The influence of different weighting schemes on the calculation of pharmacokinetic parameters for paclitaxel (Taxol®)

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Summary

Pharmacokinetic parameters are usually determined by *noncompartmental* methods or by assuming a multicompartment model in order to describe the behaviour of the drug in the patient's body. With a *noncompartmental* analysis the terminal half-life and the volume of distribution are derived from log-linear regression of the terminal elimination phase, whereas the estimation of these parameters is based on nonlinear least squares (NLS) regression when assuming a multicompartment model. The concentration of paclitaxel, a novel antitumor agent, is usually determined by a high-performance liquid chromatography (HPLC), a method that may produce higher variability with increasing concentration. Many applications are based on the so called constant coefficient of variation model, leading to weighted NLS estimation with fixed weights $1/\text{concentration}^2$ or to unweighted NLS for the log-transformed data. However, examples from the literature show that there are various strategies. Using data of a phase I clinical trial, where paclitaxel (Taxol®) was administered as 1-hour infusion to cancer patients, we considered in this paper log-linear regression of the last 3 and 4 observations, respectively, as well as a two-compartment model. It is shown that the values of half-lives and the volume of distribution depend on the model and the chosen weighting scheme. In this context we comment also on the variability of the estimated pharmacokinetic parameters. We suggest how to use information on the variability of the used HPLC-method in order to find the most appropriate statistical method.

key words: two-compartment model, weighted nonlinear-least squares, half-life, volume of distribution, high-performance liquid chromatography

1. Introduction

In oncology, main interest in phase I trials often centers around the estimation of the maximum tolerable dose (MTD) of a drug or drug combination in order to determine recommended dose for further investigations. Obviously, the estimation of MTD implies the description of observed toxicities. Especially for new drugs aspects of clinical pharmacology are also often investigated. Typically, the pharmacokinetic of the corresponding treatment is described by the area under the concentration versus time curve (AUC), the maximal concentration $C_{\max}$ and the time $t_{\max}$ when the maximal concentration is achieved. Further pharmacokinetic parameters are the terminal half-life $t_{1/2}$, the total body clearance CL, which may be interpreted as the rate at which the body is able to remove the drug, and the volume of distribution V_d, which relates a measured concentration to the total amount of drug in the body. An introduction to the principles of pharmacokinetics with an emphasize on clinical applications is given by Rowland and Tozer [1].

Based on data measured at different time points, which are usually obtained by analyzing blood samples, the AUC can be calculated by using the trapezoidal rule [2], whereas $C_{\max}$ and $t_{\max}$ are obtained directly from the data. The total clearance is given by $CL = dose(iv)/AUC$ where $dose(iv)$ is the amount of the drug administered by infusion. Considering the simple case that the patient's body represents only one compartment the elimination of the drug is assumed to be log-linear, i.e. the concentration of the drug in the body at time t is given by

$$C_p(t) = y_0 \exp(-\lambda t),$$

where y_0 denotes the concentration at time $t = 0$. Starting e.g. at t_{max} (i.e. $t_{max} = 0$) the value of y_0 is equal to C_{max}. Assuming a simple one-compartment model the half-life and the volume of distribution are given by $t_{1/2} = log(2)/\lambda$ and $V_d = CL/\lambda$ respectively, the parameter $-\lambda$ being the slope obtained by log-linear regression. Usually, however, the elimination of the drug seems to be more complicated. To cope with this situation it is convenient to assume compartmental models containing more than one compartment. An introduction to these models illustrated by several examples can be found in Seber [3]. A two-compartment model is used e.g. by Wiernik et al. [4] and by Brown et al. [5], where Taxol is given as 6-hour infusion and in Wiernik et al. [6], where Taxol is given as a 24-hour infusion. Huizing [7] used a three-compartment model in order to describe the pharmacokinetics of paclitaxel and metabolites for a 3-hour and a 24-hour infusion in platinum-pretreated ovarian cancer patients. Considering also these two infusion intervals Gianni et al. [8] investigated pharmakokinetical and pharmacodynamical features of paclitaxel by *noncompartmental* and compartmental methods. For the *noncompartmental* analysis the calculation of pharmacokinetic parameters is based on the 'LaGrange function as implemented by the computer program LAGRAN'. In this program [9] the terminal elimination constant λ, which is used to calculate $t_{1/2}$, is determined by log-linear regression of the terminal elimination phase, i.e. using the last few observed drug concentrations. Besides these different approaches there are also different strategies with respect to the estimation of the underlying parameters. Assuming a two-compartment model Brown et al. [5] used weighted nonlinear least-squares analysis with fixed weights $1/C_p(t)^2$ whereas the results in Wiernik et al. [4] are based on unweighted estimation using log-concentrations. In both situations the resulting parameters are used to estimate the ditribution and elimination half-lives. In Brown et al. [5] the model is also used in order to estimate the volume of distribution V_d and the mean clearance CL. However, the authors neither described how they calculated V_d and CL nor gave any reference for the calculation. Although Longnecker et al. [10] also assume a two-compartment model, their analysis is based on nonlinear least-squares regression using weights $1/C_p(t)$.

Using log-linear regression as well as a two-compartment model in order to analyse the data of patients who received paclitaxel as 1-hour infusion we show in this paper that the estimation of pharmacokinetic parameters strongly depends on the model and the estimation procedure. We focus on the estimation of terminal half-lives and the volume of distribution. A short introduction to the study is given in section 2. In this context we also describe the analytical method, a high performance liquid cromatography (HPLC), used in order to determine the paclitaxel plasma concentrations. In section 3 we introduce the two-compartment model and describe a method proposed by Gibaldi [11] for the estimation of the volume of distribution. Furthermore, we discuss several methods for the estimation of the underlying parameters and comment on the variability of the resulting pharmacokinetic parameters. Considering individual patients of the present study, we compare in section 4 the results obtained by unweighted and weighted analysis assuming a two-compartment model and by log-linear regression, respectively. In section 5 we show how to use information about the underlying HPLC method in order to determine an appropriate weighting scheme. A general discussion will be given in section 6.

2. Phase I clinical and pharmacokinetic study of paclitaxel (Taxol®) given as 1-hour infusion

In a multicenter phase I trial conducted at the Tumor Biology Center in Freiburg, the University Clinic in Freiburg, and the Vincentius Hospital in Karlsruhe 34 patients with different advanced incurable malignancies received a 1-hour regimen of paclitaxel (Taxol®), where the dose was escalated from 150 mg/m^2 to 250 mg/m^2 [12]. Besides the determination of the MTD and the description of toxicity, the pharmacokinetics of the 1-hour infusion was investigated [13]. Blood samples were taken before as well as 0.5, 1, 1.5, 2, 3, 4, 12 and 24 hours after starting the infusion. Paclitaxel was extracted from plasma samples by a liquid-solid extraction procedure using Sep-Pak® cartridges. The separation of paclitaxel from other co-extracted compounds was performed by a HPLC procedure. Quantification of paclitaxel amounts was done by use of calibration curves which were established

by analysing blank plasma samples spiked with known amounts of paclitaxel and 7-epi-paclitaxel as internal standard. In this paper we use a small portion of the data exclusively for illustration; a detailed analysis will be published elsewhere.
Figure 1 shows the observed paclitaxel plasma concentrations for patient 12 (dose : 225 mg/m^2) in cycle 1. We observed a very fast decrease of the plasma concentration after the cessation of the infusion indicating that log-linear regression may be inappropriate in order to describe the complete elimination phase. Indeed, restricting to values observed at and after $t_{\max}$ we obtained a bad fit with this approach. The results, which are also illustrated in figure 1, were obtained by using the SAS procedure PROG REG [14] for the log plasma concentration.

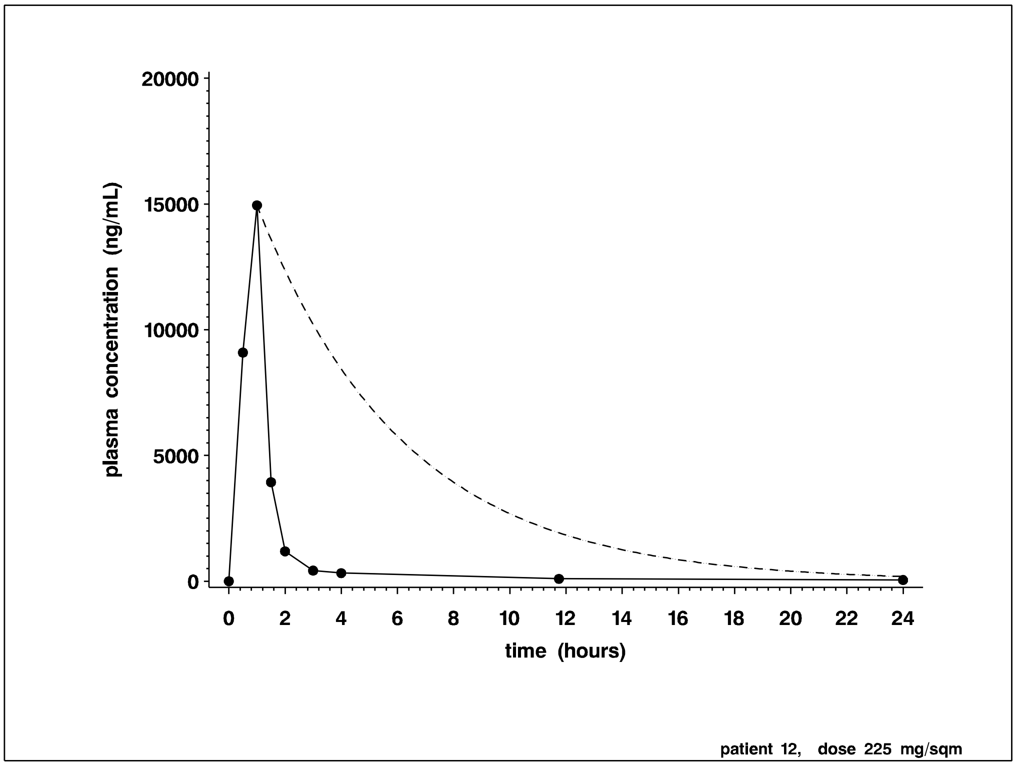


Figure 1: Observed paclitaxel concentrations (dots) and fit obtained by log-linear regression starting at $t_{\max}$ (dashed line) for patient 12

3. The two-compartment open model and its application

In the last section we have demonstrated that a naive application of *noncompartmental* methods is inappropriate in the current situation. Better results with respect to the model fit would be obtained, if we apply log-linear regression to the last three or four observations. However, concerning the number of observations used for the analysis this approach seems to be somewhat arbitrary.
Thus, it may be sensible to model the complete system including the administration and elimination of a drug.
In order to describe the behaviour of paclitaxel in the patient's body we assume a two-compartment open model [11, 15]. As shown in figure 2 the infusion of the drug at a constant rate k_0 is introduced into the central compartment from which the drug is also eliminated. Compartment-models lead to linear systems of differential equations [3], which is in case of figure 2 given by

$$\begin{aligned} \frac{\partial X_C}{\partial t} &= -(k_{el} + k_{12})X_C + k_{21}X_T + k_0 && \text{(1a)} \\ \frac{\partial X_T}{\partial t} &= k_{12}X_C - k_{21}X_T && \text{(1b)}, \end{aligned}$$

where X_C and X_T denote the amount of the drug in the central and the tissue compartment, respectively, and k_0, k_{12}, k_{21} and k_{el} are transfer rate constants as described in figure 2.

Assuming a bolus infusion the two-compartment model has to be modified in the following way: The concentration in the central compartment is modeled by $X = X_0$, where X_0 is the given dose. Thus, there is no input k_0 from the outside into the central compartment after $t = 0$ and the linear system of differential equations changes to

$$\begin{aligned} \frac{\partial X_C}{\partial t} &= -(k_{el} + k_{12})X_C + k_{21}X_T && \text{(2a)} \\ \frac{\partial X_T}{\partial t} &= k_{12}X_C - k_{21}X_T && \text{(2b)} \end{aligned}$$

Exactly the same equations would be obtained when restricting to the data obtained after time t^*, where t^* denotes the time of the cessation of the infusion. At $t = t^*$ the concentration of the drug can assumed to be greater than zero in both compartments.
A general solution of the two-compartment model can e.g. be found in chapter 8 of Riggs [16]. We used Riggs' results in order to solve the problem given by equations (2a) and (2b), the solution with respect to the central compartment is shown in the appendix.
In practice, it is common to observe the concentration of the drug in one compartment (usually the central compartment) as a function of time and to estimate the parameters of the unknown model from the behaviour of the drug. For this purpose one should have some a priori knowledge of the model, i.e. the number of compartments and the links between them. However, we should avoid too complicated models because there are usually only few observations available.

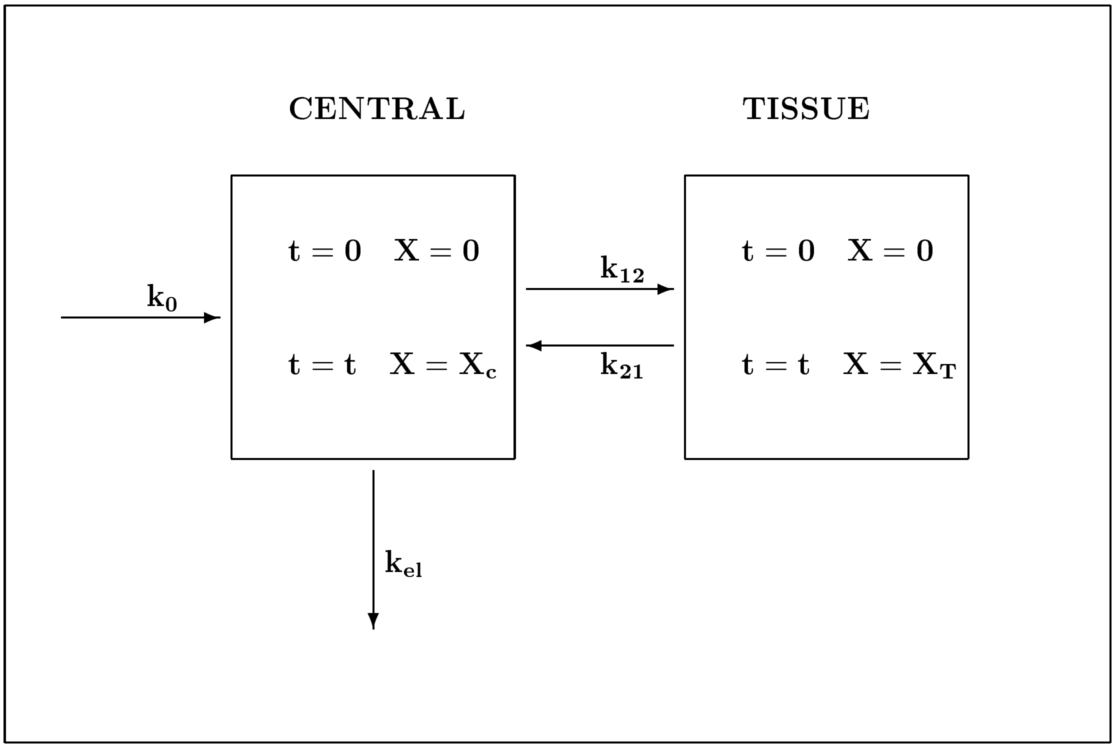


Figure 2: **Two-compartment open model**, intravenous infusion at a constant rate k_0 starting at time $t = 0$; $X = X_c$ and $X = X_T$ denote the amount of the drug of the central and tissue compartment, respectively, at time $t = t$; k_{12} is the transfer rate constant from the central to the tissue compartment; k_{21} is the transfer rate constant from the tissue to the central compartment; k_{el} is the elimination rate of the drug

The assumption of a two-compartment model seems to be sufficient in many applications, because the concentration of the drug observed after t_{max} (which is equal to $t = 0$ for a bolus infusion) can often be described well by the biexponential function

$$C_p(t) = A \exp(-\alpha t) + B \exp(-\beta t), \tag{3}$$

where $C_p(t)$ denotes the plasma concentration at time t, Thus, the concentration at $t = 0$ (or $t = t_{max} = 0$) is given by $C_p(0) = A + B$.
The plasma may be regarded as a sample of the central compartment [16] and, therefore, the apparent volume of distribution in the central compartment can be estimated by $V_c = X_0/(A + B)$. Multiplying $C_p(t)$ by V_c the amount of drug in the central compartment can be expressed by (cf. Gibaldi [11])

$$X_c(t) = X_0 \left(\frac{A}{A+B} \exp(-\alpha t) + \frac{B}{A+B} \exp(-\beta t) \right). \tag{4}$$

Comparing formula (4) with equation (A1) in the appendix we obtain

$$\frac{A}{A+B} = \frac{k_{21} - \alpha}{\beta - \alpha}.$$

Thus, k_{21} can be estimated by using the estimates of A, B, α and β. The two other transfer rate constants k_{12} and k_{el} can be derived from equations (A3) and (A4). Based on the two-compartment model Gibaldi [11] suggested to calculate the volume of distribution at *infusion equilibrium* by

$$(V_d)_{\text{inf.eq.}} = \frac{k_{21} + k_{12}}{k_{21}} V_c.$$

Furthermore, the author shows that $(V_d)_{\text{inf.eq.}}$ is identical to the steady state volume proposed by Riggs [16]. The elimination of the drug assuming a two-compartment model is described by the distribution half-life $t_{1/2,\alpha} = log(2)/\alpha$ and the elimination half-life $t_{1/2,\beta} = log(2)/\beta$, which can be obtained by using the corresponding estimates.

Weighted and unweighted analysis

As mentioned in the introduction there are various approaches in order to estimate the parameters of the biexponential function (3). In the standard situation assuming the same variability with respect to all observed plasma concentrations, the so called homoscedastic situation, the parameters are determined by using unweighted nonlinear least-squares estimation [3]. With this approach, which is often referred to as <u>o</u>rdinary <u>l</u>east <u>s</u>quares and will be, therefore, denoted by OLS, each observation obtains the same weight (w=1).

However, the assumption of homoscedascity may be violated in pharmacokinetic studies. On the one hand, the range of the plasma concentration observed e.g. 1 or 1.5 hours after starting the infusion is usually larger than the range for the smaller values obtained after 12 or 24 hours. On the other hand the concentration of a drug is often estimated via high-performance liquid chromatography (HPLC), a method that can expected to yield appropriate measurement of rather small values. Usually the variability increases with increasing values of the observed plasma concentration. Assuming unequal variances for different observations is referred to as the heteroscedastic situation. To cope with this problem it is necessary to include information about the variability leading to weighted nonlinear least-squares regression or transformation of the data. However, although the quality of the underlying analytical method is usually investigated in a validation study the information of the validation seems not to be used in order to estimate the true variability. Instead, many applications are based on commonly used strategies, which may be more or less appropriate. In pharmacokinetic studies the most common weighting scheme used with <u>n</u>onlinear <u>l</u>east <u>s</u>quares regression is based on weights $w_t = 1/\left(C_p^*(t)\right)^2$, where $C_p^*(t)$ denotes the observed plasma concentration at time t. With this approach, subsequently referred to as NLS(C^{-2}), small concentrations obtain a very large weight as compared to the huge plasma concentrations observed at or short time after $t_{\max}$. Using $w_t = 1/\,C_p^*(t)$ – in this paper denoted by NLS(C^{-1}) – leads

to a less extreme weighting.
Obviously, the use of different weighting schemes may lead to different parameter estimates of the biexponential function (3) and, therefore, to different results with respect to the half-lives and the volume of distribution. In general, as for OLS, any weighting is based on a specific model with respect to the underlying variability of the observed plasma concentrations. NLS(C^{-2}) e.g. corresponds to the constant coefficient of variation model, i.e. to the assumption that the variability increases proportionally to the concentration [17]. The same assumption is made, when using OLS of the log-concentrations [17]. For this approach, which is expected to be more robust than NLS(C^{-2}), we use the notation OLS(ln).
Considering three regression functions (a simple monoexponential decay, a Bateman function and a Michaelis-Menton-type function) and simulating four different variance models Sheiner and Beal [17] compared several approaches. They showed that NLS(C^{-2}) leads only to better results than OLS if the variability is due to the constant coefficient of variation model. In the three other variance models this weighting turned out to be very unrobust. It is well known that OLS is the best method in the homoscedastic situation, but in the simulation study it seems to be not substantally worse than the other methods in the heteroscedastic situation: If Sheiner and Beal observed an improvement of another methods as compared to OLS this seems to be rather small, wheras several methods turned out to be substantially worse for some constellations. Although Sheiner and Beal [17] did not investigate the biexponential function described in this paper, a weighted analysis based on fixed weights can expected to be unrobust with different variance models in this situation, too.

Using statistical programmes like SAS the parameters of nonlinear functions such as the biexponential function (3) are estimated by means of an iterative procedure. As described above the weights (e.g. $w_t = 1/(C_p^*(t))^2$ in many pharmacokinetical applications are fixed in advance, i.e. before starting the iterative procedure. In contrast iteratively reweighted least-squares (IRLS), which is commonly used with nonlinear regression and tends to be associated with robust regression, is based on weights that change from iteration to iteration. When using IRLS, the weights are functions of the residuals from the previous iteration such that points with larger residuals receive less weight than points with smaller residuals. Thus, untypical points tend to receive less weight than typical points. There are various approaches of IRLS defined by different functions of the residuals. In this paper we consider

$$w_k(t) = \frac{1}{1 + (\frac{u}{c})^2} \qquad \text{with } c = 2.385, \tag{5}$$

as weights for the k-th iteration. The standardised residual u is given by $u = (\hat{C}_p^{k-1}(t) - C_p^*(t))/s^{k-1}$, where $C_p^*(t)$ is the observed concentration at time t, $\hat{C}_p^{k-1}(t)$ denotes the estimated concentration and s^{k-1} is the estimated residual sum of squares in the $(k-1)$-th iteration. The IRLS method defined by the weights given in (5) is referred to as Cauchy method due to its relation to the Cauchy distribution. In this paper we use IRLS_C as notation. Details about this and other IRLS methods can be found in Holland and Welsh [18].

Besides IRLS there are further (more) robust methods used with heteroscedastic nonlinear regression. By means of a simulation study Beal and Sheiner [19] compared several approaches. Simulating seven different variance models the authors considered the same three regression functions as they did in their paper mentioned above. Assuming a constant variance OLS is (as expected) the best, but it can behave quite acceptably with three heteroscedastic variance models, too. The IRLS method used in the cited paper behaves best with those three heteroscedastic variance models, where OLS can behave rather poor. However, in some situations IRLS performs worse than OLS and both can be improved by other methods.

Variability of the estimated pharmacokinetic parameters

Presenting the results of a study usually the point estimates of pharmacokinetic parameters (e.g. half-lives and V_d) are given for all patients. However, due to the small number of observed time points, these estimates can expected to be very unstable. Based on the variance of the underlying model parameters the variance of the resulting pharmacokinetic parameters (which are functions of the model parameters) can be obtained by using the delta method [20]. Considering e.g. the elimination half-life $t_{1/2,\beta} = log(2)/\beta = g(\beta)$ derived from the two-compartment model, which is a function of β, the delta method yields the approximative variance

$$\operatorname{var}(t_{1/2,\beta}) = \left(-\frac{\log(2)}{\beta^2}\right)^2 \operatorname{var}(\beta) = (g'(\beta))^2 \operatorname{var}(\beta)\,.$$

A 95-percent confidence interval for $t_{1/2,\beta}$ can then be obtained by

$$\left[t_{1/2,\beta} - t_{n-4,0.975}\sqrt{\operatorname{var}(t_{1/2,\beta})}\,;\; t_{1/2,\beta} + t_{n-4,0.975}\sqrt{\operatorname{var}(t_{1/2,\beta})}\right], \tag{6}$$

where $t_{n-4,0.975}$ is the 0.975-quantile of the t-distribution with n-4 degrees of freedom (n is the number of time points considered for the analysis and 4 parameters in the biexponential function (3) are estimated). For the practical calculation the estimates $\hat{\beta}$ and $\widehat{\operatorname{var}}(\hat{\beta})$ are used instead of β and $\operatorname{var}(\beta)$, respectively. Obviously, replacing β by α we can use the same formulas to calculate a confidence interval for $t_{1/2,\alpha}$. For V_d the application of the delta method is more complicated, since V_d is a function of all model parameters A, B, α and β. If the elimination half-life and the volume of distribution are obtained by log-linear regression, the variability of both parameters (each is a function of the model parameter λ) can also be estimated via the delta method.

In addition – or sometimes instead of – the presentation of pharmacokinetic parameters for all patients it is common to summarize the results in terms of a mean value and the standard deviation. Doing so it is usually neglected that the point estimates themselves may have a large variability. Thus, summarizing pharmacokinetic parameters it seems to be sensible to calculate a weighted mean, where the weights are chosen according to this variability, rather than using e.g. the arithmetic mean. Considering again $t_{1/2,\beta}$, a weighted mean elimination half-life can be obtained by

$$\bar{t}^{w}_{1/2,\beta} = \frac{\sum_{i=1}^{k} \frac{1}{\operatorname{var}(t_{1/2,\beta}(i))}\, t_{1/2,\beta,}(i)}{\sum_{i=1}^{k} \frac{1}{\operatorname{var}(t_{1/2,\beta}(i))}} ,$$

where k denotes the number of patients and (i) is the index for the i-th patient $(i = 1, \ldots, k)$. Note, that values of $t_{1/2,\beta}$ with small variability obtain more weight than values with large variability. This formula is commonly used in meta-analyses, where interest centers arround the determination of a pooled estimate (e.g. the risk of a specific risk factor) based on many studies [21] The corresponding variance formula is given by

$$\operatorname{var}(\bar{t}^{w}_{1/2,\beta}) = \frac{1}{\sum_{i=1}^{k} \frac{1}{\operatorname{var}(t_{1/2,\beta}(i))}} .$$

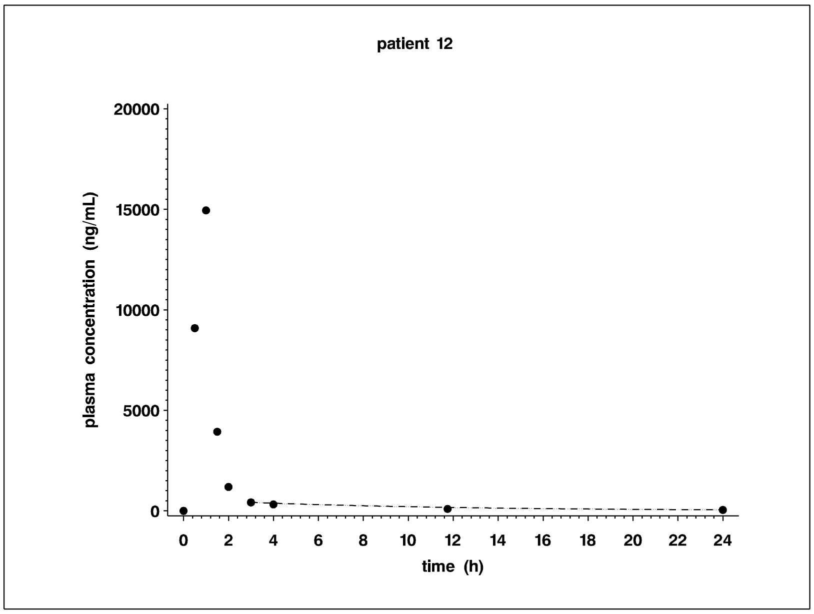
Formula (6) with $\bar{t}^{w}_{1/2,\beta}$ instead of $t_{1/2,\beta}$ and $\operatorname{var}(\bar{t}^{w}_{1/2,\beta})$ instead of $\operatorname{var}(t_{1/2,\beta})$ can be used in order to calculate a 95-percent confidence interval for the weighted mean elimination half life $\bar{t}^{w}_{1/2,\beta}$.

It should briefly be mentioned that pharmacokinetic parameters with respect to a population of patients can be obtained by using random effects models. An introduction to population pharmacokinetics and pharmacodynamics is given by Sheiner and Ludden [22].

4. Application of different approaches

Based on log-linear regression and the biexponential function (3) in this section we estimated the distribution and elimination half-lives ($t_{1/2,\alpha}$ and $t_{1/2,\beta}$) and volumes of distribution for individual patients. In order to fit the biexponential function to the observed plasma concentrations we restricted to values observed at and after t_{max}. Log-linear regression of the terminal elimination phase is based on the last 3 and 4 time points, respectively. Estimating the parameters of the biexponential function OLS, IRLS$_C$, NLS(C^{-1}), NLS(C^{-2}) and OLS(ln) were applied. For the analysis we used the SAS-procedures PROC REG for the log plasma concentrations and PROC NLIN [14] in order to fit the biexponential function. An application of the latter procedure to a pharmacokinetic study is described in Philip and Schwartz [23]. All results are illustrated with the data of five selected patients, each with a different dose level.

As shown in figure 3a for patient 12 log-linear regression using the last 4 time points leads to a good fit of the corresponding observed plasma concentrations. However, a)



b)

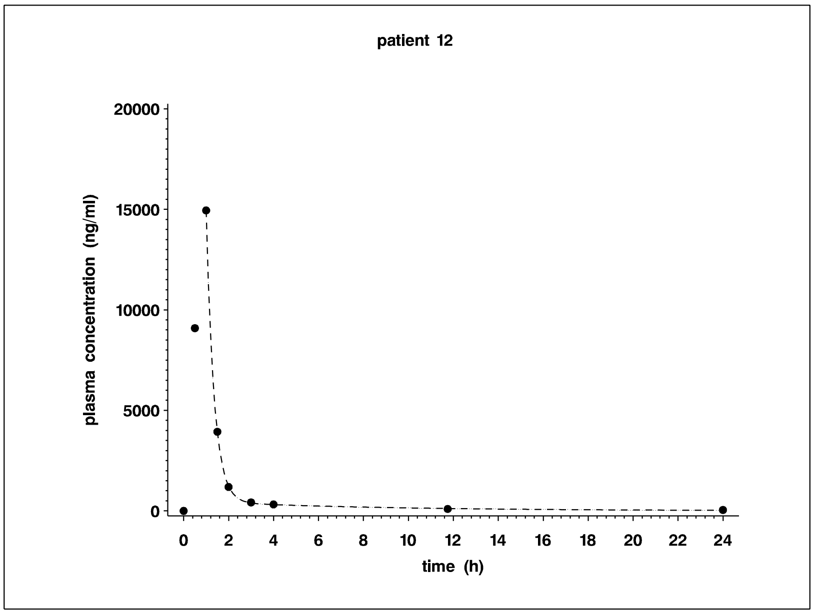


Figure 3: Observed paclitaxel concentrations (dots) and fit (dashed line) obtained by **a)** log-linear regression using the last 4 time points **b)** OLS estimation of the biexponential function starting at $t_{\max}$ for patient 12

restricting to the last 3 time points the fit is not worse. The values of the resulting estimates for the terminal half-life $t_{1/2}$ and the volume of distribution V_d, which are displayed in table 1, increased with decreasing number of time-points. For the calculation of $V_d = CL/\lambda$ (cf. introduction) we used the estimated value of the area under the curve based on the trapezodial rule without extrapolation, i.e $\mathrm{AUC}_{0\text{-}24\ \mathrm{hours}}$. The results shown in table 1 demonstrate that it is important to determine the 'correct' number of time points when applying log-linear regression: Should we use the last 3, 4 or maybe 5 time points for the analysis? Since the choice seems to be somewhat arbitrary and the calculated pharmacokinetic parameters depend on the investigators decision, it would be more appropriate to model the complete elimination phase by using multicompartment models.

Table 1: Terminal half-life $t_{1/2}$ (hours) and volume of distribution V_d (liter/m^2) for selected patients derived from log-linear regression using the last 3 and 4 time points

patient	dose (mg/m^2)	log-linear regression based on the last 4 time points		3 time points	
		$t_{1/2}$	V_d	$t_{1/2}$	V_d
3	150	5.44	120.87	6.08	135.12
5	175	5.04	81.95	5.42	88.15
10	200	6.03	111.30	6.85	126.36
12	225	6.78	122.56	7.52	135.84
24	250	3.66	25.82	3.70	26.10

Estimating the parameters of the biexponential function by OLS regression leads to a very good fit of the plasma concentrations observed at and after $t_{\max}$. The results for patient 12 are illustrated in figure 3b. Using NLS(C^{-1}) the fit to the data is not worse, whereas – for NLS(C^{-2}) – we observed a larger difference (e.g. > 1000 ng/ml for patient 3) between $\mathrm{C}_{\max}$ and the corresponding estimate resulting from the fact that the weight for the observation $\mathrm{C}_{\max}$ is approximately zero. However, all remaining observations were fitted as good as by the other strategies.
The results with respect to $t_{1/2,\alpha}$, $t_{1/2,\beta}$ and $(V_d)_{\mathrm{inf.eq.}}$ are summarized in tables 2, 3 and 4, respectivley. The values resulting from OLS and IRLS_C correspond very well, whereas we obtain subtantial differences for patients 3 and 24 when using NLS(C^{-1}). As compared to OLS the determination of the pharmacokinetical parameters by NLS(C^{-2}) leads to different results for all patients considered in this paper. With this approach the values of the terminal elimination phase obtain a very large weight as comparged to the huge concentrations observed short time after $t_{\max}$ and, therefore, the values of $t_{1/2,\beta}$ get larger, too. We also observed a higher volume of distribution for all patients. The results obtained by OLS(ln) differ only

slightly from those resulting from NLS(C^{-2}). This is not astonishing, because both strategies are based on the assumption of the constant coefficient of variation model [17]. The pharmacokinetical parameters obtained by log-linear regression of the last 3 or 4 observations are similar to those derived from NLS(C^{-2}). However, these result could have been expected in advance, because NLS(C^{-2}) puts more weight on the second exponential term of the biexponential function. It should be stressed that the similarity of results cannot be a justification for a specific model.

Table 2: Distribution half-life $t_{1/2,\alpha}$ (hours) for selected patients derived from the two-compartment model using different estimation procedures

patient	dose (mg/m^2)	OLS	IRLS$_C$	NLS(C^{-1})	NLS(C^{-2})	OLS(ln)
3	150	0.12	0.12	0.19	0.29	0.29
5	175	0.39	0.39	0.38	0.52	0.50
10	200	0.32	0.32	0.32	0.36	0.36
12	225	0.24	0.24	0.24	0.26	0.25
24	250	0.24	0.23	0.26	0.27	0.26

Table 3: Elimination half-life $t_{1/2,\beta}$ (hours) for selected patients derived from the two-compartment model using different estimation procedures

patient	dose (mg/m^2)	OLS	IRLS$_C$	NLS(C^{-1})	NLS(C^{-2})	OLS(ln)
3	150	1.32	1.32	3.22	5.42	5.58
5	175	4.61	4.58	4.37	6.26	5.95
10	200	5.17	5.13	5.12	6.88	6.74
12	225	5.63	5.59	5.61	7.21	7.49
24	250	2.64	2.39	3.87	3.68	3.68

Table 4: Volume of distribution $(V_d)_{inf.eq.}$ (liter/m^2) for selected patients derived from the two-compartment model using different estimation procedures

patient	dose (mg/m^2)	OLS	IRLS$_C$	NLS(C^{-1})	NLS(C^{-2})	OLS(ln)
3	150	57.95	57.92	93.93	134.98	130.11
5	175	74.23	73.83	72.28	88.96	85.56
10	200	86.25	85.65	86.66	107.50	105.06
12	225	92.49	91.67	92.95	114.34	116.55
24	250	27.12	25.67	33.74	32.54	32.37

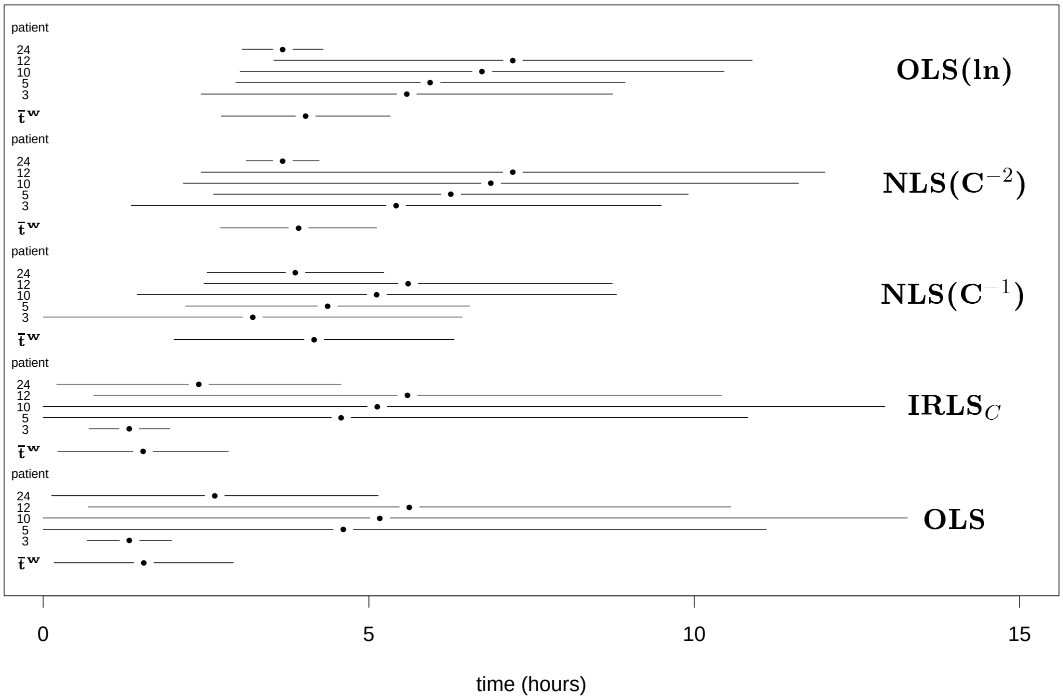


Figure 4: Estimated elimination half-lives $t_{1/2,\beta}$ (dots) and 95 percent confidence intervals for selected patients based on the delta method (lines) and summary in terms of the weighted mean elimination half-life $\bar{t}^w = \bar{t}^w_{1/2,\beta}$

So far we have compared the different approaches described in this paper by comparing the point estimates of half-lives and V_d. Keeping in mind that these estimates are based on a few observations we should also investigate their variability. In this paper we restrict to the elimination half-life $t_{1/2,\beta}$. The 95 percent confidence intervals obtained by using formula (6) are displayed in figure 4. All confidence intervals indicate an extremely large variability of the point estimates. Although we observed large differences in $t_{1/2,\beta}$ between OLS, NLS(C^{-1}) and NLS(C^{-1}), the corresponding confidence interval overlap. Thus, taken the variability into account, the results described above deserve very cautious interpretation. In many applications the results of different patients receiving the same dose are summarized by the mean and the (empirical) standard deviation, which is the square root of the (empirical) variance. Assuming that $t_{1/2,\beta}$ is independent of the given dose the calculation of these descriptive statistics with respect to different dose levels may be of interest, too. In general, including information about the variability of all estimated pharmacokinetic parameters (instead of neglecting it) may lead to different results. Using NLS(C^{-1}) we obtained $\bar{t}^w_{1/2,\beta} = 4.16$ as compared to $\bar{t}_{1/2,\beta} = 4.32$ resulting from the relative small variability (cf. figure 4) of the point estimate for patient 24 ($t_{1/2,\beta} = 3.87$). Besides the confidence intervals for all individuals figure 4 also contains the confidence intervals with respect to the weighted mean elimination half lives. Note, that there is hardly any difference between NLS(C^{-1}) and NLS(C^{-2}) in the mean value of the five selected patients.

5. The use of information about the underlying HPLC method to estimate the variability of the observed concentrations

The application of weighted NLS or OLS(ln) as well as the estimation of pharmacokinetic parameters by using log-linear regression of the terminal elimination phase is motivated by the assumption of an increasing variability with increasing observed concentration resulting from the *uncertainty* of the underlying analytical method. This problem can not expected to be solved by the simple application of IRLS, which tries to overcome the lack of robustness caused by single outliers in the data rather than modelling a specific heteroscedastic situation. Although many authors seem to believe in the constant coefficient of variation model, when the concentration of the drug is determined by an HPLC method, we have not found strong evidence to support this assumption in the literature. Considering several recently published studies on the pharmacokinetics of Paclitaxel (table 5) we observed various strategies with respect to the statistical analysis. In some of these papers important details are not given, although the results may change substantially when using e.g. another weighting scheme. These considerations as well as the existence of investigations like those of Sheiner and Beal [17, 19] reveal the uncertainty how to describe the variability of observed plasma concentrations. There are several methods [17, 19] (e.g. extended least squares regression) that estimate the variability of the observed plasma concentrations in addition to the parameters of the model. Besides the problems caused by the estimation of an additional parameter (usually there are only a few data points) the estimated variability may differ between patients. However, assuming a specific model with respect to the variability resulting from an analytical method should be consistent for all patients and all applications.

Usually, the HPLC method used in a pharmacokinetic study is investigated in a validation study before analysing the patients' blood samples. Therefore, it is worthwhile to use the information of this validation in order to estimate the true variability and to identify the most appropriate weighting scheme.

Table 5: Examples for different methods of pharmacokinetical analysis from the literature (concentration of the corresponding drug determined via HPLC except for Carboplatin*)

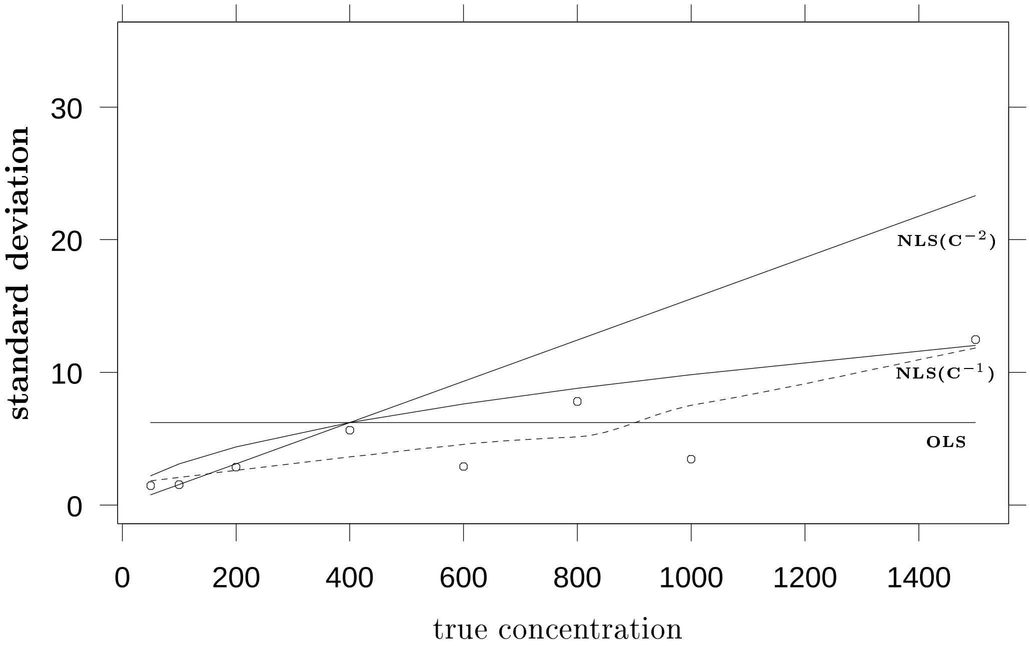
drug (administration)	reference	model	estimation procedure
Taxol (6h, iv)	Wiernik et al. [4]	2-compartment	OLS(ln)
Taxol (24h, iv)	Wiernik et al. [6]	2-compartment	OLS(ln)
Taxol (6h, iv)	Brown et al. [5]	2-compartment	NLS(C^{-2})
Taxol (1/6 h, iv)	Longnecker et al. [10]	2-compartment	NLS(C^{-1})
Taxol (1/6 h, iv)	Grem et al. [24]	noncompartmental	log-linear regression of the terminal elimination phase△
Doxorubicin (bolus iv) and Paclitaxel (3h, iv)	Gianni et al. [25]	3-compartment (DOX) noncompartmental (PTX)	unclear△ *log-linear regression of the terminal elimination phase* △♣
Paclitaxel (3h/24h, iv)	Gianni et al. [8]	noncompartmental	*log-linear regression of the terminal elimination phase* △♣
Paclitaxel (3h, iv) and Carboplatin* (30min, iv)	Huizing et al. [26]	3-compartment (PTX) 2-compartment (CP)	PTX: OLS(ln) CP: *OLS* △
Paclitaxel (3h, iv) R-Verapamil (oral)	Berg et al. [27]	2-compartment	NLS(C^{-2})
Epirubicin (bolus iv) Paclitaxel (3h, iv)	Conte et al. [28]	2-compartment (for each drug)	NLS, weights not given △ (for each drug)

△ insufficient description of the underlying method
♣ information derived from a description of the computer program LAGRAN [9]

In the present study 8 different concentrations of Paclitaxel ($conc$= 50, 100, 200, 400, 600, 800, 1000 and 1500 ng/ml) obtained by giving the corresponding amount of paclitaxel to the plasma of healthy persons were considered for validation. The same amount of 7-epi-paclitaxel was given to each of this specimen as internal standard. Then, the resulting blood samples were devided into small portions. To obtain information about the precision of the observed plasma concentrations, the HPLC-analysis was repeated three times within one day (within-days precision, WDP) as well as on six different days (day-to-day precision, DDP).

To get the most appropriate strategy for estimating the parameters of the biexponential function, we considered the analytical results for 400 ng/ml as a baseline. The standard deviation corresponding to this baseline, which will be denoted by std_{400}, is estimated by a weighted mean of the empirical standard deviations for WDP and DDP, where the weights were choosen according to the number of repetitions. Based on std_{400} we calculated those standard deviations that would be assumed for the other concentrations when using different weighting schemes (i.e. assuming different variability models): Obviously, in the homoscedastic situation the standard deviation is equal to std_{400} for all other concentrations. Assuming the constant coefficient of variation model the standard deviation of a concentration $conc$ is obtained by $std_{400} \cdot conc$ / 400. Multiplying std_{400} by $\sqrt{conc/400}$ gives the standard deviation of concentration $conc$, when the variability assumption corresponds to NLS(C^{-1}). Then, using a smoothing line we compared the empirical standard deviations observed in the data to the different model assumptions. This is done separately for WDP and DDP, the results are displayed in figures 5a (WDP) and 5b (DDP). Considering WDP we obtained clear advantages for NLS(C^{-1}), whereas for DDP this weighting seems to be slightly better only for small concentrations. Considering concentrations larger than 400 ng/ml we should rather use NLS(C^{-2}) or OLS(ln). It should briefly be mentioned that we obtained nearly the same results when using slightly modified approaches (not shown in this paper).
After fixing the strategy for the determination of the concentration in the validation study, it is common – or at least desirable – to perform a new HPLC analysis using the known concentrations each day when analysing the clinical data. The analytical results of this run are used to determine a calibration line, which is the base for the estimation of the patient's plasma concentrations. Thus, the WDP seems to be more important with respect to the results of the study, and, therefore, we decided to use NLS(C^{-1}) for the analysis. Concerning the pharmacokinetical analysis of Taxol we only found one example in the literature [10], where the analysis is based on the same weights.

a) WDP



b) DDP

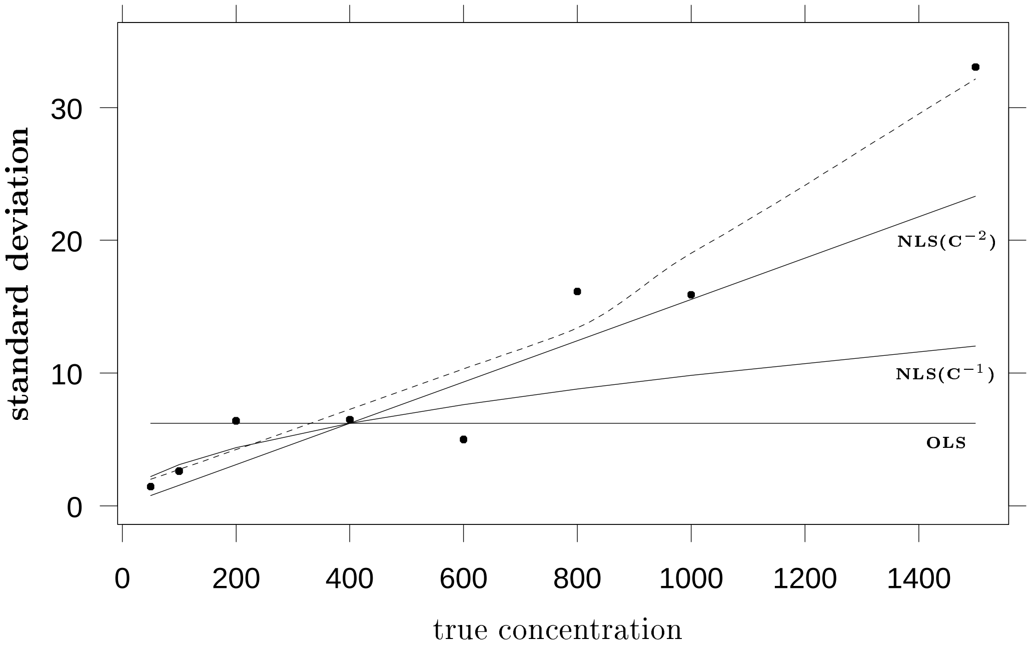


Figure 5: Within-days-precision (WDP) and day-to-day precision (DDP): Observed standard deviation (WDP: circles, DDP: dots) with smoothing line (dashed line) and assumptions according to the weights $w_t = 1$ (OLS), $w_t = 1/C(t)$ (NLS(C^{-1})) and $w_t = 1/C^2(t)$ (NLS(C^{-2}))

6. Conclusions and discussion

The estimation of pharmacokinetical parameters is often derived from the assumption of a multicompartment model or based on log-linear regression of the terminal elimination phase. In this paper we considered log-linear regression using the last 3 and 4 observations, respectively, as well as a two-compartment model, which corresponds to a biexponential function in order to describe the elimination of the drug in the central compartment. There are various strategies to estimate the unknown parameters of this biexponential function, each of these strategies is attached to a specific assumption concerning the variability of the observed concentrations.

Usually, the concentration of paclitaxel (and many other drugs) is determined by high-performance liquid chromatography (HPLC), an analytical method that can be expected to measure rather small values appropriately. Using HPLC the variability is often assumed to increase proportionally to the concentration, i.e. assuming a constant coefficient of variation, and, therefore, the statistical analysis is performed by OLS(ln) and NLS(C^{-2}), respectively (cf. section 3). However, we have not found any proof of this assumption so far. Furthermore, examples from the literature [10, 29] show that not all authors believe in the constant coefficient of variation model.

Analysing the data of patients of a phase I clinical trial, who received paclitaxel as 1-hour infusion we showed that the value of half-lives and the volume of distribution strongly depend on the estimation procedure. We observed increasing values of both pharmacokinetic parameters with decreasing number of time points when the estimation was based on log-linear regression of the so called terminal elimination phase. However, the number of observations used for the analysis is determined by the investigator rather than by any prespecified rule. Based on the estimated parameters of the biexponential function the values of the two half-lives and the volume of distribution increase with more extreme weighting schemes. Since we observed clear differences between the methods considered in this paper, it is likely to obtain incorrect estimates of pharmacokinetic parameters if the assumption with respect to the variability is not true. By means of a simulation study Sheiner and Beal [17] showed e.g. that OLS(ln) and NLS(C^{-2}) can be very unstable when the variability is unequal to the constant coefficient of variation model.

Motivated by these problems we developed an approach to use information about the HPLC method in order to estimate the variability of the observed plasma concentrations. The results can then be used to determine the most appropriate statistical analysis. In the current study we decided to estimate the parameters of the biexponential function using NLS(C^{-1}). It should be stressed that our proposal cannot guarantee to estimate the true variability appropriately. However, using information about the underlying analytical method – for HPLC the results of a validation study are usually available in all studies – seems to be better than choosing a specific strategy arbitrarily.

Considering the problems described in this paper it is obvious that pharmacokinetic

parameters obtained by using different statistical methods should not be compared as done in the literature [30, 31]. Generally, it is necessary to give a sufficient description of the underlying statistical method when reporting on (pharmacokinetic) results of a study. Unfortunately, this is not done in all publications (cf. table 5). Furthermore, it has been shown that the variability of the estimated pharmacokinetic parameters is extremely large resulting from the small number of blood samples taken. Thus, the number of observed time points should be as large as possible in order to make the analysis more robust.
According to the present study we considered the estimation of half-lives and the volume of distribution for paclitaxel. However, most problems outlined in this paper carry through to other model-based pharmacokinetic parameters and other drugs. Thus, it is important to investigate the variability of the analytical method before analysing the data of a study.

Appendix

Riggs [16] gives in chapter 8 a general solution of a two-compartment model with an exchange between and an elimination from both compartments. Riggs' formulas, which are for the fraction of the total dose, can easily be adapted to the problem described in this paper by equations (2a) and (2b). For the amount of the drug in the central compartment we obtain

$$X_c = X^0(C_1 \exp(-\alpha t) + C_2 \exp(-\beta t)),$$

where X^0 is the given dose and

$$\begin{aligned} C_1 &= \frac{k_{12} + k_{el} - k_{21} + Z}{2Z} \\ C_2 &= 1 - \frac{k_{12} + k_{el} - k_{21} + Z}{2Z} \\ \alpha &= \frac{1}{2}(k_{12} + k_{el} + k_{21} + Z) \\ \beta &= \frac{1}{2}(k_{12} + k_{el} + k_{21} - Z) \end{aligned}$$

with $Z = \sqrt{(k_{12} + k_{el} - k_{21})^2 + 4k_{12}k_{21}}$. Easy calculations lead to

$$C_1 = \frac{(k_{21} - \alpha)}{\beta - \alpha} \tag{A1}$$

$$C_2 = \frac{(k_{21} - \beta)}{\alpha - \beta} \tag{A2}$$

$$(\alpha + \beta) = k_{21} + k_{12} + k_{el} \tag{A3}$$

$$(\alpha\beta) = k_{21}k_{el} \tag{A4}$$

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