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Aspects on tolerance limit estimation -
some common approaches and flexible modeling

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Aspects on tolerance limit estimation -
some common approaches and flexible modeling

by Max Petzold

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In a dose finding study the aim is to attain a safe and efficient drug therapy in a certain population. By a dose finding study we generally refer to a study where the dosage is successively adjusted after analyzing the responding concentration in e.g. the blood. Measurements of interest are e.g. area under curve, time to peak, peak value, time to zero and tolerance limits. The estimates obtained from the pharmacokinetical data are compared with the known safety and efficiency profiles of the drug, and the dosage may be adjusted to attain a satisfactory result.

Different estimation approaches have to be used depending on the safety and efficiency profiles of the drug. When estimating tolerance limits there are roughly two different approaches, one intended for drugs with severe side effects and one intended for drugs with harmless side effects. However, sometimes the approaches are used as interchangeable resulting in misleading estimates.

It is also important to consider the data utilization. A variety of estimation techniques are used in pharmacokinetics. Some of the proposed estimators use cross sectional data and the more advanced ones use the longitudinal structure of the data to different extents. The benefits of the latter are often considerable, especially for small sample sizes common in pharmacokinetics. For crude data where we can not assume fixed regression parameters flexible regression models tend to be superior.

This licentiate thesis consists of two parts:

I. A comparative study of some approaches for constructing tolerance limits, by Max Petzold.

II. Estimating in non-linear monotone response curves using random regression techniques, by Robert Jonsson and Max Petzold.

In Report I two different estimation approaches for tolerance limits are considered: the conservative approach intended for drugs with severe side effects and the closeness approach intended for drugs with harmless side effects. It is obvious, but rarely discussed, that the conservative approach tends to result in less efficient drug therapies than the closeness approach. In the case with severe side effects this disadvantage may be well motivated by safety aspects. However, this approach is sometimes also proposed for drugs with harmless side effects. In Report I it is shown in a simulation study that the conservative approach can be considerably less efficient and some examples are given. Both parametric and non-parametric cross sectional estimators are used in the study.

In many applications data is based on repeated observations over time. In Report II this longitudinal structure of the data is taken into consideration. A flexible model which after linearization has a random intercept and a fixed slope is proposed. The model is flexible in the sense that the time dependence is modeled by t^p where t is the time value and p is an unknown parameter. A two-step estimation approach is proposed where p is estimated in the first step, and the rest of the parameters are estimated in the second step by using standard regression techniques. The effects of first estimating p upon the rest of the estimators are demonstrated by a simulation study. It is concluded that bias and precision of estimators of the variance components and the dependent variable remain quite unaffected by the two-step procedure, as well as bias of the slope and intercept parameters. However, the precision of the latter estimators may be poor for small values of p, provided that the variance of the measurement errors is large.

Acknowledgement

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A comparative study of some approaches for constructing tolerance limits

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Key words and phrases: conservative; dose finding study; non-parametric; order statistics; parametric; population pharmacokinetics; small sample; tolerance limits.

Abstract

In a dose finding study the aim is to come up with a safe and efficient drug administration. By comparing the obtained tolerance limits with predetermined desired concentration limits, i.e. the therapeutic window, one may be able to adjust the drug dosage. For drugs with a therapeutic window situated at high concentrations close to toxic levels, one has to balance between attaining a high proportion of the population at efficient high levels on one hand and the risks of an overdose on the other hand. In such cases it is important to use the proper estimation approach for the upper tolerance limit. Here a conservative estimation approach intended for a drug with potentially adverse side effects with minor overdoses is compared with an approach intended for a drug with harmless side effects. It is shown that the conservative approach can be considerably less efficient when used in the latter case, a disadvantage that is rarely discussed when proposing conservative estimators.

The properties of the two approaches are evaluated using well-known parametric and non-parametric estimators in a simulation study for small and moderate sample sizes.

1 Introduction

The question of how much drug should be used and how it should be administered for a given therapeutic purpose can not readily be answered. Basically two different methods have been used to answer the question in a population: empirical and population kinetic. The latter is to be discussed in this paper. The kinetic method is based on the hypothesis that therapeutic and toxic responses are related to the amount of drug in the body or to the plasma drug concentration. By studying pharmacokinetic data following a single dose, the tolerance limits of the concentrations in a population may be obtained. The tolerance limits can then in turn be used to modify the dosage regimen according to the known relationships among concentration levels, therapeutic response, and toxic effects.

The idea of tolerance limits and tolerance intervals has to be explained in order to get the right interpretation. Historically, *ordinary tolerance intervals* arose in response to engineers' concern with mass-production processes. For example, industry-wide specifications might dictate that any component which measures less than v_1 or greater than v_2, i.e. the *ordinary tolerance limits*, is to be considered as unsatisfactory. The variability in the interval $[v_1, v_2]$ is tolerated due to design considerations or economical reasons etc. The probability that the measurement X_i of a single component i will fall in the interval indicates how successful the production process is. As a check on the true distribution of the production process one may take sample data and estimate the *statistical tolerance interval*, which is to be compared with the ordinary tolerance interval $[v_1, v_2]$. The *statistical tolerance limits* provide information about where the population sampled is likely to be concentrated, and from this, the production process can be calibrated to get a high proportion of the population within the desired interval $[v_1, v_2]$. (Statistical) tolerance limits and tolerance intervals are frequently used today in both production and research. The terms tolerance limit and percentile may be used interchangeably depending on the area of research.

In this paper a principal question of how to obtain the upper tolerance limit for a particular drug is discussed. In section 2 a conservative estimation approach based on probability restrictions and an approach based on close-

ness of the estimators are introduced. In each approach one parametric and one non-parametric estimator are considered. The parametric estimators are based on the normal distribution and the non-parametric estimators are based on continuous data. The original drug concentration is assumed to have the lognormal distribution at each time point, but all results are based on the normal distributed logarithmic scale. All the estimators are well-known but arbitrary for the actual question. In section 3 some consequences of the two approaches when used in a dose finding study are described. The results of the simulation studies are presented in section 4 followed by a discussion in section 5. The simulation model and parameter settings can be found in the Appendix.

2 Two approaches for obtaining the tolerance limits

In this paper two approaches for obtaining the upper tolerance limit are compared: the *conservative approach* and the *closeness approach.* Note that the terminology for tolerance intervals has not been standardized and the proposed names may be interpreted differently in other papers, see Mee [7] for a discussion. In the conservative approach, let a single measurement be represented by a random variable X_i, and let w be the upper tolerance limit. Then one requires that

$$P_{X_i}\left(-\infty \leq X_i \leq w\right) \geq \beta \tag{1}$$

If $\mathbf{X} = (X_1, ..., X_n)$ represents a sample of n measurements, then the estimator of w can be written $\widehat{w} = \widehat{w}\left(\mathbf{X}\right)$. Over all possible samples one may require that

$$P_{\mathbf{X}}[P_{X_i}\left(-\infty \leq X_i \leq \widehat{w}\right) \geq \beta] \geq \gamma \tag{2}$$

The expression (2) defines a one-sided *statistical β-content tolerance interval* at the confidence level γ. The interpretation of (2) is that at least $100 \times \beta\%$ of the population is covered by the tolerance interval with a probability of

at least γ. Equality is obtained in the continuous case. The upper limit w of the β-content tolerance interval is interpreted as a conservative tolerance limit, i.e. the limit indicates what extreme value that covers at least $100 \times \beta\%$ of the population with confidence γ. Expression (1) and (2) may easily be exemplified by assuming X_i to have the exponential distribution with mean and variance λ^{-1}. From (1) we have $1 - \exp(-\lambda w) = \beta$ which defines $w = -\lambda^{-1} \ln(1-\beta)$. Here one may take $\widehat{w} = -\overline{X} \ln(1-\beta)$ which in (2) gives $P[1 - \exp(-\lambda \widehat{w}(\mathbf{X})) \geq \beta] = \gamma$, i.e. the probability over all samples should be γ. Important results in the area are due to Wilks [19], Wald [16] and Tukey [14]. In the simulation study some estimators were to be chosen. Since the principle difference between the two approaches is independent of the estimators, some well-known but arbitrary estimators were chosen. In the parametric case, under normality assumption, the conservative tolerance limit was estimated by $\widehat{w}(\mathbf{X}) = \widehat{\mu} + K\widehat{\sigma}$. The constant K is determined by $P_{\mathbf{X}}[T_{n-1}(\sqrt{n} z_{1-\beta}) \leq \sqrt{n} K] \geq \gamma$ where $T_f(\delta)$ is a non-central t variable with f degrees of freedom and non-centrality parameter δ, see Kotz&Johnson [5] for a detailed description. In the non-parametric case, Wilks' [18] conservative estimator based on the beta distributed coverage between succeeding order statistics from a continuous distribution was used. The tolerance limit is estimated by the upper order statistic of the β-content tolerance interval. The limiting order statistic is chosen with a step function of n, and the confidence level will attain its minimal level γ at the shifts of order statistics. This estimator has been proposed by e.g. Gillespie&Srinivasan [2] to obtain a normal range for screening purpose in clinical medicine, Walsh [17] to compare two samples and Nickens [8] for analyzing safety data in population pharmacokinetics. As the coverage and the confidence both tend to 1, increasingly larger sample sizes are required to achieve informative limits. The minimum required sample size for a one-sided tolerance interval would e.g. increase from 59 to 459 when both β and γ increase from 0.95 to 0.99. Sample size tables for one-sided and two-sided tolerance intervals are given by Somerville [12]. In all simulations in the present paper $\gamma = 0.95$ was used.

In the closeness approach, one requires

$$E_{\mathbf{X}}[P_{X_i}(-\infty \leq X_i \leq \widehat{w})] \approx \beta \tag{3}$$

i.e. closeness in coverage over all samples. The approximative requirement in expression (3) makes it possible to choose the estimator with minimal variance $V[\widehat{w}]$ and with small bias $E[\widehat{w} - w]$. Here, under normality assumption, the parametric estimator $\widehat{w}(\mathbf{X}) = \widehat{\mu} + \sqrt{(n+1)/n}\widehat{\sigma}t_{n-1;1-\beta}$ proposed by Wilks [18] was used. In the non-parametric case, let j and g be the integer and the decimal part of $n \times \beta$, respectively. Then the $100 \times \beta\%$ tolerance limit was estimated using the quantity $\widehat{w}(\mathbf{X}) = \left(X_{(j)} + X_{(j+1)}\right)/2$ if $g = 0$, and $\widehat{w}(\mathbf{X}) = X_{(j+1)}$ if $g > 0$. This is one out of five sample percentile definitions included in the statistical software packages SAS(r) and SPSS(r), see [11] and [13] respectively. Sample percentile definitions are frequently used to establish reference intervals in determining abnormality, see e.g. Ooi et al [9].

3 Consequences of the two approaches in a dose finding study

In a dose finding study, the obtained tolerance limits are compared with the limits of the *therapeutic window*, i.e. the desired concentration range where the chance of successful therapy is high. The information relating concentration to response can be obtained at three levels: through in vitro experiments, animal studies and investigations in human. Once the distribution of therapeutic and toxic effects at different concentration levels for a drug is obtained, it is possible to obtain a *utility curve* by weighting and adding the probabilities of the different therapeutic and toxic effects. The probabilities of adverse side effects are weighted with large negative values, and vice versa for the probability of desired effects, e.g. recovery. The utility curve has an optimum concentration at which therapeutic success is most likely, and there is a range of concentrations in which the chances of successful therapy is high. This is the *therapeutic window.* Precise limits are not definable, particularly considering the subjective nature of the utility curve,

but approximate limits will be determined by the location of the maximal efficacy and the slope of the curve. The shape of the curve may differ for different populations and even between objects, which must be taken into consideration in a study. For a further introduction to therapeutic response and toxicity, see Rowland&Tozer [10]. The utility curves and the therapeutic window for the two types of drugs discussed in this paper are shown in Figure 1. Note that the upper limit of the therapeutic window is close to toxic concentration levels and the different shapes of the two utility curves.

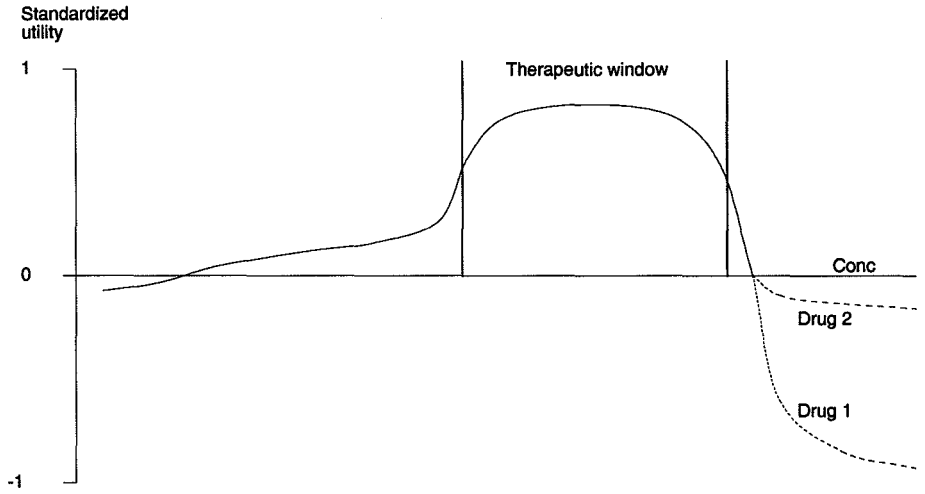

Figure 1: Utility functions of the concentration for Drug 1 and Drug 2. At positive values, the expected outcome is a successful therapy, e.g. well-being and recovery, and vice versa for negative values. The therapeutic window is the desired drug concentration interval. The weights are standardized for an outcome in the interval $[-1, 1]$.

The appropriate approach must be chosen depending on the utility function of the drug at hand. For Drug 1, the conservative approach is appropriate. When calibrating the dosage regimen for Drug 1, it is important not to underestimate the upper tolerance limit. The reason for this is that an obtained estimate lower than the upper limit of the therapeutic window indicates that the dosage should be increased. When based on an underestimate, the increased dosage will have the result that a larger than desired proportion of the population will receive unacceptably high concentrations. At these levels of concentration the risks of adverse side effects rapidly increase for Drug 1. Even a minor overdose may result in death. Using the conservative approach,

one may control for these risks. The coverage and the confidence level are chosen depending on the shape of the utility curve. However, requiring high coverages and confidence levels the dosage regimen tends to be low. This may result in a non-optimal dosage regimen where a larger than desired proportion of the population receives inefficient concentrations to the left of the therapeutic window. This is the disadvantage of the conservative approach, which may be well motivated by the side effects. With Drug 2, the expected outcome of an overdose is harmless, e.g. discomfort, and the non-optimal dosage regimen attained by the conservative approach is no longer justifiable by the side effects. In this case, the closeness approach may be more appropriate. The closeness criterion makes it possible to calibrate the dosage regimen to attain a large proportion of the population within the therapeutic window.

4 Results

Irrespective of which estimator is being used (parametric or non-parametric), the distributions of the estimates for the two approaches will principally differ in the same way. For the conservative approach the distribution will be skewed to the right due to the confidence criterion in expression (2). The skewness will increase with an increased confidence level. However, for the closeness approach the distribution of the estimates is less skewed. This is illustrated in Figure 2 where the obtained distributions of $\widehat{w}$ when estimating the 95% tolerance limit using the two non-parametric estimators are plotted. The true tolerance limit is marked with a straight vertical line at 10.67. In this particular simulation, a proportion of only 4.9% were underestimated using the conservative estimator due to the confidence criterion $\gamma \geq 95\%$. Using the closeness estimator, a proportion of 43% was underestimated. Notable is the difference in variance, which for the closeness approach was 39% of the variance for the conservative approach.

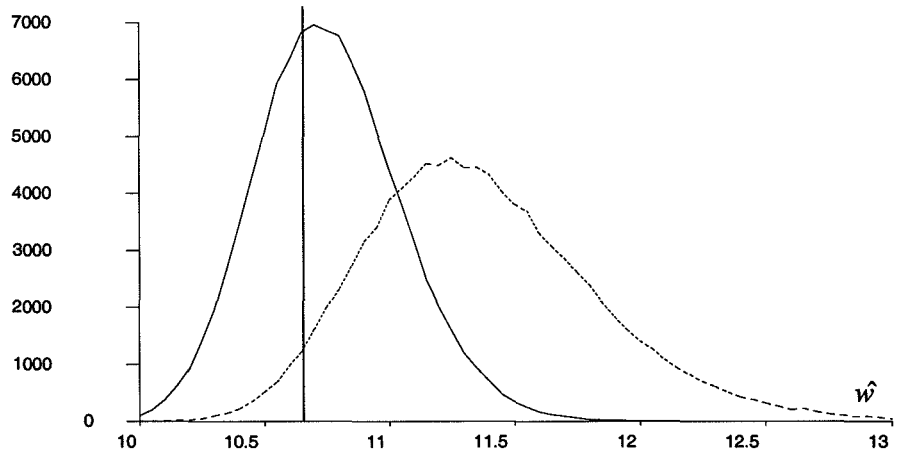

Figure 2: The distributions of the non-parametric $\widehat{w}$ when estimating the 95% tolerance limit, marked with a vertical line at 10.67,when $n = 59$. For the closeness approach, solid line, the distribution of $\widehat{w}$ is almost centered around the true limit but is highly skewed to the right for the conservative approach, dashed line. The number of replicates was 100.000.

4.1 Proportion of underestimates, bias and variance

The differences between the two approaches will principally be reflected by the proportion of underestimates and the bias when estimating the upper tolerance limit. The choice of confidence level for the conservative approach will to a large extent determine these differences with a maximum as the confidence is limiting to 1. Also the variance will be influenced in the same way by the confidence level. As could be seen from Figure 2, the differences may be considerable for the non-parametric estimators. A simulation study was carried out to illustrate how the two approaches differ for some β tolerance limits and some sample sizes using both parametric and non-parametric estimators. The sample sizes chosen are the minimal sample sizes where expression (2) still holds true for Wilks' non-parametric estimator. For these sample sizes the upper conservative limit is estimated with $X_{(n)}$. The results are given as $X/Y/Z$ in Table 1 and Table 2 where X is the proportion of underestimates and Y and Z are the bias and the variance, respectively, given as percentages of the corresponding values for the conservative approach. In Table 1, for example, it can be seen that using the closeness approach the bias and variance are 9.0% and 79% respectively compared to the use of the conservative approach for estimating the 95% tolerance limit when $n = 59$.

As expected, the bias and the variance are considerably smaller for the closeness approach than for the conservative approach. Using the closeness approach, it will be possible to obtain a relative stable estimate near the true tolerance limit. This would be preferable for drugs like Drug 2. For the two parametric estimators an increased sample size will monotonically lower the bias and the variance. However, the bias will decrease faster for the closeness approach than for the conservative approach, implying an increasing difference in bias. The opposite relation is true for the variance.

For the non-parametric estimators there will be a somewhat different relation. The bias and variance of the two non-parametric estimators will not decrease monotonically with an increasing sample size. Using order statistics as estimators the bias and variance will be determined by the positive relation between the expected value of an order statistic and the sample size. Both the non-parametric estimators use step functions of n for including and excluding order statistics. However, increasing the sample size, the bias will monotonically increase up to the next shift of order statistic, e.g. $X_{(n)}$ to $X_{(n-1)}$, where there is a decrease in one "jump" to a new lower level. This process will continue and the bias will asymptotically be zero. The variance also will not be a monotonically decreasing function of n.

Table 1: The performances of the conservative (CON) and the closeness (CLO) approach using the parametric estimators are compared. The percentage values $X/Y/Z$ are interpreted as the proportion of underestimates / the bias compared to the bias of CON / the variance compared to the variance of CON.

n	β :	97.5%	95%	90%	80%
119	CON	5.0/100/100	5.0/100/100	5.0/100/100	5.0/100/100
	CLO	44/8.6/84	46/6.5/85	47/4.5/86	48/2.6/89
59	CON	5.0/100/100	5.0/100/100	5.0/100/100	5.0/100/100
	CLO	42/12/79	44/9.0/79	46/6.2/81	48/3.5/84
29	CON	5.0/100/100	5.0/100/100	5.0/100/100	5.0/100/100
	CLO	38/16/71	41/12/71	44/8.4/73	46/5.0/76
14	CON	5.0/100/100	5.0/100/100	5.0/100/100	5.0/100/100
	CLO	33/22/61	37/17/60	41/11/61	45/6.5/65

Table 2: The performances of the conservative (CON) and the closeness (CLO) approach using the non-parametric estimators are compared. The percentage values $X/Y/Z$ are interpreted as the proportion of underestimates / the bias compared to the bias of CON / the variance compared to the variance of CON.

n	β :	97.5%	95%	90%	80%
119	CON	4.9/100/100	1.6/100/100	4.0/100/100	4.2/100/100
	CLO	43/10/37	45/5.5/42	47/5.1/71	48/2.9/84
59	CON		4.8/100/100	1.5/100/100	3.5/100/100
	CLO		43/9.5/39	45/4.9/46	47/3.8/75
29	CON			4.7/100/100	1.3/100/100
	CLO			44/8.6/42	46/3.8/51
14	CON				4.4/100/100
	CLO				45/6.9/47

4.2 Attained efficiency

Until now, focus has been on safety and the upper concentration limit of the therapeutic window. To achieve an efficient dosage regimen, i.e. a large proportion of the population within the therapeutic window, one also has to consider the lower limit. Calibrating a dosage regimen in a repeated study, one will successively increase the dosage from a low level until the obtained $100 \times \beta\%$ tolerance limit equals the upper limit of the therapeutic window. Assuming a constant variance of the concentration, this equality will be attained at different dosage levels for the two approaches. As can be seen from the comparisons of bias in the previous subsection and from Figure 2, the conservative approach is expected to reach an agreement at lower dosage levels than the closeness approach. Consequently, a higher proportion of the population will attain the inefficient concentration levels to the left of the therapeutic window for the conservative approach than for the closeness approach. This is the price of safety, which may be well motivated for Drug 1. For Drug 2, the risks of an overdose are negligible and it is then possible to use the closeness approach that does not control the risks of an overdose. The choice of approach depends now only on the expected efficiency which in some situations may be considerably higher for the closeness approach.

A simulation study of the difference in attained efficiency was performed for the parametric estimators, defining efficiency as the proportion of the population within the therapeutic window. The true 95:th percentile of the expression (5) in the Appendix was used as the fixed upper limit UL_{95} of the therapeutic window, and the lower limit LL_p was chosen as some p:th percentiles where $p < 95$. For each sample replicate the sample variance $\widehat{\sigma}^2$ was calculated. Utilizing the fact that the estimators have the same structure

$$\begin{aligned} \widehat{w}_{clo} &= \widehat{\mu} + g_{clo}(\widehat{\sigma}) \\ \widehat{w}_{con} &= \widehat{\mu} + g_{con}(\widehat{\sigma}) \end{aligned}$$

a dose finding study was imitated by calculating

$$\begin{aligned} \widehat{\mu}_{clo} &= UL - g_{clo}(\widehat{\sigma}) \\ \widehat{\mu}_{con} &= UL - g_{con}(\widehat{\sigma}) \end{aligned}$$

The parameters $\widehat{\mu}_{clo}$ and $\widehat{\mu}_{con}$ correspond to the attained doses where the estimated tolerance limit equals the upper limit of the therapeutic window in a real dose finding study. Assuming constant variance σ^2 from expression (5) the difference in efficiency D was calculated for each replicate as:

$$\widehat{D} = P\left(LL_p \leq \widetilde{X}_{clo} \leq UL_{95}\right) - P\left(LL_p \leq \widetilde{X}_{con} \leq UL_{95}\right) \tag{4}$$

where $\widetilde{X}_{clo} \sim N\left(\widehat{\mu}_{clo}; \sigma^2\right)$ and $\widetilde{X}_{con} \sim N\left(\widehat{\mu}_{con}; \sigma^2\right)$. These distributions agree with the logarithmic concentration distributions that would be obtained in a dose finding study.

As can be seen in Table 3, the closeness approach will on the average attain a larger proportion within the window. When increasing UL or decreasing the sample sizes, the differences will increase. The standard deviation seems to have a more complex dependence on UL_p and the sample size, but is not further analyzed. For the difference there will be an optimal LL_p depending on the underlying distribution. In this case the differences will decrease for increasing $p > 40$.

Table 3: The mean of $\widehat{D}$ when using the parametric estimators. The standard deviation is given within brackets.

$n \backslash p$	5	10	30
14	0.14 (0.12)	0.19 (0.11)	0.24 (0.069)
29	0.058 (0.056)	0.098 (0.056)	0.16 (0.043)
59	0.025 (0.022)	0.052 (0.026)	0.10 (0.023)
119	0.011(0.001)	0.030(0.012)	0.067(0.012)

For the non-parametric estimators the simulation study had to be done in a different way. Using the same therapeutic window as before, a successively increased dosage was simulated letting the mean value of X_i in expression (5) successively increase from a low level until the estimated tolerance limit on the average was equal to UL_{95}. This was done independently for the two approaches, and the obtained means $\widehat{\mu}_{clo}$ and $\widehat{\mu}_{con}$ were used as in the parametric case to calculate the difference in efficiency. From Table 4 it can

be seen that the difference is considerable also for relative large sample sizes when using the non-parametric estimators. The difference was found to have the same dependences on UL, LL_p and the sample size as in the parametric case.

Table 4: The mean of $\widehat{D}$ when using the parametric estimators.

$n\backslash p$	5	10	30
119	0.054	0.10	0.17
59	0.072	0.12	0.20

It is now obvious that a proper usage of the two approaches is important. To avoid overdoses of Drug 1, one has to use the conservative approach, but this approach would often be too inefficient to be motivated for Drug 2.

5 Discussion

The importance of using the appropriate efficiency and safety approach in dose finding studies was discussed. It was shown that an appropriate approach for a drug with adverse side effects with minor overdoses might be inappropriate for a drug with harmless side effects. The performance of two common approaches, the closeness and the conservative approaches, were compared for some relevant tolerance limits. Using the conservative approach, one will only risk that a certain proportion of the population attains overdoses. The drawback is that the resulting dosage tends to be low, with the consequence that an undesired large proportion of the population attains inefficient concentrations. In many papers though conservative tolerance limits are proposed without a discussion of the consequences of an inappropriate use, see e.g. the discussions in Nickens [8] and Holst&Christensen [3]. Here, efficiency was defined as the proportion of the population within the therapeutic window. A more detailed study taking the exact form of the utility curve into account would be valuable.

A simulation study showed that the estimates of the true tolerance limits obtained with the closeness approach attain relative low bias and variance. Such properties offer opportunities to adjust the dosage to obtain a large

proportion of the population within the therapeutic window, i.e. where the chance of successful therapy is high. With this approach the confidence criterion in expression (2) may not be fulfilled, and consequently it is more appropriate in dose finding studies for drugs with harmless side effects with minor overdoses. However, for diseases with very harsh outcomes, e.g. AIDS, this approach may still be motivated ethically, even if there is a high probability of adverse side effects with minor overdoses. Here the proportion within the therapeutic window is the most important property. In the opposite situation, when treating a harmless disease, no drug with any side effects may be ethical.

Although this paper does not primarily discuss the estimators, some general comments may be done. Within both approaches there are several possible estimators. In applied research the underlying data generating process is never or seldom known and distributional assumptions may be questioned. The Food and Drug Administration [1] states that distributional assumptions should be done with restraint, which motivates the consideration of non-parametric estimators in this paper. There may be drawbacks with these simple estimators, e.g. relatively high and not monotonically decreasing variance and bias. In this paper the only assumption made for the non-parametric estimators was continuous data. When making more assumptions about the data, there are of course many possibilities to perform better. One possibility to make improvements is to introduce restrictions about the shape of the data, e.g. strictly increasing or decreasing functions. Such minor restrictions open a wide field of more efficient non-parametric methods. Also under distributional assumptions the estimates may be improved by using the longitudinal structure of the data. Cross sectional analysis is still dominating in pharmacokinetics, and an important task is to introduce longitudinal models.

Acknowledgment

I wish to thank my supervisor Ph. D. Robert Jonsson for suggesting the problem and for his devoted guidance throughout this work.

Appendix: The simulation model

The following model was used in the simulations when estimating the studied tolerance limits:

$$X_i = A_i + B_i \cdot t + U_i \qquad i = 1, 2, ..., n \tag{5}$$

where

- X_i the logarithm of the drug concentration for person i.
- A_i level factor specific to person i (metabolism, weight, sex, age).
- B_i change factor specific to person i (metabolism, renal clearance).
- t the time value
- U_i error term person i.

Letting A_i and B_i have the normal distribution with the parameter settings from Table 5, $cov\,(A_i, B_i) = 0.01$ and $t = 1$, then X_i will have the normal distribution with $\mu = 9$ and $\sigma^2 = 1.03$. The software SAS(r) 6.12 IML was used for the simulations with 200.000 replicates at each sample size giving a stable outcome. Further, the computer clock was used as random seed, and for the conservative approach the confidence level γ was set to 95%.

The original concentration scale $\exp(X_i)$ will have the lognormal distribution which is a common assumption for drug concentrations. The multiplicative model $\exp(X_i)$ has been used in pharmacokinetics when estimating *area under curve*, see Jonsson [4], Mandallaz&Mau [6] and Vuorinen&Turunen [15].

Table 5: Parameter settings for the simulations.

	Mean	Variance
A_i	10	0.5
B_i	-1	0.01
U_i	0	0.5

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Estimating in non-linear monotone response curves using random regression techniques

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Abstract

Conventional estimation methods in pharmacokinetics for estimating tolerance limits make use of cross sectional data. However, utilising the longitudinal structure of the concentration data the precision may be improved considerably, especially in drug testing situations where the number of subjects seldom exceeds 20. To this end a flexible model with random intercept and fixed slope is introduced. Here, the time t is replaced by a function t^p where p is an unknown parameter. A two-step estimation approach is proposed where p is estimated in the first step, and the other parameters are estimated in the second step by using standard regression techniques. The effects of first estimating p upon the rest of the estimators are discussed and results from simulation studies are given. It is concluded that bias and precision of the estimators may remain quite unaffected by the two-step procedure. However, the precision of the slope and intercept estimators can be poor for small values of p, provided that the variance of the measurement errors is large.

1. Introduction and notations

A wide class of time-response data can be analysed by using the general model

$$F_{ij} = \exp\left\{A_j + l(B_j^{(1)}, B_j^{(2)}, ..., t_i^p) + U_{ij}\right\},\ i = 1...T,\ j = 1...n\,.$$

Here F_{ij} is the measured response at the time t_i for the j:th subject, which is composed of three random components. The random level A_j reflects factors which are specific for the j:th subject, such as body mass, and is supposed to not change with time during the interval $[t_1, t_T]$. $B_j^{(1)}, B_j^{(2)}, ...$ are random factors which express the change over time and $l(\,)$ is a linear function of $B_j^{(1)}, B_j^{(2)}, ...$ and also of functions of t_i^p, where p is an unknown parameter. U_{ij} is a residual which summarises all the effects which have not been included in the model. The multiplicative structure of (1) is motivated by the fact that in many cases the variations between subjects seem to be more apparent at times when the responses are larger. A simple special case of the model above is

$$F_{ij} = \exp\left\{A_j + \beta t_i^p + U_{ij}\right\}, \tag{1}$$

The latter contains only one factor which changes over time, and furthermore is constant for all subjects. The expression in (1) is intended for monotone responses in time, which can be found e.g. when studying the increase of a concentration in body, or the decreasing concentration of a drug at absorption site[1].

Figure 1a below shows examples of typical, decreasing realisations of (1) for some values of p, and Figure 1b shows examples of real data having a structure which agrees very well with the model in (1). Notice especially the large variations between subjects and the multiplicative structure of the data. The variance of the higher values at the start is about 4 times larger than the variance of the smaller values at the end.

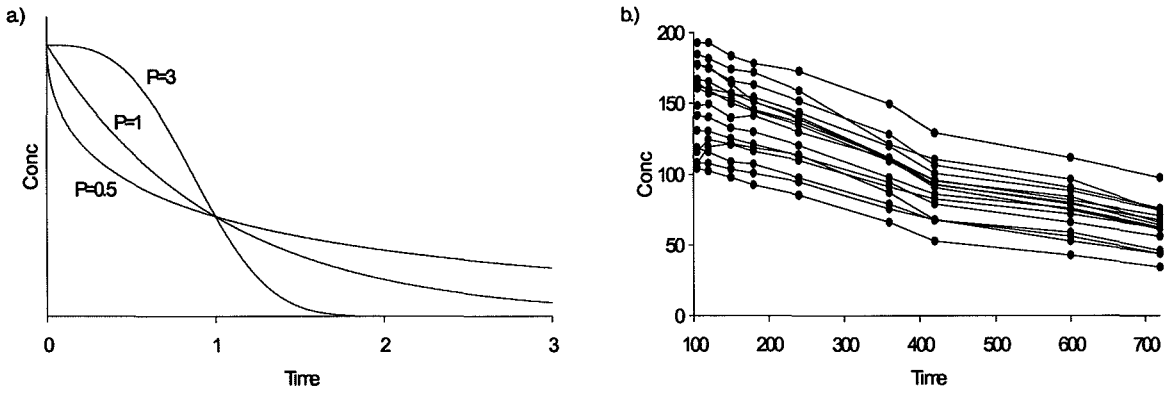

Figure 1. a) Examples of decreasing curves intended to illustrate typical realisations of (1) disregarding the random variations. b) Measurements of the concentration of a drug in the plasma from 16 humans at 9 time points. Here p was estimated as 0.92 by using methods to be considered below.

By taking the logarithm of (1) and putting $x_i = t_i^p$, the model can be written

$$Y_{ij} = A_j + \beta x_i + U_{ij} \tag{2}$$

which has the structure of a simple random effect model in which only the intercepts are variable (C.f. Ch.3 in Hsiao[2]).

Models like (1) can be used as the basis for estimating population characteristics such as the tolerance limits, in situations where series of measurements are obtained from a relatively small number of subjects. Conventional methods for estimating the tolerance limits of the response in the population only make use of the cross sectional data, and do not utilise the longitudinal structure (C.f. Nickens[3] with references). In drug testing situations where the number of subjects seldom exceeds 20, it is to be expected that much can be gained in precision if the longitudinal structure of the data is used. Estimators from longitudinal data for the tolerance limits based on the model in (1), have been presented earlier[4]. When p is allowed to vary, the model becomes more flexible. But on the other hand the estimation of p can be expected to have an influence on the precision of the rest of the estimated parameters of the model.

It is the purpose of this paper to study whether the parameters of the model in (1) can be estimated by first estimating p, and then utilising known results from the regression theory for estimating the rest of the parameters. The study is mainly based on simulations and results are presented where p ranges from 0.5 to 3. Results for $p = 3$ will also hold for values of p larger than 3. For $0 < p < 0.5$ the responses in (1) will tend very slowly to zero as t tends to infinity, and with negative values of p the responses will never reach the zero level. The latter two cases will be of less practical interest and are therefore omitted.

2. Assumptions and some results from the regression theory

In (2) it will be assumed that the A_j's are independent and identically distributed (iid) with mean and variance α and σ_A^2, respectively. The U_{ij}'s are iid with mean 0 and variance σ_U^2 and furthermore, the U_{ij}'s are independent of A_j, $j = 1...n$, $i = 1...T$. When normal distributions are assumed for A_j and U_{ij} it follows that the vector $\mathbf{y}_j = (Y_{1j}...Y_{Tj})'$ has a T-dimensional normal distribution with mean vector $((\alpha + \beta x_1)...(\alpha + \beta x_T))'$ and dispersion matrix

$$\begin{pmatrix} (\sigma_A^2 + \sigma_U^2) & \cdots & \sigma_U^2 \\ \vdots & \ddots & \vdots \\ \sigma_U^2 & \cdots & (\sigma_A^2 + \sigma_U^2) \end{pmatrix}$$

Since each Y_{ij} is normally distributed, it follows that the measured response F_{ij} has a lognormal distribution with mean (C.f. Johnson and Kotz[5])

$$\exp(\alpha + \beta x_i + (\sigma_A^2 + \sigma_U^2)/2), \text{ with } x_i = t_i^p \tag{3}$$

The latter function may be called the mean curve and shows how the mean response changes with time. The lower and upper 95% tolerance limits of the response at time t_i (i.e. the limits within which 95% of the responses are found) are easily seen to be

$$\exp\left\{\alpha+\beta x_i \pm 1.96\sqrt{\sigma_A^2+\sigma_U^2}\right\}, \text{ with } x_i = t_i^p \tag{4}$$

When the primary interest is to study how the bunch of time-response curves behave in the population, i.e. without taking the measurement errors into consideration, σ_U^2 in the last two expressions are put equal to zero.

By estimating the parameters α, β, σ_A^2 and σ_U^2 it is possible to estimate the mean curve and the tolerance interval (see Jonsson[4], for details). When p is known the optimal estimators are obtained in the following way[2]:

Let $\hat{\alpha}_j$ and $\hat{\beta}_j$ be the ordinary least squares estimators of α and β which are obtained by only using the data from the j:th subject. Then

$$\hat{\alpha}=\frac{1}{n}\sum_{j=1}^{n}\hat{\alpha}_j, \quad \hat{\beta}=\frac{1}{n}\sum_{j=1}^{n}\hat{\beta}_j . \tag{5}$$

$$\text{Put: } \bar{x} = \sum_{i=1}^{T} x_i/T, \ \bar{Y}_j = \sum_{i=1}^{T} Y_{ij}/T, \ \bar{Y} = \sum_{j=1}^{n}\bar{Y}_j/n, \ W_{YY} = \sum_{j=1}^{n}\sum_{i=1}^{T}(Y_{ij}-\bar{Y}_j)^2$$

$$W_{xY} = \sum_{j=1}^{n}\sum_{i=1}^{T}(x_i-\bar{x})(Y_{ij}-\bar{Y}_j), \quad B = \sum_{j=1}^{n}(\bar{Y}_j-\bar{Y})^2 .$$

Then

$$\hat{\sigma}_A^2 = \frac{B}{n-1}-\frac{\hat{\sigma}^2}{T}, \quad \hat{\sigma}_U^2 = \frac{W_{YY}-\hat{\beta}W_{xY}}{n(T-1)-1} . \tag{6}$$

The simulated values for the vector $\mathbf{y}_j$ were obtained by using the normality assumption with the following parameter values: $\alpha=1$, $\beta=-1$, $\sigma_A^2=0.05$ and $\sigma_U^2=0.1$. The values of the variance components were chosen by practical experience, even though the value 0.1 for the error variance is very large. The number of subjects (n) was 8 and 16, while the number of time points (T) were 5 and 10, equally spaced on the interval [1,10]. Each simulation was based on 10^4 replicates with the computer clock as random seed, and no step in order to supervise the simulated responses was taken. In this way some simulated data sets may contain realisations with very heavy fluctuations, which are far from realistic.

3. Estimation of p

The parameter p may be estimated in different ways. A Maximum Likelihood (ML) estimate of p might be found by trying to maximise the Likelihood directly, but this approach would not be suitable for a large-scale simulation study where the aim is to study the properties of the estimator. In the latter case it is preferable to work with closed expressions for the estimation equations which are as simple as possible. The Likelihood of the observations in (2) can be written (C.f. Ch 3 in Hsiao[2])

$$\left(\sigma_A^2 T+\sigma_U^2\right)^{-\frac{n}{2}} \sigma_U^{-n(T-1)} \cdot \exp\left\{\frac{-T\sum(\bar{Y}_j-\alpha-\beta\bar{x})^2}{2(\sigma_A^2 T+\sigma_U^2)}-\frac{(W_{YY}-2\beta W_{xY}+\beta^2 W_{xx})}{2\sigma_U^2}\right\}$$

where $W_{xx}=n\sum_{i=1}^{T}(x_i-\bar{x})^2$. (7)

From this it follows that the ML estimator of p is obtained by solving the equation

$$\frac{\delta}{\delta p}\left(2\beta W_{xY}-\beta^2 W_{xx}\right)=0.$$

With $x_i=t_i^p$ it is easily shown that the last expression can be written

$$\Delta(p)_{ML}=\frac{\sum_j\sum_i Y_{ij}x_i-nT\cdot\bar{Y}\cdot\bar{x}}{\sum_i x_i^2-T(\bar{x})^2}-\frac{\sum_j\sum_i Y_{ij}x_i\ln t_i-n\bar{Y}\sum_i x_i\ln t_i}{\sum_i x_i^2\ln t_i-\bar{x}\sum_i x_i\ln t_i}=0 \quad (8)$$

where $\bar{x}=\sum_i x_i/T$ and, as before $x_i=t_i^p$.

The typical behaviour of the function $\Delta(p)_{ML}$ is depicted in Figure 2a for the same data set which was depicted in Figure 1b. As can be seen there is a clear-cut local minimum at 0.93, but it is also seen that the function decreases below the latter minimum for values of p larger than 1.65. Thus, without prior information about the possible range of values for p, the ML approach may be hazardous.

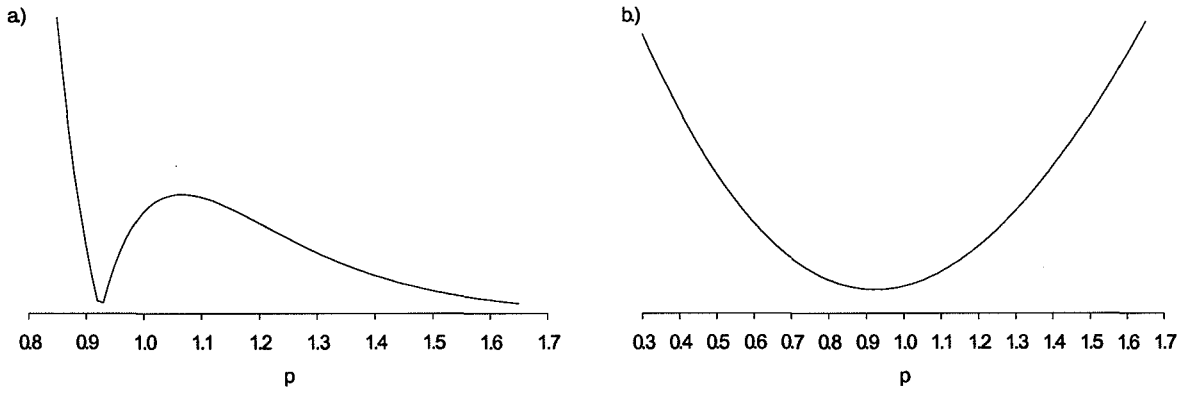

Figure 2. Typical behaviour of the functions (a) $\Delta(p)_{ML}$ in (8) and (b) $D(p)$ in (9).

Since it is possible to fit a regression plane to the values from each subject and for each value of the parameter p in the relation $x_i = t_i^p$ an alternative way of estimating p can be obtained. Let $\hat{\beta}_j(p)$ be the ordinary least squares estimator of β which is obtained for a given value of p by only fitting the model to the data from the j:th subject, $j = 1,...,n$. Each such fit also gives rise to a residual sum of squares, say $SSE_j(p)$. The simple idea is now to choose as an estimate of p, the value that minimises

$$D(p) = \frac{1}{n}\sum_{j=1}^{n}\frac{SSE_j(p)}{\sum_{i=0}^{T}(Y_{ij} - \bar{Y}_j)^2} \tag{9}$$

This estimate chooses the value of p which, on an average, gives the closest fit to the data in terms of the coefficients of determination. The behaviour of $D(p)$ is depicted in Figure 2b for the same data set which was used for the ML estimation function $\Delta(p)_{ML}$. It is seen that $D(p)$ has a U-shaped structure with an easily recognised absolute minimum value at 0.92.

The properties of the ML-estimator obtained from (8) and the properties of the estimator obtained from (9) are very similar. The distributions of the estimators are depicted in Figure 3 below. The estimates were computed from simulations with $p = 0.50$, $n = 16$ and $T = 10$. It is seen that the distributions are very similar and resembles that of the normal distribution. One difference between the two estimation procedures is that the equation (8) sometimes resulted

in abnormal solutions when p was allowed to vary within an interval, which was too large. This was never the case with the $D(p)$-estimator obtained from (9).

Table 1 below illustrates the differences between the two types of estimators when estimating p, as well as the rest of the parameters. Here $n = 8$ and $T = 5$ were used, i.e. the smallest n and T considered in this study. It is seen that the differences between the two types of estimators are negligible. For the other combinations of n and T the differences were even smaller.

Due to the similarities between the two types of estimators and since the $D(p)$-estimator is much easier to handle in simulation studies, the latter will be considered in the sequel. A grid search procedure was used to minimise (9) in the simulations.

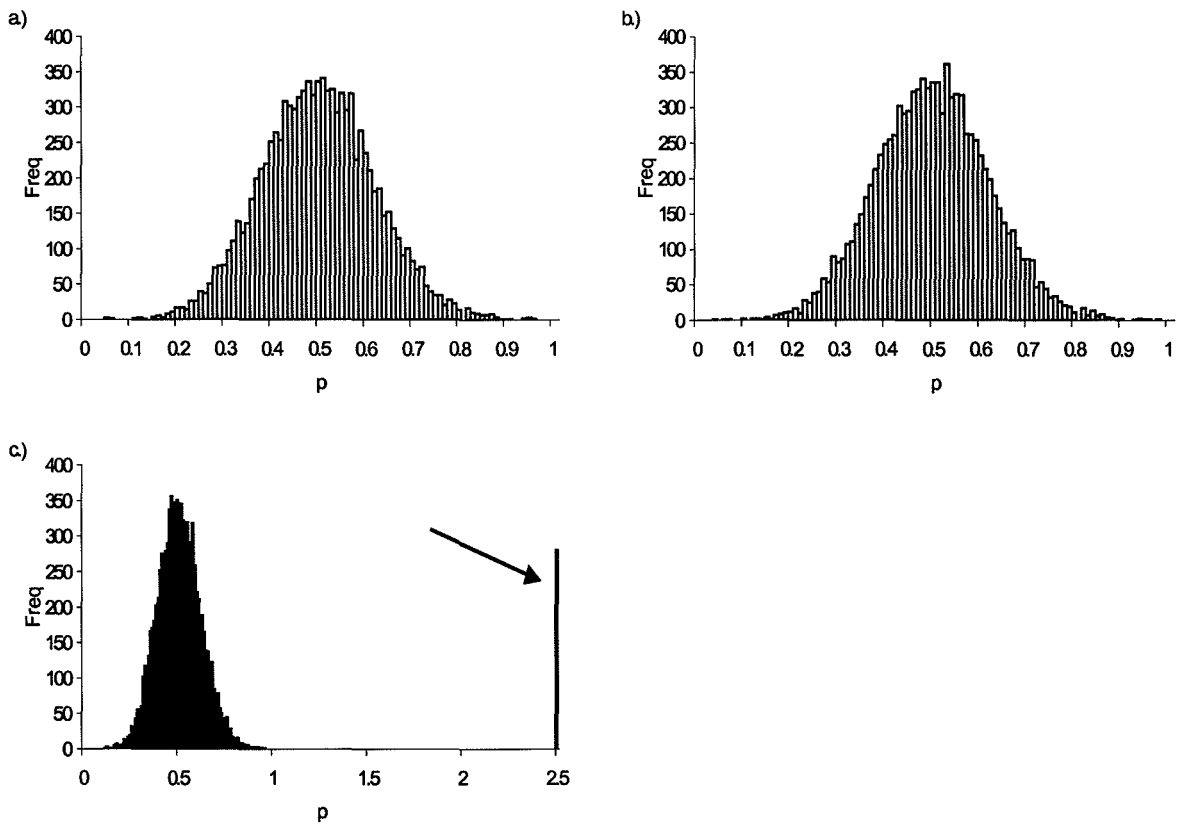

Figure 3. Distribution of the p-estimators when the true value is $p = 0.50$, $n = 16$ and $T = 10$. (a): Determined from $D(p)$ in (9). (b), (c): Determined from $\Delta(p)_{ML}$ in (8) with p varying between .01 and 1.00 and .01 and 2.5, respectively. Notice the abnormal solutions in (c).

	Relative differences (%) between the ML- and D(p) estimators									
True value of p	$\hat{p}$	$V(\hat{p})$	$\hat{\hat{\alpha}}$	$V(\hat{\hat{\alpha}})$	$\hat{\hat{\beta}}$	$V(\hat{\hat{\beta}})$	$\hat{\hat{\sigma}}_A^2$	$V(\hat{\hat{\sigma}}_A^2)$	$\hat{\hat{\sigma}}_U^2$	$V(\hat{\hat{\sigma}}_U^2)$
0.7	0.03	1.12	0.14	0.89	0.13	1.09	0.01	0.00	0.03	0.11
1.0	0.01	0.07	0.03	0.19	0.02	0.27	0.00	0.00	0.01	0.02
1.5	0.01	0.11	0.05	0.35	0.02	0.21	0.00	0.00	0.00	0.00
2.0	0.00	0.00	0.08	0.05	0.02	0.06	0.00	0.00	0.00	0.00
2.5	0.00	0.42	0.10	0.05	0.01	0.52	0.00	0.00	0.00	0.00
3.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 1. Relative differences between the ML estimator given by (8) and the D(p) estimator given by (9), expressed as the absolute value of $100 \cdot \left(\left(D(p) - ML \right) / ML \right)$ for $n = 8$, T=5. For these n and T the minimum p will be 0.7.

For values of p less than 0.5 and with $\sigma_U^2 = 0.1$, estimates of p are very unreliable for small n and T. This is essentially due to the fact that the variation of the $X_i's$ then becomes very small, as will be shown below (C.f. (11)). Values of p in the latter range are also of less practical interest as far as the model in (1) is concerned, since the concentration curves remain above the zero level for an extremely long time (C.f. Figure 1). For simplicity, only values of p from 0.5, and sometimes from 0.7, and larger will be considered here.

The iterative process of obtaining the solution of the p-estimator from (9) involves some problems, which are inevitable and are therefore worth some comments. In the next section it is shown that the p-estimator has an asymptotic normal distribution (as $n \to \infty$) with mean p and variance given by the expression in (11). The normality assumption turned out to hold even for $n = 8$. As a first step in estimating p it is recommended to first get a rough estimate of p and of the variance. From the latter one can construct an interval, $\hat{p} \pm constant \cdot \sqrt{V(\hat{p})}$, which can be supposed to cover nearly 100% of all possible p-estimates. This interval may serve as an iteration interval for the grid search. The estimate of p depends on the division of the iteration interval. A too rough division may cause a misleading too small variance. With a finer division of the iteration interval the variance first increases and then starts to decrease towards the true value.

The bias of p can be disregarded. The largest absolute bias, about 0.0026, was obtained for $p = 0.7$ which corresponded to a relative bias of 0.37%. For values of p larger than 0.7 the bias readily declined to zero (C.f. Table 2 below).

Figure 4 below illustrates how the variance of the estimator of p readily tends to zero with increasing values of p. At $p = 1.5$ the variance is about 10^{-4}, and at $p = 2.0$ it is 10^{-5} which can be seen in Table 2.

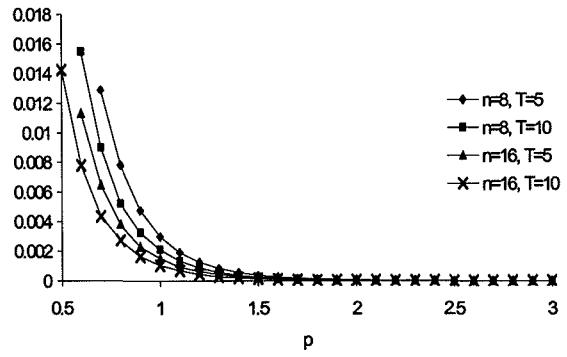

Figure 4. Variance of the $D(p)$-estimator for some values of p and the 4 possible combinations of n and T.

True value of p	$V(\hat{p})$
2.0	0.000053
2.1	0.000036
2.2	0.000024
2.3	0.000016
2.4	0.000011
2.5	0.000008
2.6	0.000005
2.7	0.000004
2.8	0.000002
2.9	0.000002
3.0	0.000001

Table 2. Variance of the $D(p)$-estimator for the larger values of p when $n = 8$ and $T = 5$.

4. Estimation of further parameters

The estimators in Sect. 2 are optimal only if p is known. When p is estimated, one may suspect that this may have effects on bias and precision of the rest of the parameters. It is the purpose of this section to study these effects on the estimates of α, β, σ_A^2, σ_U^2 and $\alpha + \beta x_i$ when the true value of p is 0.7 or larger.

Table 3 below shows the relative bias of the estimators of the latter parameters. With a few exceptions, the relative bias can be omitted. For larger values of p not shown in the table and for the other combinations of n, T and i, the relative bias is even smaller.

	Relative bias (%) when estimating:				
True value of p	α	β	σ_A^2	σ_U^2	$\alpha+\beta x_1$
0.7	-6.15	-6.16	-0.82	3.46	-0.01
1.0	-0.72	-0.84	-0.72	3.00	-0.12
1.5	-0.28	-0.15	-0.16	3.54	0.13
2.0	0.06	-0.01	-0.67	3.17	-0.07
2.5	0.12	0.00	-1.42	2.96	-0.12
3.0	0.11	0.00	-1.24	3.14	-0.11

Table 3. Relative bias (%) for the estimators of the parameters of the model in (1) when $n=8$, $T=5$ and $i=1$.

The asymptotic variances and covariances (n large) of the ML-estimators can be obtained from the expectations of the second order derivatives of the log-likelihood in (7)[2]. When p is fixed, the following expressions are obtained:

$$V(\hat{\alpha})=\frac{1}{n}\left[\sigma_A^2+\sigma_U^2\left(\frac{1}{T}+\frac{\overline{x}^2}{W_{tt}}\right)\right],\quad V(\hat{\beta})=\frac{\sigma_U^2}{nW_{tt}},\quad Cov(\hat{\alpha},\hat{\beta})=-\frac{\sigma_U^2\overline{x}}{nW_{tt}},$$

$$V(\hat{\sigma}_A^2)=\frac{2}{n(T-1)T^2}\left[(T-1)\left(\sigma_A^2T+\sigma_U^2\right)^2+\sigma_U^4\right],\quad V(\hat{\sigma}_U^2)=\frac{2\sigma_U^4}{n(T-1)}, \tag{10}$$

$$V(\hat{\alpha}+\hat{\beta}x_i)=\frac{1}{n}\left[\sigma_A^2+\frac{\sigma_U^2}{T}\right]+\frac{\sigma_U^2}{nW_{tt}}(x_i-\overline{x})^2$$

while all covariances between $(\hat{\alpha},\hat{\beta})$ and $(\hat{\sigma}_A^2,\hat{\sigma}_U^2)$ are zero.

On the other hand, when p in $x_i=t_i^p$ is estimated, the following expressions are obtained:

$$\text{Put: } M=\frac{1}{T}\sum_{i=1}^{T}x_i\ln t_i,\quad S_1=\sum_{i=1}^{T}x_i^2\ln t_i-T\cdot M\cdot\overline{x},\quad S_2=\sum_{i=1}^{T}(x_i\ln t_i)^2-T\cdot M^2$$

and let $\hat{\alpha}$, $\hat{\beta}$, $\hat{\sigma}_A^2$, $\hat{\sigma}_U^2$, $\hat{p}$ be the ML-estimators. Then, with $R_\beta = 1 - \frac{S_1^2}{W_{tt} S_2}$,

$$\left.\begin{aligned} V(\hat{\alpha}) &= \frac{1}{n}\left[\sigma_A^2 + \sigma_U^2\left\{\frac{1}{T} + \left(\frac{\bar{x}^2}{W_{tt}} + \frac{M}{S_2}\left(M - \frac{2\bar{x}S_1}{W_{tt}}\right)\right)\frac{1}{R_\beta}\right\}\right], \\ V(\hat{\beta}) &= \frac{\sigma_U^2}{nW_{tt}} \cdot \frac{1}{R_\beta} = V(\hat{\beta}) \cdot \frac{1}{R_\beta}, \\ V(\hat{p}) &= \frac{\sigma_U^2}{n\beta^2 S_2} \cdot \frac{1}{R_\beta} \end{aligned}\right\} \qquad (11)$$

and

$$\left.\begin{aligned} Cov(\hat{\alpha}, \hat{\beta}) &= \frac{\sigma_U^2}{nW_{tt}S_2} \cdot \frac{(MS_1 - \bar{x}S_2)}{R_\beta}, \\ Cov(\hat{\alpha}, \hat{p}) &= \frac{\sigma_U^2}{nW_{tt}S_2} \cdot \frac{(\bar{x}S_1 - MW_{tt})}{\beta R_\beta}, \\ Cov(\hat{\beta}, \hat{p}) &= -\frac{\sigma_U^2}{nW_{tt}S_2} \cdot \frac{S_1}{\beta R_\beta} \end{aligned}\right\} \qquad (12)$$

The variances of $\hat{\sigma}_A^2$ and $\hat{\sigma}_U^2$ are as in (10), while all covariances between $(\hat{\alpha}, \hat{\beta}, \hat{p})$ and $(\hat{\sigma}_A^2, \hat{\sigma}_U^2)$ are zero.

To study the effect of first estimating p and then estimating the rest of the parameters, it is natural to consider the ratio

$$R = \frac{\text{Variance of parameter when } p \text{ is known}}{\text{Variance of parameter when } p \text{ is estimated}} \qquad (13)$$

When estimating σ_A^2 and σ_U^2, the ratio in (13) was very close to 1 as expected. The smallest and largest values of R, for all possible combinations of n, T and p, were 0.96 and 1.06. However, when estimating α and β the value of R was below 1. From Figure 5a it can be

seen that R is slowly increasing for larger p when estimating α. When estimating β the value of R is about 10^{-2} for all values of p. The very poor variance of the β-estimator is quite remarkable. From (11) it can be seen that the variance of the β-estimator is proportional to the variance of $\hat{p}$, and from Table 2 the latter was smaller than about 10^{-5} for p larger than 2.0. However, this relatively small variance is not small enough to improve the variance of the β-estimator. Notice that the expressions for the mean curve and the tolerance limits in (3) and (4) do not involve α and β, but $\alpha+\beta x_i$. From Figure 5b it is seen that the estimator of the latter quantity behaves much better than when α and β are estimated separately.

The above results were obtained by using the relatively large value 0.1 of σ_U^2. For smaller values of σ_U^2, say smaller than 0.01, the ratio R will be closer to 1 when estimating α, β and $\alpha+\beta x_i$. In fact, simulations by using the parameter values, which were estimated from the data set depicted in Figure 1b (where σ_U^2 was estimated as 0.0034), suggest that the two-step procedure described in this paper may be used even when the number of subjects (n) is 2.

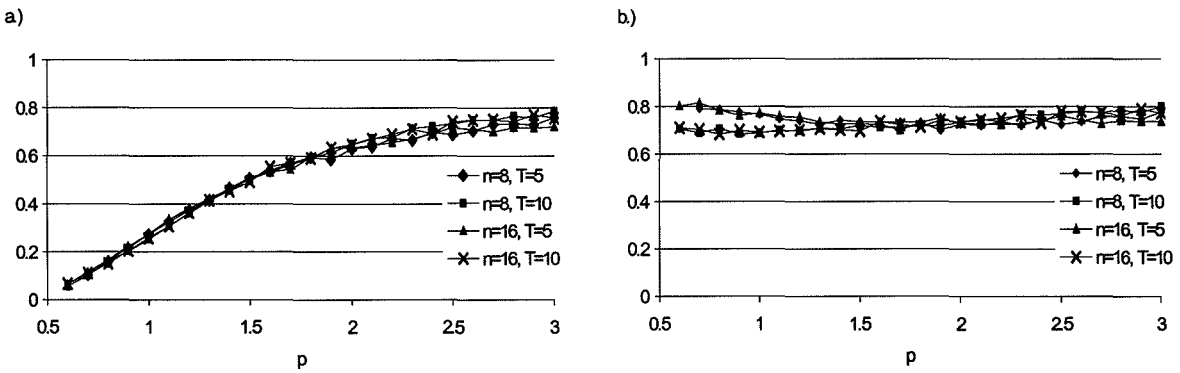

Figure 5. Values of the ratio R in (13) when estimating (a) α, (b) $\alpha+\beta x_1$ where $x_1 = 1^p$.

5. Conclusions

There is a great need for statistical methods that utilise the longitudinal structure of the data. For instance, in pharmacokinetics one often uses a relatively small number of patients from which data can be collected at many time points. In such situations it seems natural to try to use all the information contained in the sample when constructing e.g. tolerance limits, rather than using conventional methods, which only make use of cross sections over the subjects.

Objections may be raised against the use of structural models for longitudinal data, like the one in (1). But if they are flexible enough to fit well to the data there should be no reasons for not using them. This paper has presented a model for monotone time-response data, containing a parameter p, which has to be estimated as a first step. The rest of the parameters can then be estimated by standard regression techniques in a second step. Whether this works or not, depends essentially on the magnitude of p and of the variance of the measurement errors. The two-step procedure works well for small values of the latter variance. But for large values of the variance simulation studies have shown that the procedure only works well when estimating $\alpha + \beta x_i$, i.e. the quantity of main interest in some situations.

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