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Application of regression transformations to the determination of reaction orders in stability studies

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Abstract

Box–Cox and link function transformations can assist in the determination of reaction orders in pharmaceutical studies. © 1998 Elsevier Science B.V.

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1. Introduction

Rate processes are of fundamental concern to everyone working with pharmaceuticals. They are important in stability and incompatibility studies, in dissolution studies and in absorption, distribution and elimination processes.

The overall order of a reaction is the sum of the exponents of the concentration terms that afford a linear plot. The order with respect to each reactant is the exponent of the individual concentration term. In general, when one of the reactants of a given reaction is present in such great excess that its concentration may be considered constant or nearly constant, the reaction will be of overall pseudo-order. Once the reaction order is known, one can calculate the reaction rate constant, for example, to determine how fast a drug decomposes.

Reactions can be classified into zero, first and second order, and the order can be determined by a substitution method, graphical method or half-life method (Martin et al., 1970). In the case of the graphical method, the zero order reaction will plot as a straight line of drug concentration versus time. In a first order reaction the straight line is obtained by plotting the logarithm of the drug concentration versus time, and in a second order

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reaction it is obtained by plotting the inverse of the drug concentration versus time.

The Box–Cox transform is a statistical technique to determine a transformation for the drug concentration so that, after the transformation, the relationship between the transformed drug concentration is linear with respect to time within normal errors (Aitkin et al., 1989). Below we compare this technique with the more traditional ones used in pharmaceutical studies. The Box–Cox transform changes the error structure of the model. An alternative approach is based on the transform of the link function. We compare this approach to the Box–Cox transform models.

2. Experimental

2.1. Kinetic studies

Kinetic studies were performed with salbutamol sulphate solutions prepared in buffer solutions. The effect of drug concentration was studied by placing the samples in a preheated oven for varying periods of time. At appropriate times, two to six samples were removed from the oven and the salbutamol concentration of each sample was determined by high performance liquid chromatography (HPLC). The results have been published elsewhere (Mälkki and Tammilehto, 1990). The data are represented in Appendix A.

Fig. 1 shows the salbutamol concentration as a function of heating time. We have also plotted a smoothed line. The figure should be read from left to right and bottom to top. At the lowest concentration level the relationship is almost linear, while at the other levels the relationship between time and concentration is nonlinear.

3. The Box–Cox transformation technique

The standard linear regression problem for the above data is the following. Model the measurements with the equation

$$y_{ij} = \mu + \alpha_j + \beta t + \epsilon_{ij}, \quad (1)$$

where y_{ij} is the i^{th} measured concentration at the drug concentration level j, $j = 1, 2, 3, 4$ with $i = 1,\ldots, 16$ for the levels $j = 1, 3, 4$ and $i = 1,\ldots, 21$ for the level $j = 2$, and t is the time; here the measurement errors ϵ_{ij} are assumed to be independent and normally distributed with mean equal to 0 and the unknown variance equal to σ^2.

The Box–Cox transformation technique for the model in Eq. (1) is the following. Transform the response variables y_{ij} with a transformation $g_\lambda(y)$, where $g_\lambda(y) = \dfrac{y^\lambda - 1}{\lambda}$, for $\lambda \neq 0$ and $g_\lambda(y) = \log(y)$ for $\lambda = 0$. With this definition of $g_\lambda(y)$ we get that $g_\lambda(y)$ as a function of λ is continuous at the point $\lambda = 0$. This property is needed to derive some of the statistical properties explained below. Note that the value $\lambda = -1$ corresponds to the inverse transformation of y, the value $\lambda = 0$ corresponds to the logarithmic transformation of y and the value $\lambda = 1$ corresponds to the model in Eq. (1), i.e. no transformation for the response variable y. The transformation is found by maximizing the profile likelihood in λ: We fix a λ, compute the parameter estimates μ, α_j, β and σ^2 for the model in Eq. (1) with y_{ij}^λ replacing y_{ij}, and use the obtained estimates to compute the value of the log-likelihood for the transformed model. Since the parameter estimates depend on the transformation parameter λ, the profile likelihood is a function of λ. The maximum is sought over a dense grid, and using the asymptotic likelihood theory, one can compute an asymptotic confidence interval for the maximum (see Aitkin et al., 1989, Section 3.1., for details).

The model in Eq. (1) cannot, however, be applied for all concentration levels simultaneously, and below we determine the reaction order separately for each of the four levels.

4. Link function transformations

The model in Eq. (1) implies that

$$\mu_{ij} \doteq Ey_{ij} = \mu + \alpha_j + \beta t$$

where Ey_{ij} is the mean of the random variable y_{ij}. The link function transformation assumes that the expectation of the random variable is some non-

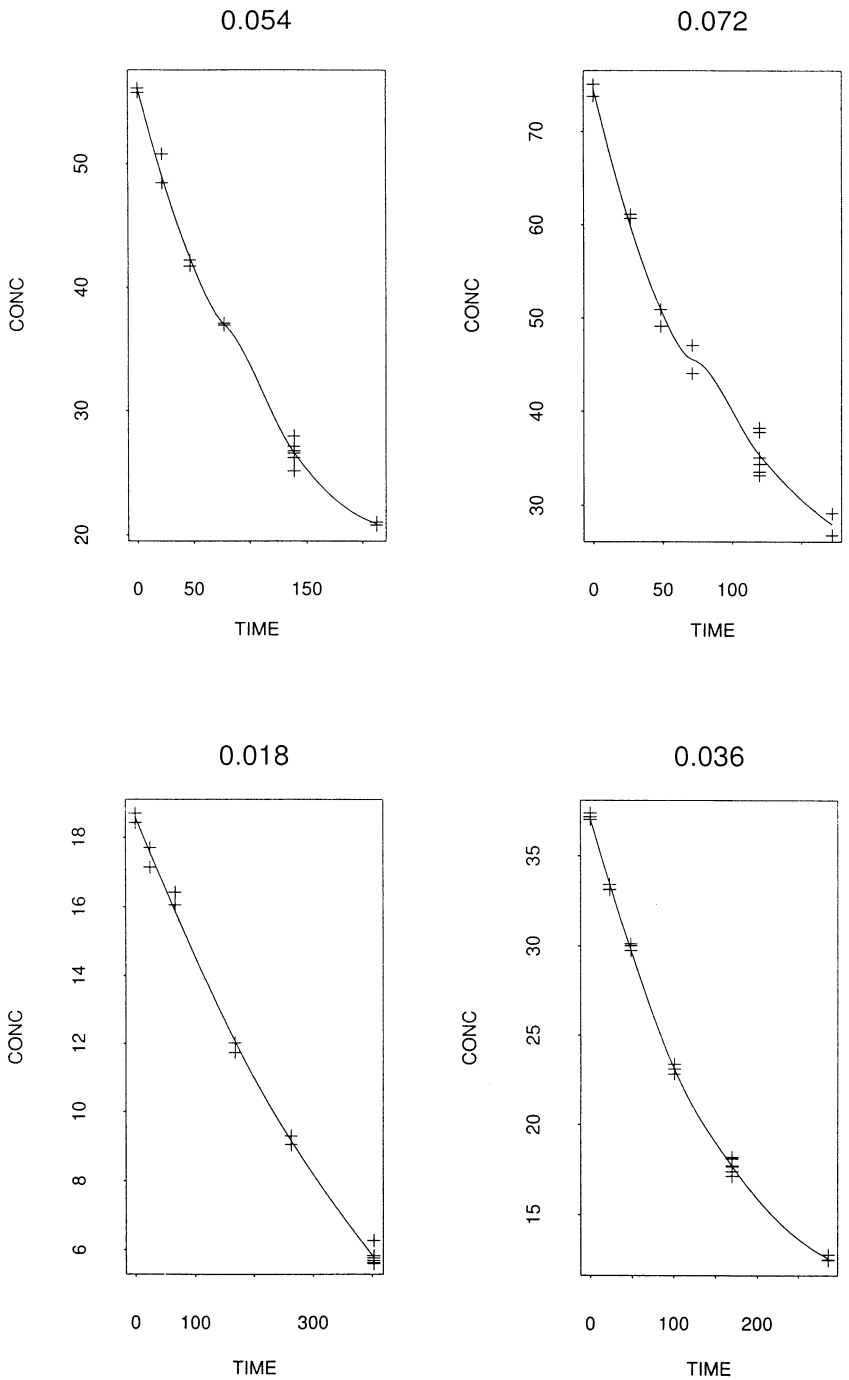

Fig. 1. The decomposition of salbutamol at four different drug concentrations (0.018, 0.036, 0.054 and 0.072 M) at elevated temperature.

linear function of the liner predictor (in our model in Eq. (1) this predictor is $\mu + \alpha_j + \beta t$). More formally, denote the mean by μ and the linear predictor by $X\beta$. Then the link function transform model is summarized by

$$Y \sim N(g_\lambda(\mu), \sigma^2) \quad \text{and} \quad g_\lambda(\mu) = X\beta. \tag{2}$$

Here the function g_λ is as above and $N(\eta, \sigma^2)$ is the normal distribution with mean μ and variance σ^2. Note that the model in Eq. (2) does not change the variance after the transformation, but the Box–Cox transform changes the variance.

The link function transformation can be statistically compared to the Box–Cox transformation using the profile likelihood values $-2 \log \text{pl}(\lambda)$ (Aitkin et al., 1989).

5. Results and discussion

5.1. Graphical technique

In practice the graphical technique is sometimes applied as follows: compute the correlation coefficients between the transformed concentration and time, and then choose the largest correlation coefficient to determine the transformation.

Table 1 shows the correlation coefficients between the transformed concentration and time. From it we can see that the transformation is logarithmic for the lowest level of the drug concentration and inverse for the remaining three levels.

In Table 2 we give the regression information after the transformations. We then use the slope in the usual way to determine the reaction rate constant. Note that here the logarithmic transformation is a so called natural logarithm and not the base 10 logarithm. See Martin et al. (1970) for more details.

Table 1
Correlation coefficient between the transformed concentration and time

Concentration level (M)	Linear	Logarithmic	Inverse
0.018	0.9825464	−0.9945478	0.9825464
0.036	−0.9674535	−0.9927489	0.9979245
0.054	−0.9705963	−0.9903622	0.9953571
0.072	−0.9607391	−0.9837569	0.9860962

Table 2
Regression information after the transformation

Concentration level (M)	Intercept	Slope	Transformation
0.018	2.951	−0.003	Logarithmic
S.E.	0.015	0.6e^{-4}	
0.036	0.026	0.2e^{-3}	Inverse
S.E.	0.4e^{-3}	0.3e^{-5}	
0.054	0.017	0.1e^{-3}	Inverse
S.E.	0.4e^{-3}	0.3e^{-5}	
0.072	0.013	0.1e^{-3}	Inverse
S.E.	0.001	0.6e^{-5}	

5.2. Box–Cox technique

The Box–Cox transformations were computed using S-Plus (Venables and Ripley, 1994). They could also have been computed with other statistical programs such as SAS, SPSS, BMDP and Glim.

Fig. 2 shows the Box–Cox likelihood plots. The lines correspond to a 95 per cent confidence interval for the maximum value of λ.

From the information in Fig. 2 we can conclude that, for the first level, the transformation is not clear; however, the maximum is closer to the value 0, which suggests a logarithmic transformation. Note that 0 is not contained in the confidence interval. For the next level the maximum is close to the value -1, but this value again lies outside the confidence interval. For the last two levels the maximum value can be taken as -1, which indicates the inverse transformation. In reality, the order of reactions may be somewhere between first and second order due to the fact that decomposition kinetics are usually very complicated.

5.3. Link function transformations

Link function transformations for the four concentration levels were computed using logarithm and inverse functions as transformations. The computations were done by Glim.

Table 3 shows the R^2-values of the different transformation models for the four different con-

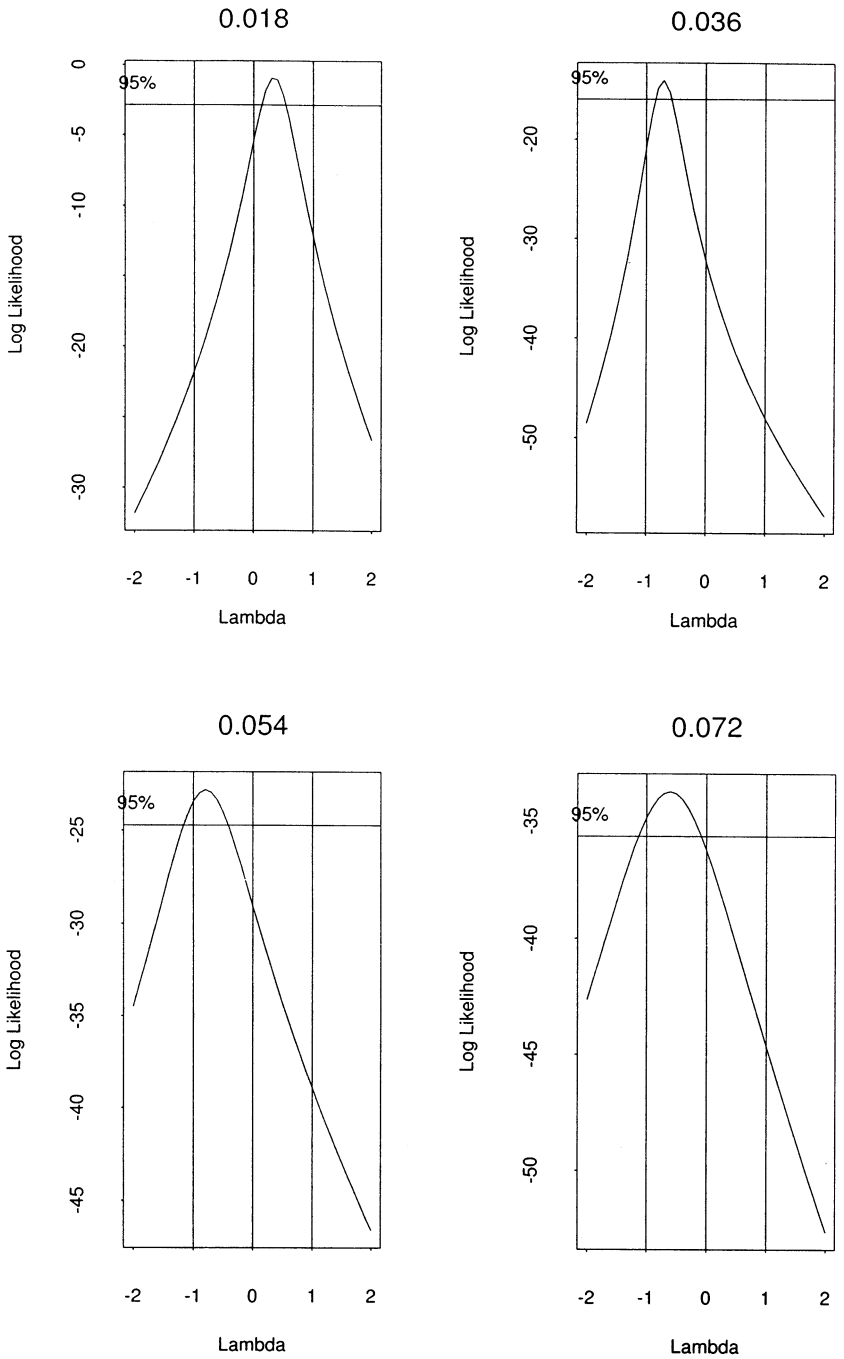

Fig. 2. Box–Cox likelihood plots for four different concentrations of salbutamol (0.018, 0.036, 0.054 and 0.072 M).

Table 3
R^2 values for different models

Concentration level (M)	Log (y)	Log (mean)	Inverse (y)	Inverse (mean)
0.018	0.995	0.995	0.965	0.967
0.036	0.986	0.992	0.996	0.994
0.054	0.981	0.988	0.991	0.992
0.072	0.968	0.973	0.972	0.988

Table 4
Profile likelihood values ($-2\text{pl}(\lambda)$) for different models

Concentration level (M)	Log (y)	Log (mean)	Inverse (y)	Inverse (mean)
0.018	10.83	11.78	43.60	42.11
0.036	64.37	52.65	41.89	45.24
0.054	58.08	52.09	46.85	46.43
0.072	72.45	72.30	69.67	59.54

centration levels. For the lowest level this Table indicates the logarithmic transform and for the three other levels inverse transform gives higher R^2-values.

Table 4 shows the profile likelihood values $-2\log \text{pl}(\lambda)$, where $\text{pl}(\lambda)$ is the value of the profile likelihood with the parameter value $\lambda \in \{-1, 0\}$ for the four different levels and the four different models. The values of $-2\log \text{pl}(\lambda)$ were computed by Glim. Both techniques suggest logarithmic transformation for the lowest concentration level and inverse transformation for the three highest levels. The Box–Cox transform gives the minimum for the two lowest levels and the link function transform gives the minimum for the two highest levels. Note that the differences between the Box–Cox transform and the corresponding link function transform are not statistically significant except for the highest concentration level.

5.4. Discussion

The Box–Cox transform and link function transform can be used as an additional instrument to determine the reaction order. These methods clearly provide useful additional information for the determination problem. However, the distributional properties of the profile likelihood are derived asymptotically, and this should be kept in mind while inspecting the confidence intervals or testing hypotheses between the different transforms.

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Appendix A. Data

Level (M)	Time (h)	Concentration (mM)	Level (M)	Time (h)	Concentration (mM)
0.018	0	18.430	0.018	0	18.708
0.018	24.42	17.714	0.018	24.42	17.142
0.018	66.47	16.055	0.018	66.47	16.424
0.018	167.72	12.027	0.018	167.72	11.743
0.018	262.72	9.297	0.018	262.72	9.052
0.018	402.78	5.591	0.018	402.78	5.759
0.018	402.78	5.686	0.018	402.78	5.626

0.018	402.78	5.828	0.018	402.78	6.270
0.036	0	37.168	0.036	0	37.378
0.036	0	37.018	0.036	23.03	33.132
0.036	23.03	33.428	0.036	23.03	33.160
0.036	48.30	30.126	0.036	48.30	29.754
0.036	48.30	30.018	0.036	100.67	23.386
0.036	100.67	22.84	0.036	100.67	23.126
0.036	170.03	17.732	0.036	170.03	18.19
0.036	170.03	18.098	0.036	170.03	17.144
0.036	170.03	17.396	0.036	170.03	17.666
0.036	286.31	12.798	0.036	286.31	12.486
0.036	286.31	12.530	0.054	0	56.138
0.054	0	55.775	0.054	21.70	48.463
0.054	21.70	50.805	0.054	46.33	41.712
0.054	46.33	42.192	0.054	76.85	36.925
0.054	76.85	37.068	0.054	138.75	27.955
0.054	138.75	27.132	0.054	138.75	26.221
0.054	138.75	26.595	0.054	138.75	26.772
0.054	138.75	25.170	0.054	212.63	21.046
0.054	212.63	20.800	0.072	0	73.784
0.072	0	75.072	0.072	26.47	61.116
0.072	26.47	60.692	0.072	48.13	49.120
0.072	48.13	50.924	0.072	71.05	44.072
0.072	71.05	47.068	0.072	119.47	33.552
0.072	119.47	35.080	0.072	119.47	33.200
0.072	119.47	34.400	0.072	119.47	37.796
0.072	119.47	38.232	0.072	172.46	26.816
0.072	172.46	29.164			

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