avoid this problem, it would have been preferable to increase the dosage or to perform directly a steady state evaluation. However, increasing the dosage could bias the result if the linearity of the kinetics is not respected for one of the two formulations, and the steady state evaluation is more expensive, more time consuming and sometimes not acceptable in healthy volunteers for certain drugs.

All these observations done in this analysis are certainly not a big surprise for those who have some experience in this field as they are rather obvious by intuition. However, from our experience, using systematically values below the recommended LOQ must not be a general rule during pharmacokinetic evaluation. For numerous studies, this does not bring any additional information and does not justify the necessary additional cost and time to obtain and validate a good accuracy under the LOQ during the sample assay. Moreover, using concentrations below the LOQ is only justified when, at the time of the study, the bioanalytical method is not sensitive enough, consequently to time pressure (first step of development) or insuperable analytical problem.

CONCLUSION

These results based on real data, suggest that in some situations and under certain conditions, such as homogeneous population, sufficient subject number, sufficient sampling times and low bias of the concentration, it is possible to get valuable and more reliable kinetic information by using concentrations

obtained with a poor precision (>20%). This is specially true for the parameters depending directly on the last part of the elimination phase, such as $t1/2\beta$ and AUC, but also for parameters depending to a lesser extent, on the last part of the elimination phase, such as $t1/2\alpha$ and AUC_{tn}. Moreover, the mean concentration time curves is by far best defined by using all the concentrations whatever their precisions. So under certain circumstances the use of concentrations below the LOQ to analyse pharmacokinetic studies appears highly recommended. However, this should not be the rule, especially when this does not bring any additional information, or when it is possible to increase the sensitivity of the bioanalytical assay. Finally, when such concentrations are used, this assumes that the range of reported concentrations would be fairly validated regarding accuracy.

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REFERENCES

1. A. B. J. Nix, D. W. Wilson. Assay detection limits: concept, definition and estimation. *Eur. J. Clin. Pharmacol* **39**:203–206 (1990).
2. V. P. Shah, K. K. Midha, S. Dighe, I. J. McGilveray, J. P. Skelly, A. Yacobi, T. Layloff, C. T. Viswanathan, C. E. Cook, R. D. McDowall, K. A. Pittman, S. Spector. Analytical methods validation: bioavailability, bioequivalence and pharmacokinetic studies. *Eur. J. Drug Metab.* **16**:249–255 (1991).
3. J. O. Westgard, P. L. Barry. Cost effective quality control: managing the quality and productivity of analytical processes, AACC Press, Washington DC, 1986, pp. 33–64.
4. A. J. Jackson. Inappropriate inclusion of non-quantifiable plasma concentrations in the estimation of extent of absorption. *Biopharm. Drug Disp.* **13**:629–634 (1992).
5. P. Francheteau, J. L. Steimer, C. Dubray, D. Lavène. Mathematical model for in vivo pharmacodynamics integrating fluctuation to the response: application to the prolactin suppressant effect of the dopaminomimetic drug DCN 203–922. *J. of Pharm. and Biopharm.* 19(**3**):287 (1991).
6. H. Zehender, M. D. Cabiac, J. Denouël, J. Faergemann, P. Donastsch, K. Kutz, H. Humbert. Elimination kinetics of terbinafine (lamisil) from human plasma and tissues following multiple dose administration of 250 mg daily during four, twelve and fourty-eight weeks and comparison with three main metabolites. *Drug Invest.* 8(**4**):203–210 (1994).
7. D. A. Graves. Failure of single dose kinetics to predict steady-state. *Drug Intell. Clin. Pharm.* **22**:917–918 (1988).
8. H. T. Karnes, G. Shiu, V. P. Shah. Validation of bioanalytical methods. *Pharm. Res.* **8**:421–426 (1991).
9. W. A. Sadler, M. H. Smith. Use and abuse of imprecision profiles: some pitfalls illustrated by computing and plotting confidence intervals. *Clin. Chem.* **36**:1346–1350 (1990).
10. M. Gibaldi, D. Perrier. Pharmacokinetics, 2nd edition, Marcel Dekker Inc, New-York, 1983.
11. L. B. Sheiner. ELSFITT (Version 3.1). A program for the extended least Squares fit to individual pharmacokinetic data. Users manual. April 1985. Technical report of the division of Clinical Pharmacology, University of California, San Francisco, CA 94143, USA.
12. D. A. Graves, C. S. Locke, Jr., K. T. Muir, R. P. Miller. The influence of assay variability on pharmacokinetic parameter estimation. *J. Pharmacokin. Biopharm.* **17**:571–592, 1989.
13. H. Passing, W. Bablok. A new biometrical procedure for testing the equality of measurements from two different analytical methods. Application of linear regression procedures for method comparison studies in clinical chemistry. Part I. *J. Clin. Chem. Clin. Biochem.* **21**:709–720, 1983.
14. F. Y. Bois, T. N. Tozer, W. W. Hauck, M. L. Chen, R. Patnaik, R. L. Williams. Bioequivalence: performance of several measures of extent of absorption. *Pharm. Research* **11(5)**:715 (1994).
15. S. E. Tett, D. J. Cutler. Apparent dose-dependence of chloroquine pharmacokinetics due to limited assay sensitivity and short sampling times. *Eur. J. Clin. Pharmacol.* **31**:729–731 (1987).