



Residual fraction of the area under the curve as a qualitative criterion in pharmacokinetic studies

Tomasz Grabowski¹, Jerzy J. Jaroszewski², Piotr Jakubowski²

¹Centre of Pharmacokinetics Research FILAB, Ravimed Sp. z o.o., Polna 54, PL 05-119 Łajski, Poland

²Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Warmia and Mazury, Oczapowskiego 13, PL 10-718 Olsztyn, Poland

Correspondence: Tomasz Grabowski, e-mail: t.grabowski@filab.com.pl

Abstract:

The aim of the present study was to determine whether the residual area under the curve ($AUC_{res\%}$; expressed as % of total value of AUC) could be used as a parameter for the qualitative evaluation of pharmacokinetic studies. We propose new criteria for the qualitative evaluation of pharmacokinetic analysis. Two sets of hypothetical data that illustrate the relationship between concentration and time were used for the analysis of drug pharmacokinetics. Non-compartmental analysis was applied for the calculations. The results obtained from the hypothetical data were compared with those obtained from an *in vivo* study in which 3-week-old broiler chickens were administered 10 mg/kg b.w. enrofloxacin intravenously (*iv*) or *per os* (*po*). In the first set of data (A–D), $AUC_{res\%}$ values were as follows: A = 16.29% and B = 20.79% for *iv* administration and C = 29.61% and D = 27.90% for *po* administration. In the next set of data (E–G), $AUC_{res\%}$ values after oral administration were 25.30% (E), 23.18% (F), and 20.79% (G). The $AUC_{res\%}$ values after *iv* administration of enrofloxacin were similar to *po* administration; the range of *iv* and *po* administration values were 14.35% to 17.50% and 11.14% to 28.33% of the total AUC, respectively. The analysis of the hypothetical data indicates that $AUC_{res\%}$ is not an optimal method for the evaluation of pharmacokinetic studies.

Key words:

AUC_{res} , K_{el} , bioequivalence, pharmacokinetics, extrapolated area, truncated area

Abbreviations: $AUC_{0-\infty}$ – area under the curve from 0 to infinity, AUC_{res} – value of the residual area under the curve, $AUC_{res, min}$ – minimal value of the residual area under the curve, $AUC_{res\%}$ – residual observed part of the area under the curve (expressed as % of $AUC_{0-\infty}$), $AUC_{res\%, pred}$ – residual predicted part of the area under the curve (expressed as % of $AUC_{0-\infty}$), b.w. – body weight, $C_{last, obs}$ – last observed concentration, C_{max} – peak concentration, C-T – concentration- time, CV% – percent of variability coefficient, DMPK – drug metabolism and pharmacokinetics, HLOQ – higher limit of quantitation, HPLC – high performance liquid chromatography, *iv* – intravenous administration, K_{el} – elimination phase rate constant, LLOQ – lower limit of quantitation, MRT_{0-last} – mean residence time between 0 and $t_{last, obs}$, *po* – *per os* administration, S – analytical model sensitivity, $t_{1/2}$ – elimination phase half-life time, $t_{last, obs}$ – last observed concentration of C-T curve

Introduction

In vivo pharmacokinetic studies have three main aspects of study design: the design of the clinical component, optimization of an analytic method, and the adjustment of an appropriate mathematical model [17, 18, 23, 25, 32]. Toxicokinetic and bioequivalence pilot studies are routinely performed on new medicinal products. In these studies, optimization of the experimental components is a main aim; this includes the clinical component (the number of volunteers or laboratory animals), determination of the sampling points

(to obtain an optimal curve of concentration vs. time (C-T) relationship), the bioanalytical component (optimization of the higher limit of quantitation (HLOQ) and lower limit of quantitation (LLOQ)), choice of an analytical method and its validation, and finally, the analysis of pharmacokinetic parameters (calculation model) [5, 6, 15, 16, 24, 26, 30]. These experimental concerns apply to classical *in vivo* pharmacokinetic studies following a single drug administration, studies of toxicokinetic, and comparative studies, including bioavailability or bioequivalence analysis [1, 10, 13].

High performance liquid chromatography (HPLC) is among the most commonly used methods for the chemical analysis of drugs and is an important tool for the study of pharmacokinetics. HPLC gives precise results and has a high degree of data repeatability. To evaluate and describe analytical techniques and validation procedures, the sensitivity and LLOQ are calculated in addition to other parameters [8, 11, 12]. These calculations simultaneously describe a method as an independent parameter and constitute elements of the method validation [2]. A calibration curve may be used to determine the sensitivity of the analytical method. The calibration curve is described by the regression equation and the coefficient of determination [21]. Method sensitivity is expressed by the value of the tangent to the angle of the calibration curve slope. The curve illustrates the operating range of an analytic method and allows the determination of the limits of linearity in the range of LLOQ-HLOQ in the analytical method [8]. Both LLOQ and HLOQ values should be determined in a range demarcated by the concentrations between the last observed concentration of the C-T curve ($C_{\text{last, obs}}$) and the peak concentration (C_{max}). This should take into consideration the variability of the concentration values in a given experimental group; this variability is expressed by the percent of variability coefficient (CV%) and the limits of acceptance, which result from the validation principles for bioanalytical methods ($\text{LLOQ} < C_{\text{last, obs}} - (C_{\text{last, obs}} \times \text{CV}\% \times 0.20)$; $\text{HLOQ} > C_{\text{max}} + (C_{\text{max}} \times \text{CV}\% \times 0.15)$) (Tab. 1). The LLOQ and elimination phase rate constant (K_{el}) values allow assignment of the AUC_{res} value. In particular, bioequivalence studies relate the quality of the analytical method (expressed as HLOQ and LLOQ values) with the value of K_{el} , allowing for a quality estimation of the experiment according to the 80:20 rule [1, 3, 7]. In a drug metabolism and pharmacokinetics (DMPK) study, it is assumed that sampling in the clinical phase should

be performed sufficiently long enough to take full advantage of the capabilities of an analytical method. This means that the final sample time point should contain a drug concentration higher than the LLOQ of the analytical method. Sampling that is too short results in the omission of a portion of the C-T points; confirms an elimination phase and error in the calculation of the K_{el} . Due to imprecise calculation of the area under the curve from zero to infinity ($\text{AUC}_{0-\infty}$), an error appears in other AUC-associated pharmacokinetic parameters, including the volume of distribution and drug clearance. Over-sampling should also not be performed to avoid extending beyond the limit of determinability for the analytic method.

One of the qualitative evaluation parameters associated with pharmacokinetic studies is the residual portion of the area under the curve (AUC_{res}); this is defined as the area calculated for the fragment of the C-T curve that extends beyond the time of the final sample concentration ($C_{\text{last, obs}}$). According to the guidelines of some drug registering agencies, the value of the residual observed part of the area under the curve ($\text{AUC}_{\text{res}\%}$) in DMPK studies should be less than 20% of the AUC total value from zero to infinity (80:20 rule) [3]. This rule poses a significant problem for drugs with a long half-life (in the elimination phase) and drug for which the concentration following a single administration is maintained for several days in the elimination phase. The 80:20 rule cannot be applied in these cases because the experimental sampling would be significantly extended without an increase in the quality of the DMPK calculation. This occurs mainly with drugs that have an extensive and

Tab. 1. Current and proposed assumptions of bioanalytical method optimization that allow low residual area under the curve (AUC_{res}) values

| Present rules | Proposed relationships |
|---|---|
| $\text{HLOQ} > C_{\text{max}}$ | $\text{HLOQ} > C_{\text{max}} + (C_{\text{max}} \times \text{CV}\% \times 0.15)$ |
| $\text{LLOQ} < C_{\text{last, obs}}$ | $\text{LLOQ} < C_{\text{last, obs}} - (C_{\text{last, obs}} \times \text{CV}\% \times 0.20)$ |
| $\text{AUC}_{\text{res, min}} = C_{\text{last, obs}} / K_{\text{el}}$ | $\text{AUC}_{\text{res, min}} = [C_{\text{last, obs}} \times (\text{CV}\% + 0.20)] / K_{\text{el}}$ |

CV% – percent of variability coefficient; 0.15 – acceptable deviation from a calibration curve in a bioanalytical method; $\pm 15\% = 0.15$ for concentration points beyond LLOQ; 0.2 – acceptable deviation from a calibration curve in a bioanalytical method; $\pm 20\% = 0.2$ for concentration points equal to LLOQ

complex distribution and redistribution processes, such as hepato-intestinal circulation. Knowledge of the $AUC_{res\%}$ as quality criterion is still limited. Few publications (excluding formal guidance) indicate the truncated area calculation as the appropriate drug pharmacokinetics analysis for drugs with a long half-life (elimination half-life > 24 h) [19, 20, 22]. Therefore, some registration agencies allow experimental sampling up to 72 h for drugs with a long elimination half-life, regardless of the value of the residual area [2, 4, 7, 9].

The aim of the present study was to determine the applicability of the $AUC_{res\%}$ value as a parameter for the evaluation of pharmacokinetic studies. Two sets of hypothetical data were used in this study and they consisted of a single drug administration differing only in the route of administration. A secondary purpose of the study was to establish whether the calculation methods employed elicit similar or different results of $AUC_{res\%}$ between the hypothetical and experimental data sets. The results obtained from the hypothetical data were compared with a representative data set from an *in vivo* study.

Materials and Methods

WinNonlin 5.01 software was used for the calculation of the pharmacokinetic parameters. Two sets of data illustrating the C-T relationship were used for the analysis of drug pharmacokinetics. Non-compartmental analysis is a common objective procedure for the determination of pharmacokinetic calculations; this analysis is typically used in clinical trial (I–IV) phase studies. The elimination rate constant, determined by the calculated residual parameters, was analyzed using the last four C-T points.

The first set of data (A–D) measured changes in drug concentration following a single intravenous (*iv*) administration (A and B) and *per os* (*po*) administration (C and D). Samples collected at 0.1, 0.5, 1, 1.5, 2, 2.5, 4, 6, 8, 12, 24, and 48 h had a concentration of 200, 150, 75, 35, 12, 10, 7, 5, 4, 3.5, 2, and 1.5 ng/ml for A, 100, 75, 50, 25, 12, 10, 7, 5, 4, 3.5, 2, and 1.5 ng/ml for B, 0, 5, 8, 15, 12, 10, 7, 5, 4, 3.5, 2, and 1.5 ng/ml for C, and 1, 8, 12, 25, 20, 10, 7, 5, 4, 3.5, 2, and 1.5 ng/ml for D, respectively.

The second set of data (E–G) measured changes in drug concentration following a single *po* administration of a drug in three formulations. Samples collected at 0.1, 0.5, 1, 1.5, 2, 2.5, 4, 6, 8, 12, 24, and 48 h had a concentration of 1, 5, 10, 20, 45, 25, 7, 5, 4, 3.5, 2, and 1.5 ng/ml for E, 1, 10, 20, 50, 45, 25, 7, 5, 4, 3.5, 2, and 1.5 ng/ml for F, and 5, 20, 40, 80, 45, 25, 7, 5, 4, 3.5, 2, and 1.5 ng/ml for G, respectively.

It was assumed that all the data sets were obtained for the same drug and the only difference between them were the C-T values. The elimination phase was identical in each set. The four final C-T points and the samples obtained between 8 and 24 h were used for the calculation of K_{el} and $AUC_{res\%}$. The drug concentrations proposed for the hypothetical data had a similar C-T curve slope to that observed in the *in vivo* enrofloxacin data set.

To verify the results obtained from the hypothetical data, data sets from *in vivo* experiments were used (Jakubowski and Jaroszewski, unpublished data), in which 10 mg/kg b.w. of enrofloxacin (Enrobioflox 5% injection; Vetoquinol Biowet, Poland) was administered *iv* to a single 3-week-old broiler chicken. The resulting calculations of the observed and predicted residual area were obtained in accordance with the directives in force [3, 7]. The predicted C_{last} was based on the regression line derived from a minimum of 3 or 4 data points in the elimination phase. Three to four points in the final portion of the C-T curve, which was determined from various modifications, were used for the calculation of K_{el} . The plasma concentrations of enrofloxacin were obtained using HPLC with fluorescence detection. The sampling time points for both the *iv* and *po* administration were 0.08, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, and 24 h and 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, and 24 h, respectively. Animal procedures were approved by the Olsztyn Local Ethics Commission on Experimental Animal Care (Agreement No. 14/2006).

Results

Differences between the *iv* and *po* drug administration from the first set of data are presented in Figure 1 and the pharmacokinetic parameters are shown in Table 2. In contrast to the C_{max} , the $AUC_{res\%}$ was lower for the *iv* administration than the *po* administration. In addi-

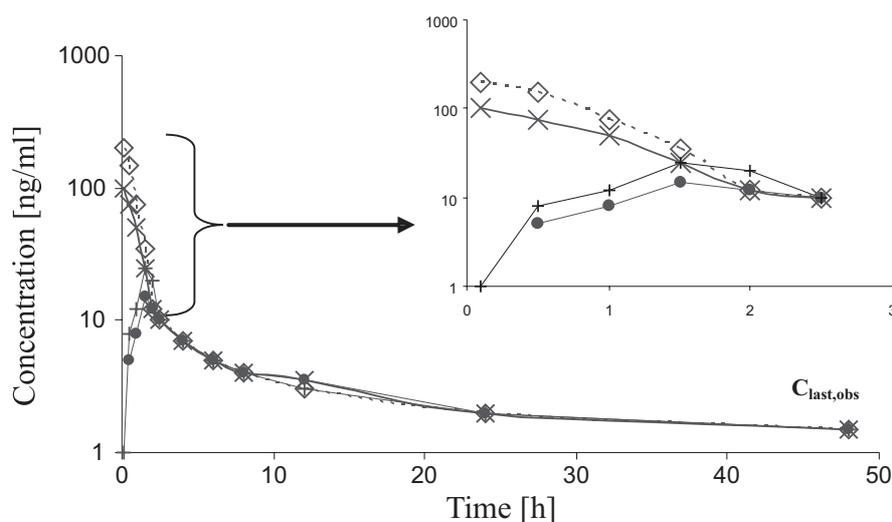


Fig. 1. Drug concentration changes over time for hypothetical data sets **A** (\diamond), **B** (\times), **C** ($+$), and **D** (\bullet). The inserted graph illustrates differences between the hypothetical data sets during the absorption phase (oral administration) and the distribution phase (intravenous administration) up to 2.5 h after drug administration

Tab. 2. The pharmacokinetic parameters obtained for four sets (**A–D**) of hypothetical data after the intravenous (**A, B**) and oral (**C, D**) administration of a drug

| Series | C_{max} [ng/ml] | t_{max} [h] | t_{last} [h] | $C_{last,obs}$ [ng/ml] | K_{el} [h ⁻¹] | $MRT_{0-tlast}$ [h] | $AUC_{0-tlast}$ [ng·ml ⁻¹ ·h] | $AUC_{0-\infty}$ [ng·ml ⁻¹ ·h] | $AUC_{res\%}$ [%] | $AUC_{res\%}^1$ [%] |
|----------|----------------------|------------------|-------------------|---------------------------|--------------------------------|------------------------|---|--|----------------------|------------------------|
| A | 200.00 | 0.10 | 48.00 | 1.50 | 0.0244 | 7.63 | 315.50 | 376.90 | 16.29 | 9.73 |
| B | 100.00 | 0.10 | 48.00 | 1.50 | 0.0244 | 10.13 | 233.87 | 295.28 | 20.79* | 12.70 |
| C | 15.00 | 1.50 | 48.00 | 1.50 | 0.0244 | 15.91 | 146.00 | 207.40 | 29.61* | 18.90 |
| D | 25.00 | 1.50 | 48.00 | 1.50 | 0.0244 | 14.75 | 158.65 | 220.05 | 27.90* | 17.66 |

* According to valid directives, the experiment is negatively verified. $AUC_{res\%}^1$ – the residual area calculated for time points K_{el} of 8, 12, and 24 h

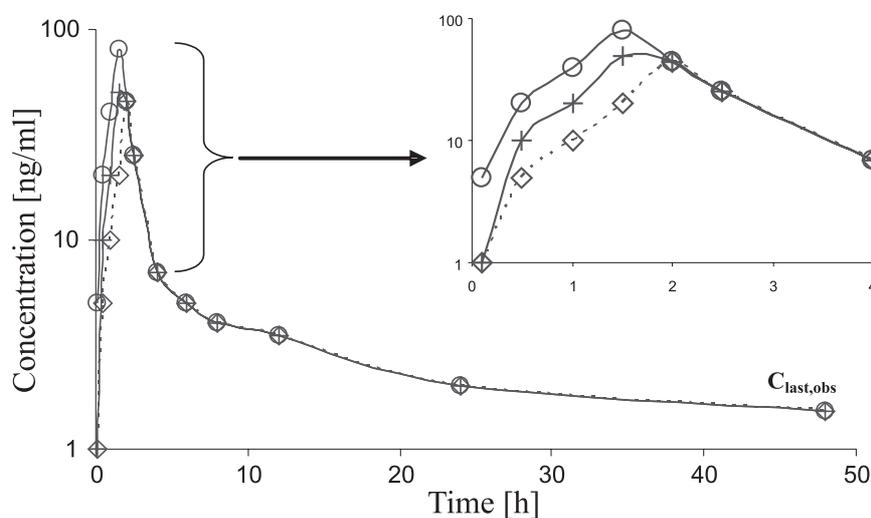


Fig. 2. Drug concentration changes over time for the hypothetical data sets **E** (\circ), **F** ($+$), and **G** (\square). The inserted graph illustrates the differences between the hypothetical data during the absorption phase up to 2 h after a drug administration

Tab. 3. The pharmacokinetic parameters obtained for three sets (E, F, and G) of hypothetical data after oral administration of a drug

| Series | C_{max} [ng/ml] | t_{max} [h] | t_{last} [h] | $C_{last,obs}$ [ng/ml] | K_{el} [h ⁻¹] | $MRT_{0-tlast}$ [h] | $AUC_{0-tlast}$ [ng·ml ⁻¹ ·h] | $AUC_{0-\infty}$ [ng·ml ⁻¹ ·h] | $AUC_{res\%}$ [%] | $AUC_{res\%}^1$ [%] |
|--------|----------------------|------------------|-------------------|---------------------------|--------------------------------|------------------------|---|--|----------------------|------------------------|
| E | 45.00 | 2.00 | 48.00 | 1.50 | 0.0244 | 13.23 | 181.25 | 242.65 | 25.30 * | 15.81 |
| F | 50.00 | 1.50 | 48.00 | 1.50 | 0.0244 | 11.92 | 203.50 | 264.90 | 23.18 * | 14.32 |
| G | 80.00 | 1.50 | 48.00 | 1.50 | 0.0244 | 10.52 | 234.00 | 295.40 | 20.79 * | 12.69 |

* According to valid directives, the experiment is negatively verified. $AUC_{res\%}^1$ – the residual area calculated for time points K_{el} of 8, 12, and 24 h

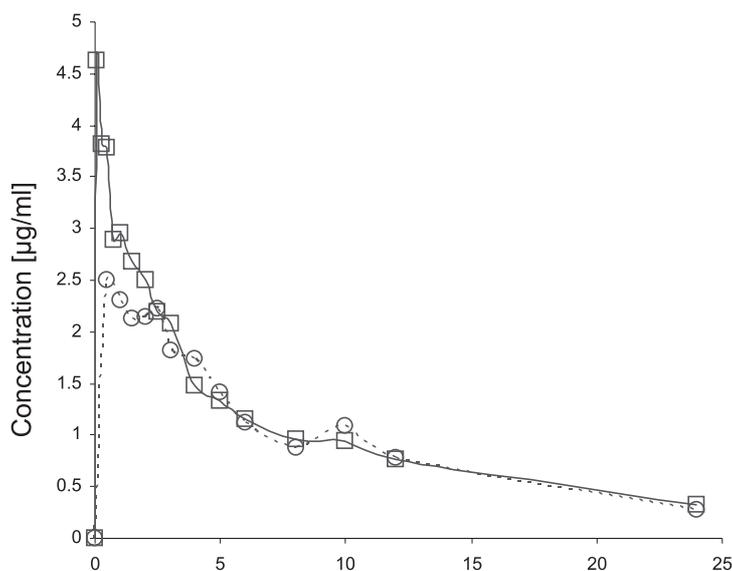


Fig. 3. Enrofloxacin plasma concentrations in 3-week-old broiler chicken treated intravenously (□) or *per os* (o) with the drug at a dose of 10 mg/kg b.w.

Tab. 4. Values of the residual area calculated in six different variants after intravenous (*iv*) or *per os* (*po*) administration of enrofloxacin (at a dose of 10 mg/kg b.w.) in 3-week-old broiler chicken

| Data | Enrofloxacin in broiler chicken (<i>po</i> administration) | | | | | | M | SD | RSD [%] |
|---------------------------|---|-------|--------|-------|--------|-------|-------|-------|---------|
| Number points K_{el} | 3.00 | 4.00 | 3.00 | 3.00 | 3.00 | 4.00 | | | |
| Lower point K_{el} [h] | 10.00 | 8.00 | 8.00* | 8.00 | 6.00* | 6.00 | | | |
| Higher point K_{el} [h] | 24.00 | 24.00 | 24.00* | 12.00 | 12.00* | 12.00 | | | |
| $AUC_{res\%}$ | 11.14 | 12.54 | 13.46 | 28.33 | 17.39 | 21.21 | 17.35 | 6.97 | 40.19 |
| $AUC_{res\%,pred}$ | 11.03 | 12.88 | 13.77 | 46.35 | 22.95 | 32.76 | 23.29 | 14.69 | 63.08 |
| | Enrofloxacin in broiler chicken (<i>iv</i> administration) | | | | | | | | |
| Number points K_{el} | 3.00 | 4.00 | 3.00 | 3.00 | 3.00 | 4.00 | | | |
| Lower point K_{el} [h] | 10.00 | 8.00 | 8.00* | 8.00 | 6.00* | 6.00 | | | |
| Higher point K_{el} [h] | 24.00 | 24.00 | 24.00* | 12.00 | 12.00* | 12.00 | | | |
| $AUC_{res\%}$ | 14.35 | 14.97 | 15.41 | 17.50 | 15.53 | 16.48 | 15.71 | 1.18 | 7.54 |
| $AUC_{res\%,pred}$ | 14.29 | 15.06 | 15.48 | 20.28 | 15.89 | 18.22 | 16.53 | 2.36 | 14.25 |

* The denoted point ranges were analyzed (excluding the point at hour 10)

tion, drug mean residence time (MRT) was lower for the *iv* administration than for the *po* administration. The AUC between points $t = 0$ and $t_{\text{last, obs}}$ and the AUC between points $t = 0$ to infinity were higher for the *iv* administration than for the *po* administration. The K_{el} , calculated on the based on the four final measurement points, was the same for all the data.

In the second set of data, the only difference in the output was the drug absorption phase (Fig. 2). The pharmacokinetic parameters for this data set are presented in Table 3. The values of the $\text{AUC}_{\text{res}\%}$ were higher than 20% for each *po* administration. The K_{el} , calculated based on the final four measurement points, was the same for all the hypothetical data sets.

The residual area, calculated from the K_{el} in the range between 8 and 24 h, was 10.82, 13.98, 23.44, and 19.09% for the hypothetical data sets A, B, C, and D, respectively. The area for hypothetical data sets E, F, and G was 15.81, 14.32, and 12.69%, respectively. The residual area exceeded 20% only in data C.

The plasma concentrations from broiler chickens treated with an *iv* and *po* enrofloxacin administration are shown in Figure 3. The residual area values in the *po* and *iv* administration experiments reached 11.14–28.33% and 14.35–17.50% of the total AUC, respectively; the exact value depended on the selected range, number, and type of points (Tab. 4). In relation to $\text{AUC}_{\text{res}\%, \text{pred}}$, these values ranged between 11.03% and 46.35% for the *po* administration and between 14.29% and 20.28% for the *iv* administration. The pharmacokinetic parameters of the *iv* and *po* enrofloxacin administration are presented in Table 5.

Discussion

Despite differences in the absorption and distribution phases in the first data set (A–D), the elements had different qualifications; this suggests that the use of

an improper analytical method (differences in $C_{\text{last, obs}}$) or an erroneous C-T curve (in the elimination phase) did not negatively influence the evaluation of the oral administration data. The experiment was negatively evaluated due to the difference in area in the initial stages of distribution and the low area value obtained after oral administration. However, this evaluation is not associated with the final section of the curve. The analysis of the hypothetical data is related to the same analytical method used in both experiments. The experiment is verified positively or negatively depending on the administration route. Therefore, the evaluation method is not objective. The $\text{AUC}_{\text{res}\%}$ value is independent of the drug administration route and it does not evaluate the effects of drug administration route on the pharmacokinetic properties of the substance.

Of the hypothetical data E, F, and G, only experiment G was positively qualified according to the current criteria. We assumed that the results of all three hypothetical data groups were obtained using the same analytical method. Hypothetical data groups used the same subject or the same animal laboratory and had large intra-individual variability. The current criteria (residual area 20% of the total area) disqualified two of the experiments despite the fact that the ratio of the residual area to the total area is dependent only on changes in the absorption and distribution phase. A negative evaluation of an experiment also means that in this case, these data that have variability in the absorption phase, which are valuable from the cognitive point of view, are disqualified. The initial course of the C-T curve modifies the value of the total area and, subsequently, the ratio of the residual area to the total area; therefore, the $\text{AUC}_{\text{res}\%}$ value, (based only on the elimination rate constant) represents only AUC in the elimination phase but not in the whole experiment.

The 80:20 rule, as a guideline for the evaluation of a DMPK experiment, is not an optimal method for the evaluation of the experiment correctness. The AUC_{res}

Tab. 5. The pharmacokinetic parameters obtained after intravenous (*iv*) and *per os* (*po*) administration of enrofloxacin at a dose of 10 mg/kg b.w. in 3-week-old broiler chicken

| Dosing | C_{max} [$\mu\text{g}/\text{ml}$] | t_{max} [h] | t_{last} [h] | $C_{\text{last, obs}}$ [$\mu\text{g}/\text{ml}$] | K_{el} [h^{-1}] | $\text{MRT}_{0-t_{\text{last}}}$ [h] | $\text{AUC}_{0-t_{\text{last}}}$ [$\mu\text{g}\cdot\text{ml}^{-1}\cdot\text{h}$] | $\text{AUC}_{0-\infty}$ [$\mu\text{g}\cdot\text{ml}^{-1}\cdot\text{h}$] |
|-----------|---|-------------------------|--------------------------|---|--|---|---|--|
| <i>iv</i> | 4.62 | 0.08 | 24.00 | 0.32 | 0.0721 | 7.20 | 25.21 | 29.64 |
| <i>po</i> | 2.51 | 0.50 | 24.00 | 0.27 | 0.0831 | 7.75 | 22.91 | 26.19 |

value determined by the final concentration point $C_{last, obs}$ and K_{el} , is only associated with two pharmacokinetic parameters ($AUC_{res} = C_{last, obs}/K_{el}$). The $AUC_{res\%}$ value associated with the $AUC_{0-\infty}$ analysis ($AUC_{res\%} = C_{last, obs}/(K_{el} \times AUC_{0-\infty}) \times 100\%$) affects analysis quality based on the route of administration, rate of absorption, and distribution and redistribution processes, in addition to other variables. This is confirmed by the analysis of the residual area after *po* and *iv* enrofloxacin administration in broiler chicken. The formula used for the calculation of the residual area is imprecise; the formula assumes that $C_{last, obs}$ is always a point located on a linear curve and matches 3–4 points of the final section of the curve. In practical terms, the final point is usually characterized by the largest deviation from the matching curve. This means that the calculations derived from this method are erroneous. Analysis of the C-T curve generated from the enrofloxacin administration to broiler chicken demonstrated that atypical concentration changes during the elimination phase caused an additional error, resulting in variable interpretation of the directives and variable data.

The assumptions that are made when calculating the residual area, which determine the positive or negative qualification of drug pharmacokinetics, are not optimal for the evaluation of the entire study. This is because the parameter depends on factors other than the quality of the analytical method and sampling method used in the study. In addition, the parameter depends on the drug administration route, in addition to other variables; this was demonstrated by using a hypothetical data set presenting typical time-dependent variations in drug concentration after a single oral or intravenous administration of a drug. According to the assumptions, the choice of DMPK sampling scheme and the bioanalytical method should be dependent on the residual area. In practical terms, the parameter is associated with, at most, the final four sampling points of the elimination rate constant analysis and is completely unrelated to the accuracy and precision of the bioanalytical studies. Experimental sampling control was the reason for introduction of the residual area as an evaluation parameter for the DMPK experiment. Insufficient sampling frequency results in improper C-T points in the end stage of the elimination phase, used for the calculation of the elimination rate constant. This leads to an erroneous rate constant value for the elimination phase, and therefore, causes underestimation of drug half-life.

On the other hand, drugs with an extremely long elimination profile cannot be verified by the evaluation of the residual area with respect to the entire experiment. Analysis of the hypothetical data proved that the same bioanalytical method and the same experimental system, differing only by the drug administration route, may be verified negatively or positively, regardless of the analysis of the final section of the C-T curve. The selection of sampling data was dependent on the method used for calculating the elimination rate constant and included the final 3 or 4 data points [2]; these points were selected by a trained analyst. In practical terms, this procedure allows liberal interpretation of the guidelines provided by registration agencies. Therefore, the value of the residual area may be calculated differently, as shown in our study with hypothetical data and *in vivo* data. Because visual evaluation of the C-T curve cannot precisely separate the elimination phase from the distribution phase, the $MRT_{0-tlast}$ value is suggested as a separation criterion. This value indicates the mean time drug molecules stay within an organism [14]. Therefore, it may be assumed that the point equal to $MRT_{0-tlast}$ is located within the elimination phase. Because the MRT time point is equal to 63.2%, the drug was eliminated from the body, the time between $MRT_{0-tlast}$ and $t_{last, obs}$ is the optimal range for K_{el} analysis [27–29, 31]. It may also be assumed that a DMPK experiment was ended at a proper time point, and the elimination rate constant was determined properly, (qualifying positively the experiment with optimal sampling during the absorption and distribution phase) if experiment meets the following premises:

- the linear equation is based on three to four C-T points of a curve measuring the drug concentration changes over time in the elimination phase;
- the slope of the fitted line (based on the final four observed C-T points) is equivalent to the K_{el} used for the calculation of elimination phase $t_{1/2}$;
- the differences between observed and theoretical concentrations (based on the linear equation) are below 20% for at least three of four C-T points. This is in accordance with a 20% deviation for accuracy, precision, and nominal LLOQ concentration in the bioanalytical method validation procedure [1];
- at least two of the final C-T points used for the slope calculation are located in a time point $\geq MRT_{0-tlast}$ ($\geq 63.2\%$ of eliminated drug).

These rules positively qualify both the hypothetical and the *in vivo* data; they are not affected by the route

of administration in the same value as the 80:20 rule. The 80:20 rule negatively verifies the *po* enrofloxacin due to the high AUC_{res} value. The sampling schedule in the *iv* administration experiment resulted in a different quality analysis; however, the elimination profile of the drug was independent of application or dose. Quality analysis of experiments (*iv* and *po*) according to the new rules allows proper performance. In both cases (*iv* and *po*), the analysis was performed based on 4 C-T points, which fulfills the conditions described in subsections a, b, and c for a single time point located before the drug MRT. The interpretation of this analysis affirms the two-three points of C-T; this is the basis for determination of drug K_{el} and is in the portion of the kinetics in which 2/3 of the drug was eliminated (subsection a and d). The determination of K_{el} using this procedure does not create erroneous estimation of the elimination kinetics (subsection b). The analytical method used in the experiment is characterized by suitably low LLOQ value. This finding reflects the ability to determine the proper number of C-T points with a sufficiently low level of concentrations (linear course of the fragment of the curve 20% of standard deviation; subsections c and d). In addition, the range of C-T points used in the K_{el} analysis is controlled by a suitable LOQ and the omitted falsification of late phase distribution (subsection d).

In conclusion, our study indicates that $AUC_{res\%}$ analysis is not the optimal method for the evaluation of pharmacokinetic studies. The analysis of hypothetical data confirms that the residual area is a parameter dependent on the drug administration route, despite it not being an independent parameter used for the evaluation of drug administration route.

Acknowledgments:

We thank Dr. hab. Wiesław Raszewski for critical reading and the financial support of the manuscript. We also thank Prof. Dr. Witold Gumułka for helpful discussions and critique of the manuscript.

References:

1. Anonymous: Bioanalytical Method Validation. FDA, 2001, 1–20.
2. Anonymous: Bioavailability and Bioequivalence Studies for Orally Administered Drug Products – General Considerations. FDA, 2000, 1–23.
3. Anonymous: Bioequivalence Guidance. Docket No. 94D-0401, FDA, 2006, 6–28.
4. Anonymous: Bioequivalence Requirements for Long Half-life Drugs. Health Canada, 2005, 1–2.
5. Anonymous: Discussion Paper. Bioequivalence Requirements: Highly Variable Drugs and Highly Variable Drug Products: Issues and Options. Health Canada, 2003, 1–14.
6. Anonymous: Food-Effect Bioavailability and Fed Bioequivalence Studies. FDA, 2002, 1–8.
7. Anonymous: Guideline on the investigation of bioequivalence. CPMP/EWP/QWP/1401/98 Rev. 1, 2008, 3–29.
8. Anonymous: Note for Guidance on the Investigation of Bioavailability and Bioequivalence. CPMP/EWP/QWP/1401/98, 1998, 1–19.
9. Anonymous: Note for Guidance on the Investigation of Bioavailability and Bioequivalence. EMEA, 2001, 2–22.
10. Anonymous: Note for Guidance on Toxicokinetics: A Guidance For Assessing Systemic Exposure In Toxicology Studies. CPMP/ICH/384/95, 1995, 1–12.
11. Anonymous: Note for Guidance on Validation of Analytical Methods: Definitions and Terminology. CPMP/ICH/381/95, 1995, 1–6.
12. Anonymous: Note for Guidance on Validation of Analytical Procedures: Methodology. CPMP/ICH/281/95, 1996, 1–10.
13. Anonymous: Statistical Approaches to Establishing Bioequivalence. FDA, 2001, 1–45.
14. Gibaldi M, Perrier D: Pharmacokinetics, 2nd edn., Marcel Dekker Inc., New York, 1982.
15. James CA, Hill HM: Procedural elements involved in maintaining bioanalytical data integrity for good laboratory practices studies and regulated clinical studies. AAPS J, 2007, 9, 123–127.
16. Kelley M, DeSilva B: Key elements of bioanalytical method validation for macromolecules. AAPS J, 2007, 9, 156–163.
17. Mackie C, Wuyts K, Haseldonckx M, Blokland S, Gysenberg P, Verhoeven I, Timmerman P, Nijssen M: New model for intravenous drug administration and blood sampling in the awake rat, designed to increase quality and throughput for in vivo pharmacokinetic analysis. J Pharmacol Toxicol Methods, 2005, 52, 293–301.
18. Medina L, Garcia L, Medina J, Diaz-Oliveros J, Jung H: Implementation of a system for evaluating and ensuring quality of bioequivalence studies. Proc West Pharmacol Soc, 1988, 41, 175–177.
19. Marzo A: Open questions on bioequivalence: an updated reappraisal. Curr Clin Pharmacol, 2007, 2, 179–189.
20. Marzo A, Ceppimonti N, Vuksic D: Experimental, extrapolated and truncated areas under the concentration-time curve in bioequivalence trials. Eur J Clin Pharmacol, 1999, 55, 627–631.
21. Peng GW, Chiov WL: Analysis of drugs and other toxic substances in biological samples for pharmacokinetics studies. J Chromatogr B Biomed Appl, 1990, 51, 3–50.
22. Sathe P, Venitz J, Lesko L: Evaluation of truncated areas in the assessment of bioequivalence of immediate release formulations of drugs with long half-lives and of C_{max} with different dissolution rates. Pharm Res, 1999, 16, 939–943.
23. Shah VP, Midha KK, Dighe S, McGilveray IJ, Skelly JP, Yacobi A, Layloff T et al.: Analytical methods valida-

-
- tion: bioavailability, bioequivalence and pharmacokinetic studies. Conference report. *Eur J Drug Metab Pharmacokinet*, 1991, 16, 249–255.
24. Shah VP: The history of bioanalytical method validation and regulation: evolution of a guidance document on bioanalytical methods validation. *AAPS J*, 2007, 9, 43–47.
 25. Siderov J, Brien JE, Morgan DJ, Zalberg J, Cosolo W: Quality of pharmacokinetic research in oncology. *Br J Cancer*, 1995, 72, 792–794.
 26. Viswanathan CT, Bansal S, Booth B, DeStefano AJ, Rose MJ, Sailstad J, Shah VP et al.: Quantitative bioanalytical methods validation and implementation: best practices for chromatographic and ligand binding assays. *AAPS J*, 2007a, 24, 1962–1973.
 27. Veng-Pedersen P: Mean time parameters in pharmacokinetics: Definition, computation and clinical implications (part I). *Clin Pharmacokinet*, 1989, 17, 345–366.
 28. Veng-Pedersen P: Mean time parameters in pharmacokinetics: Definition, computation and clinical implications (part II). *Clin Pharmacokinet*, 1989, 17, 424–440.
 29. Veng-Pedersen P, Gillespie W: Mean residence time in peripheral tissue. A linear disposition parameter useful for evaluating a drug's tissue distribution. *J Pharmacokinet Biopharm*, 1984, 12, 535–543.
 30. Viswanathan CT, Bansal S, Booth B, DeStefano AJ, Rose MJ, Sailstad J, Shah VP et al.: Workshop/Conference report – quantitative bioanalytical methods validation and implementation: best practices for chromatographic and ligand binding assays. *AAPS J*, 2007, 9, 30–42.
 31. Weiss M: Use of gamma distributed residence times in pharmacokinetics. *Eur J Clin Pharmacol*, 1983, 25, 695–702.
 32. White LO: Assays for therapeutic monitoring and pharmacokinetic investigations of aminoglycosides: quality aspects. *Ther Drug Monit*, 1998, 20, 464–468.

Received:

January 16, 2009; in revised form: October 7, 2009.