Extending existing structural identifiability analysis methods to mixed-effects models

David L. I. Janzén[a,b,c,*], Mars Jirstrand[b], Michael J. Chappell[c], Neil D. Evans[c]

[a] *AstraZeneca RD, Gothenburg, Sweden, Cardiovascular and Metabolic Disease Innovative Medicines Drug Metabolism and Pharmacokinetics*
[b] *Department of Systems and Data Analysis, Fraunhofer-Chalmers Centre, Chalmers Science Park, SE-412 88 Gothenburg*
[c] *School of Engineering, University of Warwick, Coventry, CV4 7AL, United Kingdom*

Abstract

The concept of structural identifiability for state-space models is expanded to cover mixed-effects state-space models. Two methods applicable for the analytical study of the structural identifiability of mixed-effects models are presented. The two methods are based on previously established techniques for non-mixed-effects models; namely the Taylor series expansion and the input-output form approach. By generating an exhaustive summary, and by assuming an infinite number of subjects, functions of random variables can be derived which in turn determine the distribution of the system's observation function(s). By considering the uniqueness of the analytical statistical moments of the derived functions of the random variables, the structural identifiability of the corresponding mixed-effects model can be determined. The two methods are applied to a set of examples of mixed-effects models to illustrate how they work in practice.

Keywords: Structural Identifiability, Mixed-Effects modelling, Taylor series expansion approach, Input-Output form approach.

1. Introduction

Structural identifiability analysis tests if the parameters in a given model structure can be uniquely determined with a given input design together with

*David L. I. Janzén
Email address: `david.janzen@astrazeneca.com` (David L. I. Janzén)

noise-free, continuous output function(s). If there exists a unique set of parameters for the output solution then the model is called *structurally globally identifiable*, if there exists a countable number of sets of parameters for an output the model is called *structurally locally identifiable* and if there exist uncountable numbers of parameter sets for a model output the model is called *structurally unidentifiable* [1, 2].

Several methods have been developed for performing structural identifiability analysis including the Taylor series approach [3], the Laplace transformation approach [4], the similarity transformation approach [5], the Exact Arithmetic Rank (EAR) approach [6], differential algebra based approaches [7], input-output approaches [8], and the profile likelihood approach [9]. These methods were originally developed to study structural identifiability in systems of ordinary differential equations with no statistical element included. Additional important publications regarding structural identifiability include [10, 11, 12, 13, 14, 15].

An area where mathematical modelling and simulation plays an important role is in drug discovery and development in the pharmaceutical industry. One of the motivations for using modelling in drug discovery and development is to detect and quantify variations in both pharmacokinetics in a population, i.e., how the drug is distributed in the body, and in pharmacodynamics, i.e., what effect the drug has on the body. This is essential for instance when finding personalised dosing regimes and optimal dosing for different subgroups in the population. It is not uncommon that the pharmacokinetic properties and the pharmacodynamic response for a particular treatment varies between different patients, or groups of patients with different covariates (sex, age, weight, etc), or even between different treatment occasions. In order to predict such future scenarios with confidence having a structurally identifiable model is central. To model this, a so called mixed-effects framework is commonly used [16]. In such a framework, all subjects in a population share the same structural model and parametrisation, but not the same parameter values. By postulating the form of the distribution of the model parameters in a statistical model, the inference problem is expanded to include variance parameters as well as the structural parameters.

However, the addition of a statistical model means that existing structural identifiability methods are not directly applicable to mixed-effects models. Although there has been some work done on the problem of structural identifiability in mixed-effects models including [17], [18], [19], [20] and [21], the main efforts of developing methods to analyse structural identifiability have so far been focused on non-mixed-effects, or fixed-effects systems. The two methods presented in this paper are related to the Laplace transform approach for mixed-effects system presented in [21] via the generation of the exhaustive summary explained below. However, the Laplace transform for mixed-effects systems presented in [21] is only applicable to linear systems whereas the two approaches presented in this paper are appblicable to nonlinear system as well.

In this paper, we first define what we mean by structural identifiability in systems of ordinary differential equations and mixed-effects systems, respectively. Then, we present two existing structural identifiability analysis methods and how they can be extended to mixed-effects models. Lastly, we apply these methods to a set of mixed-effects models to illustrate how the methods work in practice.

2. Structural identifiability

2.1. State-space model

Consider a model written in the following state-space form

$$\begin{aligned} \dot{\boldsymbol{x}}(t,\boldsymbol{\theta}) &= \boldsymbol{f}(\boldsymbol{x}(t,\boldsymbol{\theta}),\boldsymbol{u}(t),\boldsymbol{\theta}) \quad \boldsymbol{x}(t_0) = \boldsymbol{x}_0(\boldsymbol{\theta}) \\ \boldsymbol{y}(t,\boldsymbol{\theta}) &= \boldsymbol{h}(\boldsymbol{x}(t,\boldsymbol{\theta}),\boldsymbol{u}(t),\boldsymbol{\theta}) \end{aligned} \tag{1}$$

where $\boldsymbol{x}(t,\boldsymbol{\theta}) \in \mathbb{R}^n$ is the state vector, $\boldsymbol{u}(t) \in \mathbb{R}^q$ is the input vector, $\boldsymbol{\theta} \in \mathbb{R}^p$ is the vector of the model parameters, $\boldsymbol{y}(t,\boldsymbol{\theta}) \in \mathbb{R}^m$ is the output vector, t denotes time and $\boldsymbol{f}$ and $\boldsymbol{h}$ are smooth functions.

Let the generic parameter vector $\boldsymbol{\theta}$ belong to a feasible parameter space $\boldsymbol{\Theta}$, i.e., $\boldsymbol{\theta} \in \boldsymbol{\Theta}$. Let $\boldsymbol{y}(t,\boldsymbol{\theta})$ be the output function from the state-space model (1).

Further, consider a parameter vector $\bar{\boldsymbol{\theta}}$ where $\boldsymbol{y}(t, \boldsymbol{\theta}) = \boldsymbol{y}(t, \bar{\boldsymbol{\theta}})$ for all t. If this equality, in a neighbourhood $N \in \boldsymbol{\Theta}$ of $\boldsymbol{\theta}$, implies that $\boldsymbol{\theta} = \bar{\boldsymbol{\theta}}$ then the model is *structurally locally identifiable*. If $N = \boldsymbol{\Theta}$ then the model is *structurally globally identifiable*. For a structurally unidentifiable parameter, θ_i, every neighbourhood N around θ_i has a parameter vector $\bar{\boldsymbol{\theta}}$ where $\theta_i \neq \bar{\theta}_i$ that gives rise to identical input-output relations [1].

2.2. Mixed-effects state-space model

By a mixed-effects state-space model, subsequently denoted mixed-effects model, we mean a system written in the following form

$$\begin{aligned} \dot{\boldsymbol{x}}_i(t, \boldsymbol{\phi}_i) &= \boldsymbol{f}(\boldsymbol{x}_i(t, \boldsymbol{\phi}_i), \boldsymbol{u}_i(t), \boldsymbol{\phi}_i) \quad \boldsymbol{x}_i(t_0) = \boldsymbol{x}_0(\boldsymbol{\phi}_i) \\ \boldsymbol{y}_i(t, \boldsymbol{\phi}_i) &= \boldsymbol{h}(\boldsymbol{x}_i(t, \boldsymbol{\phi}_i), \boldsymbol{u}_i(t), \boldsymbol{\phi}_i) \end{aligned} \tag{2}$$

where $\boldsymbol{\phi}_i = g(\boldsymbol{\theta}, \boldsymbol{\eta}_i, \mathbf{C}_i)$ are the parameters for the i:th subject, $\boldsymbol{\eta}_i \sim N(\mathbf{0}, \boldsymbol{\Omega})$ are the random effects where N denotes a normal distribution, $\boldsymbol{\Omega}$ is the covariance matrix of the random effects $\boldsymbol{\eta}_i$, $\boldsymbol{\theta}$ is a vector of the population parameters and $\mathbf{C}_i$ are the covariates vector for the different subjects in the population.

As mixed-effects models give individual trajectories, the structural identifiability concept needs to be extended from considering the uniqueness of model parameters given a set of output signals to considering the uniqueness of model parameters given a set of distributions of the output signals, i.e., whether different parameter values may result in different or identical distributions of the same given output signal(s).

Let $p(\boldsymbol{y}_{\{\boldsymbol{\theta}, \boldsymbol{\Omega}\}}, t)$ denote the distribution of the output signals $\boldsymbol{y}$ at time t. Let the generic parameter vector and matrix $\{\boldsymbol{\theta}, \boldsymbol{\Omega}\}$ belong to a feasible parameter space $\{\boldsymbol{\theta}, \boldsymbol{\Omega}\} \subset \boldsymbol{\Theta}$, and consider the following two sets of parameters $\{\boldsymbol{\theta}, \boldsymbol{\Omega}\}$ and $\{\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\Omega}}\}$. If $p(\boldsymbol{y}_{\{\boldsymbol{\theta}, \boldsymbol{\Omega}\}}, t) = p(\boldsymbol{y}_{\{\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\Omega}}\}}, t)$ for all t implies that $\{\boldsymbol{\theta}, \boldsymbol{\Omega}\} = \{\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\Omega}}\}$ in a neighbourhood $N \subset \boldsymbol{\Theta}$ then the model is *structurally locally identifiable*, and if $N = \boldsymbol{\Theta}$ the model is *structurally globally identifiable*. For a *structurally unidentifiable* parameter, θ_i, or ω_i, every neighbourhood N around θ_i, or ω_i, has a parameter vector/matrix $\bar{\boldsymbol{\theta}}$, or $\bar{\boldsymbol{\Omega}}$, where $\theta_i \neq \bar{\theta}_i$, or $\omega_i \neq \bar{\omega}_i$, that gives rise to the same distribution of identical input-output relations.

3. Methods

In this section two structural identifiability analysis methods that were originally developed for non-mixed-effects state-space systems will be presented. It will be shown how these methods can be extended to also study structural identifiability in mixed-effects models by considering functions of random variables.

In a structural identifiability analysis the model structure itself is analysed to see whether it allows for unique parameter estimates or otherwise. In such an analysis, assumptions on having ideal experimental conditions are made. For a fixed-effects state-space model, such ideal experimental conditions include noise-free and continuous-time data. In a mixed-effects system, ideal experimental conditions also include having data from an infinite number of subjects. In some sense, this concept is similar to the parallel experiment approach presented in [11] since each subject could be viewed as a single experiment resulting in an infinite number of parallel experiments. As a consequence, the output signal(s) are continuous both in time as well as their distribution at all time points. The distribution of the output signal(s) depends on the distribution of the model parameters. Therefore, in order to study the structural identifiability of a mixed-effects model, the distributions of the model parameters must be studied analytically.

3.1. Functions of random variables

In this paper we relate the structural identifiability problem in mixed-effects systems to functions of random variables $Z_k(\boldsymbol{\theta}, \boldsymbol{\eta})$.

Let $\boldsymbol{Z}(\boldsymbol{\theta}, \boldsymbol{\eta}) = (Z_1(\boldsymbol{\theta}, \boldsymbol{\eta}), Z_2(\boldsymbol{\theta}, \boldsymbol{\eta}), \ldots)^T$ be a vector of functions of random variables. In our analysis we assume full knowledge of all of the statistical moments and covariances of $\boldsymbol{Z}(\boldsymbol{\theta}, \boldsymbol{\eta})$. We are interested in whether the statistical moments and covariance matrix of $\boldsymbol{Z}(\boldsymbol{\theta}, \boldsymbol{\eta})$ determines $\{\boldsymbol{\theta}, \boldsymbol{\Omega}\}$ uniquely, or otherwise. By calculating different orders m of the statistical moments and covariance of $\boldsymbol{Z}(\boldsymbol{\theta}, \boldsymbol{\eta})$, introducing alternative parameters $\{\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\Omega}}\}$, equating these such that

$$\mathbb{E}[\boldsymbol{Z}^m(\boldsymbol{\theta},\boldsymbol{\eta})] = \mathbb{E}[\boldsymbol{Z}^m(\bar{\boldsymbol{\theta}},\bar{\boldsymbol{\eta}})] \tag{3}$$

$$\mathrm{Cov}(\boldsymbol{Z}(\boldsymbol{\theta},\boldsymbol{\eta})) = \mathrm{Cov}(\boldsymbol{Z}(\bar{\boldsymbol{\theta}},\bar{\boldsymbol{\eta}})) \tag{4}$$

and solving for $\boldsymbol{\theta}$ and $\boldsymbol{\Omega}$ the uniqueness or otherwise of the parameters can be determined. By $\mathbb{E}[\boldsymbol{Z}^m(\boldsymbol{\theta},\boldsymbol{\eta})]$ we mean the m:th statistical moment element-wise in $\boldsymbol{Z}(\boldsymbol{\theta},\boldsymbol{\eta})$.

As an example, consider the case of two functions of random variables $\boldsymbol{Z}$. To ensure positivity both functions are lognormally distributed. The associated covariance matrix $\boldsymbol{\Omega}$ is full. We therefore have the following:

$$\boldsymbol{Z} = \begin{pmatrix} Z_1 \\ Z_2 \end{pmatrix} = \begin{pmatrix} \theta_1 e^{\eta_1} \\ \theta_2 e^{\eta_2} \end{pmatrix} \tag{5}$$

$$\boldsymbol{\eta} \sim N(\mathbf{0},\boldsymbol{\Omega}) \qquad \boldsymbol{\Omega} = \begin{pmatrix} \omega_1 & \omega_{12} \\ \omega_{12} & \omega_2 \end{pmatrix} \tag{6}$$

with unknown parameter vector $\boldsymbol{\theta} = (\theta_1, \theta_2, \omega_1, \omega_2, \omega_{12})$. The first moment for $\boldsymbol{Z}$ is

$$\mathbb{E}[\boldsymbol{Z}] = \begin{pmatrix} \theta_1 e^{\frac{\omega_1}{2}} \\ \theta_2 e^{\frac{\omega_2}{2}} \end{pmatrix}. \tag{7}$$

The covariance matrix for $\boldsymbol{Z}$ is given by

$$\mathrm{Cov}(\boldsymbol{Z}) = \mathbb{E}[\boldsymbol{Z}\boldsymbol{Z}^T] - \mathbb{E}[\boldsymbol{Z}]\mathbb{E}[\boldsymbol{Z}]^T =$$
$$= \begin{pmatrix} \mathbb{E}[Z_1^2] - \mathbb{E}[Z_1]^2 & \mathbb{E}[Z_1 Z_2] - \mathbb{E}[Z_1]\mathbb{E}[Z_2] \\ \mathbb{E}[Z_1 Z_2] - \mathbb{E}[Z_1]\mathbb{E}[Z_2] & \mathbb{E}[Z_2^2] - \mathbb{E}[Z_2]^2 \end{pmatrix}$$

where the diagonal elements, i.e., the variances of Z_1 and Z_2, are given by

$$\mathbb{E}[Z_1^2] - \mathbb{E}[Z_1]^2 = \theta_1^2 e^{2\omega_1} - \theta_1^2 e^{\omega_1} \tag{8}$$

$$\mathbb{E}[Z_2^2] - \mathbb{E}[Z_2]^2 = \theta_2^2 e^{2\omega_2} - \theta_2^2 e^{\omega_2} \tag{9}$$

and the off-diagonal element, i.e., the covariances between Z_1 and Z_2, are given by

$$\mathbb{E}[Z_1 Z_2] - \mathbb{E}[Z_1]\mathbb{E}[Z_2] = \theta_1\theta_2 e^{\frac{1}{2}(2\omega_{12}+\omega_1+\omega_2)} - \theta_1\theta_2 e^{\frac{\omega_1}{2}+\frac{\omega_2}{2}}. \tag{10}$$

Setting up an equation system as in (3)–(4) from (7)–(10) we obtain:

$$\theta_1 e^{\frac{\omega_1}{2}} = \bar{\theta}_1 e^{\frac{\bar{\omega}_1}{2}} \tag{11}$$

$$\theta_1^2 e^{2\omega_1} - \theta_1^2 e^{\omega_1} = \bar{\theta}_1^2 e^{2\bar{\omega}_1} - \bar{\theta}_1^2 e^{\bar{\omega}_1} \tag{12}$$

$$\theta_2 e^{\frac{\omega_2}{2}} = \bar{\theta}_2 e^{\frac{\bar{\omega}_2}{2}} \tag{13}$$

$$\theta_2^2 e^{2\omega_2} - \theta_2^2 e^{\omega_2} = \bar{\theta}_2^2 e^{2\bar{\omega}_2} - \bar{\theta}_2^2 e^{\bar{\omega}_2} \tag{14}$$

$$\theta_1\theta_2 e^{\frac{1}{2}(2\omega_{12}+\omega_1+\omega_2)} - \theta_1\theta_2 e^{\frac{\omega_1}{2}+\frac{\omega_2}{2}} = \bar{\theta}_1\bar{\theta}_2 e^{\frac{1}{2}(2\bar{\omega}_{12}+\bar{\omega}_1+\bar{\omega}_2)} - \bar{\theta}_1\bar{\theta}_2 e^{\frac{\bar{\omega}_1}{2}+\frac{\bar{\omega}_2}{2}} \tag{15}$$

which has only one solution, namely $\theta_1 = \bar{\theta}_1$, $\theta_2 = \bar{\theta}_2$, $\omega_1 = \bar{\omega}_1$, $\omega_2 = \bar{\omega}_2$ and $\omega_{12} = \bar{\omega}_{12}$. This means that the distribution of $\boldsymbol{Z}$ is uniquely determined by the parameters $\{\boldsymbol{\theta}, \boldsymbol{\Omega}\}$.

To study structural identifiability in a mixed-effects system using functions of random variables, the corresponding *exhaustive summary* [22] for the mixed-effects system must be found. The exhaustive summary is a vector $\sigma(\boldsymbol{\theta})$ which contains all information about the model parameters $\boldsymbol{\theta}$ that can be extracted from the knowledge of the input and output signal(s) [23]. The functions of random variables can be generated from the exhaustive summary for the corresponding non-mixed-effects system, i.e., where $\boldsymbol{\Omega} = \mathbf{0}$. Once the functions of the random variables for the mixed-effects system have been found the structural identifiability of the mixed-effects system can be considered. In Sections 3.2–3.3 it will be shown how the functions of random variables for the mixed-effects system can be found using established techniques for performing a structural identifiability analysis of non-mixed-effects systems.

3.2. Taylor series expansion approach

The Taylor series expansion approach for the study of the structural identifiability of state-space systems was first presented in [3]. In this method, the model output $y(t, \boldsymbol{\theta})$ is expanded around a known time point where there is information about the state, typically at $t = 0$, as

$$y(t, \boldsymbol{\theta}) = y(0, \boldsymbol{\theta}) + y^{(k)}(0, \boldsymbol{\theta})\frac{t^k}{k!} + \dots \tag{16}$$

where $k = 1, 2, \dots$ Since all coefficients in the Taylor series expansion are unique for a particular model output, the uniqueness of the model parameters can be determined from these coefficients. The exhaustive summary is therefore the coefficients in the Taylor series expansion and by equating them as

$$\sigma_k(\boldsymbol{\theta}) = y^{(k)}(0, \boldsymbol{\theta}) \qquad k = 1, 2, \dots \tag{17}$$

$$\sigma_k(\boldsymbol{\theta}) = \sigma_k(\bar{\boldsymbol{\theta}}) \qquad k = 1, 2, \dots \tag{18}$$

and solving for $\boldsymbol{\theta}$ the structural identifiability of the state-space model can be determined.

In the mixed-effects case, under the assumption of an infinite number of subjects, the coefficients $\sigma_k(\boldsymbol{\theta})$ become distributed over the population. This distribution depends on the underlying statistical model. Therefore, the coefficients in the Taylor series expansion can, in the mixed-effects case, be regarded as functions of random variables and given by

$$Z_k(\boldsymbol{\theta}, \boldsymbol{\eta}) = \sigma_k(\boldsymbol{\theta}, \boldsymbol{\eta}) \qquad k = 1, 2, \dots. \tag{19}$$

For a non-mixed-effects system, there exist different upper bounds for k depending on the form of the system. For linear systems the upper bound is $2n-1$ [24], for bilinear systems $2^{2n}-1$ [25], for homogeneous polynomial systems $\frac{q^{2n}-1}{q-1}$ [25] and for a generic state-space model on the form (1) the upper bound is $n+p$ [26].

A natural question is what upper bounds hold in the mixed-effects case. When studying the structural identifiability of a mixed-effects model we are studying the distribution of the exhaustive summary $\sigma(\boldsymbol{\theta})$ of the corresponding non-mixed-effects model. If the bounds are higher in the mixed-effects case we would instead study the distribution of $\sigma^*(\boldsymbol{\theta})$, a vector containing redundant information on $\boldsymbol{\theta}$ at the individual level. The distributions of $\sigma^*(\boldsymbol{\theta})$ would therefore contain redundant information about $\{\boldsymbol{\theta}, \boldsymbol{\Omega}\}$. If the bounds instead are lower for the mixed-effects case, then we study the distribution of $\sigma^{**}(\boldsymbol{\theta})$, a vector containing some, but not all information on $\boldsymbol{\theta}$ that is required to deduce structural identifiability from the output at an individual level. In other words, neither $\sigma^*(\boldsymbol{\theta})$ nor $\sigma^{**}(\boldsymbol{\theta})$ are exhaustive summaries. Therefore, the upper bounds for the Taylor series expansion in the non-mixed-effects case are the same for the mixed-effects case. To clarify, the number of statistical parameters are not included in p when computing the upper bounds. Instead, p still represent the number of parameters in the non-mixed-effects model.

Again, by combining (3)–(4) with the exhaustive summary from the Taylor series coefficients (19) the structural identifiability of mixed-effects models can be studied.

3.3. Input-Output form approach

A second way of generating the exhaustive summary of a system and extending it to functions of random variables is to rewrite the model in an input-output form. Starting with a non-mixed-effects state-space system, this can be done by iteratively differentiating the output function $\boldsymbol{y}$ and substituting in place of all of the model states $\boldsymbol{x}$ [8]. A system rewritten in an input-output form has the following differential algebraic polynomial form

$$\sum_{k=1}^{l} \sigma_k(\boldsymbol{\theta}) g_k(y, \dot{y}, \ddot{y}, \dots) = 0. \tag{20}$$

Together with the initial conditions of the system, (20) determines uniquely the solution of the model output [8]. Therefore, by setting up the equation system

$$\sigma_k(\boldsymbol{\theta}) = \sigma_k(\bar{\boldsymbol{\theta}}) \qquad k = 1, 2, \dots, l \tag{21}$$

$$y(0, \boldsymbol{\theta}) = y(0, \bar{\boldsymbol{\theta}}) \tag{22}$$

$$y^{(k)}(0, \boldsymbol{\theta}) = y^{(k)}(0, \bar{\boldsymbol{\theta}}) \qquad k = 1, 2, \dots, n-1 \tag{23}$$

and solving for $\boldsymbol{\theta}$ the structural identifiability of the system can be determined. Note that for this to be true the terms in $g_k(y, \dot{y}, \ddot{y}, \dots)$ must all be linearly independent.

In a mixed-effects model, using the same reasoning as for the Taylor series approaches, the input-output form (20) becomes

$$\sum_{k=1}^{l} \sigma_k(\boldsymbol{\theta}, \boldsymbol{\eta}) g_k(y, \dot{y}, \ddot{y}, \dots) = 0. \tag{24}$$

and the full set of functions of random variables is therefore given by

$$Z_k(\boldsymbol{\theta},\boldsymbol{\eta}) = \sigma_k(\boldsymbol{\theta},\boldsymbol{\eta}) \qquad k = 1, 2, \ldots, l \tag{25}$$

$$Z_{l+1}(\boldsymbol{\theta},\boldsymbol{\eta}) = y(0,\boldsymbol{\theta},\boldsymbol{\eta}) \tag{26}$$

$$Z_{l+1+r}(\boldsymbol{\theta},\boldsymbol{\eta}) = y^{(r)}(0,\boldsymbol{\theta},\boldsymbol{\eta}) \qquad r = 1, 2, \ldots, n-1. \tag{27}$$

Again, by using (3)–(4) with (25)–(27) the structural identifiability of mixed-effects models can be studied.

4. Examples

In this section the Taylor series expansion and the input-output approaches will be applied to generate the functions of random variables $\boldsymbol{Z}$ that can be used to study the structural identifiability of three exemplar mixed-effects models.

4.1. Taylor series approach: Linear one-compartment model

To demonstrate that covariance parameters also are included in the structural identifiability analysis, consider the following simple linear one-compartment model

$$\dot{x}_1 = -\theta_{10}x_1 \qquad x_1(0) = D \tag{28}$$

$$y = \theta_c x_1 \tag{29}$$

with unknown parameter vector (θ_{10}, θ_c) where θ_{10} denotes the rate of elimination and θ_c denotes the scaling of the output, and known initial condition, i.e., dose D. This model could for instance be used to describe the elimination of a drug from the blood plasma while measuring the concentration in the blood plasma. The first and second coefficients in the Taylor series expansion around $t = 0$ are

$$\sigma_1 = y(0) = \theta_c D \tag{30}$$

$$\sigma_2 = \dot{y}(0) = -\theta_{10}\theta_c D. \tag{31}$$

It can be seen directly from (30)–(31) that both θ_c and θ_{10} can be uniquely determined in a non-mixed effects framework. Introducing lognormally distributed random effects on the structural parameters θ_c and θ_{10} to ensure positivity the following two functions of random variables are derived:

$$Z_1 = \theta_c e^{\eta_c} D \tag{32}$$

$$Z_2 = -\theta_{10} e^{\eta_{10}} \theta_c e^{\eta_c} D \tag{33}$$

where the random effects vector $\boldsymbol{\eta} = (\eta_c, \eta_{10})$ is normally distributed with full covariance matrix

$$\boldsymbol{\Omega} = \begin{pmatrix} \omega_{10} & \omega_{10c} \\ \omega_{10c} & \omega_c \end{pmatrix}. \tag{34}$$

The unknown parameters in the mixed-effects model are $\boldsymbol{\theta} = (\theta_{10}, \theta_c, \omega_{10}, \omega_c, \omega_{10c})$. First we consider the first two moments of Z_1 which are given by

$$\mathbb{E}[Z_1(\boldsymbol{\theta}, \boldsymbol{\eta})] = D\theta_c e^{\frac{\omega_c}{2}} \tag{35}$$

$$\mathbb{E}[Z_1^2(\boldsymbol{\theta}, \boldsymbol{\eta})] = D^2\theta_c^2 e^{\omega_c}\left(e^{\omega_c} - 1\right). \tag{36}$$

By equating and solving $\mathbb{E}[Z_1^m(\boldsymbol{\theta}, \boldsymbol{\eta})] = \mathbb{E}[Z_1^m(\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\eta}})]$ for $m = 1, 2$ we obtain that $\theta_c = \bar{\theta}_c$ and $\omega_c = \bar{\omega}_c$ is the only solution. Let $\boldsymbol{Z} = (Z_1, Z_2)^T$ and consider the covariance matrix of $\boldsymbol{Z}$, given by

$$\begin{aligned}
&\mathrm{Cov}(\boldsymbol{Z}) = \mathbb{E}[\boldsymbol{Z}\boldsymbol{Z}^T] - \mathbb{E}[\boldsymbol{Z}]\mathbb{E}[\boldsymbol{Z}]^T = \\
&= \begin{pmatrix} \mathbb{E}[Z_1^2] - \mathbb{E}[Z_1]^2 & \mathbb{E}[Z_1 Z_2] - \mathbb{E}[Z_1]\mathbb{E}[Z_2] \\ \mathbb{E}[Z_1 Z_2] - \mathbb{E}[Z_1]\mathbb{E}[Z_2] & \mathbb{E}[Z_2^2] - \mathbb{E}[Z_2]^2 \end{pmatrix}
\end{aligned}$$

where the diagonal elements, i.e., the variances of Z_1 and Z_2, are given by

$$\mathbb{E}[Z_1^2] - \mathbb{E}[Z_1]^2 = D^2\theta_c^2 e^{2\omega_c} - D^2\theta_c^2 e^{\omega_c} \tag{37}$$

$$\mathbb{E}[Z_2^2] - \mathbb{E}[Z_2]^2 = D^2\theta_c^2\theta_{10}^2 e^{2(\omega_c + 2(\omega_{10c} + \omega_{10}))} - D^2\theta_c^2\theta_{10}^2 e^{\omega_c + 2\omega_{10c} + 2\omega_{10}} \tag{38}$$

and the off-diagonal elements, i.e., the covariance between Z_1 and Z_2, is given by

$$\mathbb{E}[Z_1 Z_2] - \mathbb{E}[Z_1]\mathbb{E}[Z_2] = -D^2\theta_c^2\theta_{10} e^{2\omega_c + 2\omega_{10c} + \omega_{10}} + D^2\theta_c^2\theta_{10} e^{\omega_c + \omega_{10c} + \omega_{10}}. \tag{39}$$

By equating and solving $\text{Cov}(\boldsymbol{Z}(\boldsymbol{\theta}, \boldsymbol{\eta})) = \text{Cov}(\boldsymbol{Z}(\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\eta}}))$, and using the previous result $\theta_c = \bar{\theta}_c$ and $\omega_c = \bar{\omega}_c$, we have that the only solution is $\theta_{10} = \bar{\theta}_{10}$, $\omega_{10} = \bar{\omega}_{10}$ and $\omega_{10c} = \bar{\omega}_{10c}$. All model parameters, including the covariance parameters ω_{10c}, have been shown to be uniquely determined and the mixed-effects model (28)–(29) is therefore structurally globally identifiable.

4.2. Input-Output form approach: Non-linear two compartment model

In this example we consider a model with slightly higher complexity involving two compartments with both a non-linear and linear elimination from the observed compartment. This model could be used to describe how a drug distributes between the plasma compartment x_1 and the rest of the body x_2 while being eliminated from the plasma compartment by different routes, i.e., one non-linear route and one linear route. The model structure is given by the following:

$$\dot{x}_1 = -(\frac{\theta_{Vmax}}{\theta_{ED50} + x_1} + \theta_e + \theta_{12})x_1 + \theta_{21}x_2 \tag{40}$$

$$\dot{x}_2 = \theta_{12}x_1 - \theta_{21}x_2 \tag{41}$$

$$x_1(0) = D \tag{42}$$

$$x_2(0) = 0 \tag{43}$$

$$y = \theta_c x_1 \tag{44}$$

with the unknown parameter vector $\boldsymbol{\theta} = (\theta_{12}, \theta_{21}, \theta_e, \theta_c, \theta_{Vmax}, \theta_{ED50})$ where θ_{12} and θ_{21} denotes the transport rate between the compartments, θ_e and θ_c is the elimination rate and the output scaling respecitively, θ_{Vmax} and θ_{ED50} is the maximum elimination rate and the concentration level where half of the maximum elimination rate is reached respectively, and known initial conditions, i.e., D and 0. To generate the input-output form of the model the symbolic computational software Maple was used. The model rewritten in input-output form is

$$\begin{aligned}
&\theta_e\theta_{21}y^3 + 2\,y^2\theta_{21}\theta_c\theta_e\theta_{ED50} + y^2\theta_{21}\theta_c\theta_{Vmax}+\\
&y^2\dot{y}\theta_e + \ddot{y}y^2 + y^2\dot{y}\theta_{21} + y^2\dot{y}\theta_{12}+\\
&y{\theta_c}^2\theta_{21}{\theta_{ED50}}^2\theta_e + 2\,\ddot{y}\theta_c\theta_{ED50}y+\\
&2\,y\theta_{12}\dot{y}\theta_{ED50}\theta_c + y{\theta_c}^2\theta_{21}\theta_{ED50}\theta_{Vmax}+\\
&2\,y\theta_{21}\dot{y}\theta_{ED50}\theta_c + 2\,y\theta_e\dot{y}\theta_{ED50}\theta_c + \ddot{y}{\theta_{ED50}}^2{\theta_c}^2+\\
&{\theta_c}^2\theta_e{\theta_{ED50}}^2\dot{y} + {\theta_c}^2\theta_{12}{\theta_{ED50}}^2\dot{y} + {\theta_c}^2\theta_{21}{\theta_{ED50}}^2\dot{y}+\\
&{\theta_c}^2\theta_{Vmax}\theta_{ED50}\dot{y} = 0 \qquad (45)
\end{aligned}$$

and the initial condition for the output function is

$$y(0) = \theta_c D \qquad (46)$$

$$\dot{y}(0) = -\theta_c(\frac{\theta_{Vmax}}{\theta_{ED50} + D} + \theta_e + \theta_{12})D. \qquad (47)$$

Here we have chosen to add lognormal random effects to ensure positivity on all structural parameters with a diagonal covariance matrix $\mathbf{\Omega}$. The following functions from the coefficients of the input-output form (45) can then be generated

$$Z_1 = (\theta_{ED50}e^{\eta_{ED50}})^2(\theta_c e^{\eta_c})^2 \tag{48}$$

$$Z_2 = \theta_{21}e^{\eta_{21}}\theta_e e^{\eta_e} \tag{49}$$

$$Z_3 = 2\theta_{ED50}e^{\eta_{ED50}}\theta_c e^{\eta_c} \tag{50}$$

$$Z_4 = \theta_c e^{\eta_c}\theta_{21}e^{\eta_{21}}\theta_{Vmax}e^{\eta_{Vmax}} + 2\theta_c e^{\eta_c}\theta_{21}e^{\eta_{21}}\theta_e e^{\eta_e}\theta_{ED50}e^{\eta_{ED50}} \tag{51}$$

$$\begin{aligned} Z_5 = {} & (\theta_c e^{\eta_c})^2\theta_{21}e^{\eta_{21}}\theta_e e^{\eta_e}(\theta_{ED50}e^{\eta_{ED50}})^2+ \\ & (\theta_c e^{\eta_c})^2\theta_{21}e^{\eta_{21}}\theta_{Vmax}\theta_{ED50}e^{\eta_{ED50}} \end{aligned} \tag{52}$$

$$Z_6 = \theta_{12}e^{\eta_{12}} + \theta_e e^{\eta_e} + \theta_{21}e^{\eta_{21}} \tag{53}$$

$$\begin{aligned} Z_7 = {} & 2\theta_c e^{\eta_c}\theta_{ED50}e^{\eta_{ED50}}\theta_{21}e^{\eta_{21}} + 2\theta_c e^{\eta_c}\theta_{ED50}e^{\eta_{ED50}}\theta_e e^{\eta_e}+ \\ & 2\theta_c e^{\eta_c}\theta_{ED50}e^{\eta_{ED50}}\theta_{12}e^{\eta_{12}} \end{aligned} \tag{54}$$

$$\begin{aligned} Z_8 = {} & (\theta_c e^{\eta_c})^2\theta_{ED50}^2\theta_{12}e^{\eta_{12}} + (\theta_c e^{\eta_c})^2(\theta_{ED50}e^{\eta_{ED50}})^2\theta_e e^{\eta_e}+ \\ & (\theta_c e^{\eta_c})^2\theta_{ED50}e^{\eta_{ED50}}\theta_{Vmax}e^{\eta_{Vmax}} + (\theta_c e^{\eta_c})^2(\theta_{ED50}e^{\eta_{ED50}})^2\theta_{21}e^{\eta_{21}} \end{aligned} \tag{55}$$

$$Z_9 = \theta_c e^{\eta_c}D \tag{56}$$

$$Z_{10} = -\theta_c e^{\eta_c}\left(\frac{\theta_{Vmax}e^{\eta_{Vmax}}}{\theta_{ED50}e^{\eta_{ED50}} + D} + \theta_e e^{\eta_e} + \theta_{12}e^{\eta_{12}}\right)D. \tag{57}$$

170 It is clear that both the model written in an input-output form (45) and the subsequent functions of random variables (48)–(57) are already quite complex expressions. The details of the full analysis can be found in Appendix A for brevity. We will now give an overview of how the analysis was performed.

First we consider Z_9 by setting up the following system

$$\mathbb{E}[Z_9(\boldsymbol{\theta}, \boldsymbol{\eta})] = \mathbb{E}[Z_9(\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\eta}})] \tag{58}$$

$$\mathbb{E}[Z_9^2(\boldsymbol{\theta}, \boldsymbol{\eta})] = \mathbb{E}[Z_9^2(\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\eta}})] \tag{59}$$

which has has the single solution

$$\theta_c = \bar{\theta}_c \tag{60}$$

$$\omega_c = \bar{\omega}_c. \tag{61}$$

Equating and solving

$$\mathbb{E}[Z_3(\boldsymbol{\theta},\boldsymbol{\eta})] = \mathbb{E}[Z_3(\bar{\boldsymbol{\theta}},\bar{\boldsymbol{\eta}})] \tag{62}$$

$$\mathbb{E}[Z_3^2(\boldsymbol{\theta},\boldsymbol{\eta})] = \mathbb{E}[Z_3^2(\bar{\boldsymbol{\theta}},\bar{\boldsymbol{\eta}})] \tag{63}$$

using (60)–(61) we obtain one solution, namely

$$\theta_{ED50} = \bar{\theta}_{ED50} \tag{64}$$

$$\omega_{ED50} = \bar{\omega}_{ED50}. \tag{65}$$

By equating and solving the following:

$$\mathbb{E}[Z_6(\boldsymbol{\theta},\boldsymbol{\eta})] = \mathbb{E}[Z_6(\bar{\boldsymbol{\theta}},\bar{\boldsymbol{\eta}})] \tag{66}$$

$$\mathbb{E}[Z_8(\boldsymbol{\theta},\boldsymbol{\eta})] = \mathbb{E}[Z_8(\bar{\boldsymbol{\theta}},\bar{\boldsymbol{\eta}})] \tag{67}$$

$$\mathbb{E}[Z_8^2(\boldsymbol{\theta},\boldsymbol{\eta})] = \mathbb{E}[Z_8^2(\bar{\boldsymbol{\theta}},\bar{\boldsymbol{\eta}})] \tag{68}$$

using the previous results from (60)–(61) and (64)–(65) we get one solution, namely

$$\theta_{Vmax} = \bar{\theta}_{Vmax} \tag{69}$$

$$\omega_{Vmax} = \bar{\omega}_{Vmax}. \tag{70}$$

Solving the equation system

$$\mathbb{E}[Z_2(\boldsymbol{\theta},\boldsymbol{\eta})] = \mathbb{E}[Z_2(\bar{\boldsymbol{\theta}},\bar{\boldsymbol{\eta}})] \tag{71}$$

$$\mathbb{E}[Z_5(\boldsymbol{\theta},\boldsymbol{\eta})] = \mathbb{E}[Z_5(\bar{\boldsymbol{\theta}},\bar{\boldsymbol{\eta}})] \tag{72}$$

$$\mathbb{E}[Z_5^2(\boldsymbol{\theta},\boldsymbol{\eta})] = \mathbb{E}[Z_5^2(\bar{\boldsymbol{\theta}},\bar{\boldsymbol{\eta}})] \tag{73}$$

using the previous results from (60)–(61), (64)–(65) and (69)–(70) yields only one solution

$$\theta_{21} = \bar{\theta}_{21} \tag{74}$$

$$\omega_{21} = \bar{\omega}_{21}. \tag{75}$$

Using (74)–(75) the following equation system

$$\mathbb{E}[Z_2(\boldsymbol{\theta},\boldsymbol{\eta})] = \mathbb{E}[Z_2(\bar{\boldsymbol{\theta}},\bar{\boldsymbol{\eta}})] \tag{76}$$

$$\mathbb{E}[Z_2^2(\boldsymbol{\theta},\boldsymbol{\eta})] = \mathbb{E}[Z_2^2(\bar{\boldsymbol{\theta}},\bar{\boldsymbol{\eta}})] \tag{77}$$

has the unique solution

$$\theta_e = \bar{\theta}_e \tag{78}$$

$$\omega_e = \bar{\omega}_e. \tag{79}$$

Finally, we use (74)–(75) and (78)–(79) to solve

$$\mathbb{E}[Z_6(\boldsymbol{\theta}, \boldsymbol{\eta})] = \mathbb{E}[Z_6(\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\eta}})] \tag{80}$$

$$\mathbb{E}[Z_6^2(\boldsymbol{\theta}, \boldsymbol{\eta})] = \mathbb{E}[Z_6^2(\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\eta}})] \tag{81}$$

which has the unique solution

$$\theta_{12} = \bar{\theta}_{12} \tag{82}$$

$$\omega_{12} = \bar{\omega}_{12}. \tag{83}$$

From (60)–(61),(64)–(65), (69)–(70), (74)–(75), (78)–(79) and (82)–(83) we can conclude that the mixed-effects model (40)–(44) has $\boldsymbol{\theta} = \bar{\boldsymbol{\theta}}$ and $\boldsymbol{\Omega} = \bar{\boldsymbol{\Omega}}$ and is therefore structurally globally identifiable.

4.3. An unidentifiable mixed-effects model

In this last example, we consider a one-compartment model with linear elimination rate θ_{10} and unknown scaling parameters θ_F and θ_c for both the input u and output y. This model represent the scenario when both the dose of the drug and its volume of distribution is unknown in addition to an unknown rate of elimination. As will be shown below, this results in an structurally unidentifiable model. The model structure is given by

$$\dot{x}_1 = -\theta_{10} x_1 + \theta_F u \quad x_1(0) = 0 \tag{84}$$

$$y = \theta_c x_1 \tag{85}$$

with unknown parameter vector $\boldsymbol{\theta} = (\theta_c, \theta_{10}, \theta_F)$, and known input and output functions u and y. By calculating the time derivative of the output signal y and substituting it for the model state x_1, the model (84)–(85) can be rewritten in the following input-output form

$$\dot{y} + \theta_{10} y - \theta_c \theta_F u = 0. \tag{86}$$

It is clear from (86) that the structural part of the model is unidentifiable since only the product $\theta_c\theta_F$ can be determined but not their individual contribution. By introducing lognormally distributed random effects, ensuring positivity, with a diagonal covariance matrix $\mathbf{\Omega}$ to all model parameters the corresponding functions of random variables are given by

$$Z_1(\boldsymbol{\theta},\boldsymbol{\eta}) = \theta_{10}e^{\eta_{10}} \tag{87}$$

$$Z_2(\boldsymbol{\theta},\boldsymbol{\eta}) = \theta_c e^{\eta_c}\theta_F e^{\eta_F}. \tag{88}$$

Calculating and equating the first and second statistical moments of Z_1 as $\mathbb{E}[Z_1^m(\boldsymbol{\theta},\boldsymbol{\eta})] = \mathbb{E}[Z_1^m(\bar{\boldsymbol{\theta}},\bar{\boldsymbol{\eta}})]$ for $m=1,2$ yields

$$\theta_{10}e^{\frac{\omega_{10}}{2}} = \bar{\theta}_{10}e^{\frac{\bar{\omega}_{10}}{2}} \tag{89}$$

$$\theta_{10}^2 e^{\omega_{10}}\left(e^{\omega_{10}}-1\right) = \bar{\theta}_{10}^2 e^{\bar{\omega}_{10}}\left(e^{\bar{\omega}_{10}}-1\right) \tag{90}$$

which has only one solution, namely

$$\theta_{10} = \bar{\theta}_{10} \tag{91}$$

$$\omega_{10} = \bar{\omega}_{10}. \tag{92}$$

Calculating and equating the first and second statistical moments of Z_2 as $\mathbb{E}[Z_2^m(\boldsymbol{\theta},\boldsymbol{\eta})] = \mathbb{E}[Z_2^m(\bar{\boldsymbol{\theta}},\bar{\boldsymbol{\eta}})]$ for $m=1,2$ yields

$$\theta_c\theta_F e^{\frac{1}{2}(\omega_c+\omega_F)} = \bar{\theta}_c\bar{\theta}_F e^{\frac{1}{2}(\bar{\omega}_c+\bar{\omega}_F)} \tag{93}$$

$$\theta_c^2\theta_F^2 e^{\omega_c+\omega_F}\left(e^{\omega_c+\omega_F}-1\right) = \bar{\theta}_c^2\bar{\theta}_F^2 e^{\bar{\omega}_c+\bar{\omega}_F}\left(e^{\bar{\omega}_c+\bar{\omega}_F}-1\right). \tag{94}$$

With the substitution $\beta_\theta = \theta_c\theta_F$ and $\beta_\omega = \omega_c+\omega_F$ the equation system for the statistical moments of Z_2 becomes

$$\beta_\theta e^{\frac{1}{2}\beta_\omega} = \bar{\beta}_\theta e^{\frac{1}{2}\bar{\beta}_\omega} \tag{95}$$

$$\beta_\theta^2 e^{\beta_\omega}\left(e^{\beta_\omega}-1\right) = \bar{\beta}_\theta^2 e^{\bar{\beta}_\omega}\left(e^{\bar{\beta}_\omega}-1\right) \tag{96}$$

which has only one solution, namely $\beta_\theta = \bar{\beta}_\theta$ and $\beta_\omega = \bar{\beta}_\omega$. From this it is easy to see that only the product $\theta_c\theta_F$ and the sum $\omega_c+\omega_F$ are structurally globally identifiable but not their individual contribution. The mixed-effects model (84)–(85) is therefore structurally unidentifiable.

5. Discussion

The two presented methods for the study of the structural identifiability for mixed-effects models are similar in the sense that they both are used to generate functions of random variables. However, there are a few differences between them that should be mentioned.

While the Taylor series expansion and the input-output approaches are applicable to both linear and nonlinear systems, the Taylor series expansion suffers from computational problems even for relatively simple model structures. The input-output approach can handle more complex model structures than the Taylor series approach, but there is still a limit of how complex the models can be in order for a analytical approach to be feasible. With the input-output approach it is necessary the check of linear independence among the terms. This can be done by computing the Wronskian [27], a potentially computationally demanding task. A structural identifiability analysis for a mixed-effects system is often more computationally demanding than its corresponding non-mixed-effects system since the use of random effects introduces the covariance matrix $\mathbf{\Omega}$ with unknown variance parameters, and with a non-diagonal covariance matrix, unknown covariance parameters as well. Nevertheless, the presented methods are still useful since many mixed-effects models used within the pharmaceutical industry are relatively simple in structure, e.g., compartmental models [28, 29, 30].

The two methods presented in this manuscript, the input-output form approach and the Taylor series expansion approach, are related to the Laplace transform approach presented in [21] since all three methods derives the exhaustive summary, a vector which contains all information about the model parameters for a given set of input and output functions. However, while the Taylor series expansion approach and the input-output form approach can be applied to both linear and nonlinear models the Laplace transform approach in [21] is limited to linear models only.

A future research topic that has come out of this work is with regard to the possible existence of upper and lower bounds of the order of statistical moments of the functions of random variables needed to determine structural identifiability. Consider the case of some Z_i being either normally, or lognormally distributed. The first two moments fully characterise the distribution and the upper and lower bound is therefore two. This was the case in the last example in Section 4.3 where both of the functions of random variables (87)–(88) are lognormally distributed and therefore only the first two statistical moments were considered. As the required relevant upper bound for structural identifiability was reached it can be concluded that the model is structurally unidentifiable. For a non-standard distribution for Z_i, the lower bound is the same as the number of unknown parameters in Z_i given that those unknown parameters do not appear in any Z_j where $j \neq i$. However, deriving an upper bound for non-standard distributions will almost certainly require dividing them up in different groups of distributions, a task for ongoing research. A consequence of having unknown upper bounds is that it makes proving that a model is structurally unidentifiable impossible, since it is not known what the required number of moments are.

Lastly, the presented methods open up the possibility of studying the structural identifiability of mixed-effects models analytically. This could potentially have a big impact on modelling in the pharmaceutical industry since such types of models are routinely used. Knowing whether the structural and variance parameters are structurally identifiable or otherwise increases the confidence in the model parameter estimates, leaving only the experimental data as a source of uncertainty for the model parameters. The presented methods should ideally also be integrated into the model development process as well as experimental design in order to answer questions such as what to measure and what administration routes to use in order to ensure structural identifiability? For other sources on how structural identifiability analysis affect experimental design see [14, 31, 11, 16, 32].

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Appendix A. Appendix A: Nonlinear two-compartment model

In this Appendix a more detailed analysis of the nonlinear two compartment model using the input-output approach presented in Section 4.2 is provided. The model structure is given by the following

$$\dot{x}_1 = -(\frac{\theta_{Vmax}}{\theta_{ED50} + x_1} + \theta_e + \theta_{12})x_1 + \theta_{21}x_2 \tag{A.1}$$

$$\dot{x}_2 = \theta_{12}x_1 - \theta_{21}x_2 \tag{A.2}$$

$$x_1(0) = D \tag{A.3}$$

$$x_2(0) = 0 \tag{A.4}$$

$$y = \theta_c x_1 \tag{A.5}$$

with the unknown parameters $\boldsymbol{\theta} = (\theta_{12},\, \theta_{21},\, \theta_e,\, \theta_c,\, \theta_{Vmax},\, \theta_{ED50})$ and known initial conditions, i.e, D. The model rewritten in an input-output form is

$$\begin{aligned}
&\theta_e\theta_{21}y^3 + 2\,y^2\theta_{21}\theta_c\theta_e\theta_{ED50} + y^2\theta_{21}\theta_c\theta_{Vmax} + \\
&y^2\dot{y}\theta_e + \ddot{y}y^2 + y^2\dot{y}\theta_{21} + y^2\dot{y}\theta_{12} + \\
&y{\theta_c}^2\theta_{21}{\theta_{ED50}}^2\theta_e + 2\,\ddot{y}\theta_c\theta_{ED50}y + \\
&2\,y\theta_{12}\dot{y}\theta_{ED50}\theta_c + y{\theta_c}^2\theta_{21}\theta_{ED50}\theta_{Vmax} + \\
&2\,y\theta_{21}\dot{y}\theta_{ED50}\theta_c + 2\,y\theta_e\dot{y}\theta_{ED50}\theta_c + \ddot{y}{\theta_{ED50}}^2{\theta_c}^2 + \\
&{\theta_c}^2\theta_e{\theta_{ED50}}^2\dot{y} + {\theta_c}^2\theta_{12}{\theta_{ED50}}^2\dot{y} + {\theta_c}^2\theta_{21}{\theta_{ED50}}^2\dot{y} + \\
&{\theta_c}^2\theta_{Vmax}\theta_{ED50}\dot{y} = 0
\end{aligned} \tag{A.6}$$

and the initial condition for the output function is

$$y(0) = \theta_c D \tag{A.7}$$

$$\dot{y}(0) = -\theta_c(\frac{\theta_{Vmax}}{\theta_{ED50} + D} + \theta_e + \theta_{12})D. \tag{A.8}$$

The coefficients in the input-output relation (A.6) and the initial condition (A.7) are given by

$$\sigma_1(\boldsymbol{\theta}) = \theta_{ED50}^2\theta_c^2 \tag{A.9}$$

$$\sigma_2(\boldsymbol{\theta}) = \theta_{21}\theta_e \tag{A.10}$$

$$\sigma_3(\boldsymbol{\theta}) = 2\theta_{ED50}\theta_c \tag{A.11}$$

$$\sigma_4(\boldsymbol{\theta}) = \theta_c\theta_{21}\theta_{Vmax} + 2\theta_c\theta_{21}\theta_e\theta_{ED50} \tag{A.12}$$

$$\sigma_5(\boldsymbol{\theta}) = \theta_c^2\theta_{21}\theta_e\theta_{ED50}^2 + \theta_c^2\theta_{21}\theta_{Vmax}\theta_{ED50} \tag{A.13}$$

$$\sigma_6(\boldsymbol{\theta}) = \theta_{12} + \theta_e + \theta_{21} \tag{A.14}$$

$$\sigma_7(\boldsymbol{\theta}) = 2\theta_c\theta_{ED50}\theta_{21} + 2\theta_c\theta_{ED50}\theta_e + 2\theta_c\theta_{ED50}\theta_{12} \tag{A.15}$$

$$\begin{aligned}\sigma_8(\boldsymbol{\theta}) = {}&\theta_c^2\theta_{ED50}^2\theta_{12} + \theta_c^2\theta_{ED50}^2\theta_e + \theta_c^2\theta_{ED50}\theta_{Vmax} + \\ &\theta_c^2\theta_{ED50}^2\theta_{21}\end{aligned} \tag{A.16}$$

$$\sigma_9(\boldsymbol{\theta}) = \theta_c D. \tag{A.17}$$

Lognormally distributed random effects were added to all parameters. From this the following functions of random variables are derived

$$Z_1 = (\theta_{ED50} e^{\eta_{ED50}})^2 (\theta_c e^{\eta_c})^2 \tag{A.18}$$

$$Z_2 = \theta_{21} e^{\eta_{21}} \theta_e e^{\eta_e} \tag{A.19}$$

$$Z_3 = 2\theta_{ED50} e^{\eta_{ED50}} \theta_c e^{\eta_c} \tag{A.20}$$

$$\begin{aligned} Z_4 = {} & \theta_c e^{\eta_c} \theta_{21} e^{\eta_{21}} \theta_{Vmax} e^{\eta_{Vmax}} + \\ & 2\theta_c e^{\eta_c} \theta_{21} e^{\eta_{21}} \theta_e e^{\eta_e} \theta_{ED50} e^{\eta_{ED50}} \end{aligned} \tag{A.21}$$

$$\begin{aligned} Z_5 = {} & (\theta_c e^{\eta_c})^2 \theta_{21} e^{\eta_{21}} \theta_e e^{\eta_e} (\theta_{ED50} e^{\eta_{ED50}})^2 + \\ & (\theta_c e^{\eta_c})^2 \theta_{21} e^{\eta_{21}} \theta_{Vmax} \theta_{ED50} e^{\eta_{ED50}} \end{aligned} \tag{A.22}$$

$$Z_6 = \theta_{12} e^{\eta_{12}} + \theta_e e^{\eta_e} + \theta_{21} e^{\eta_{21}} \tag{A.23}$$

$$\begin{aligned} Z_7 = {} & 2\theta_c e^{\eta_c} \theta_{ED50} e^{\eta_{ED50}} \theta_{21} e^{\eta_{21}} + \\ & 2\theta_c e^{\eta_c} \theta_{ED50} e^{\eta_{ED50}} \theta_e e^{\eta_e} + \\ & 2\theta_c e^{\eta_c} \theta_{ED50} e^{\eta_{ED50}} \theta_{12} e^{\eta_{12}} \end{aligned} \tag{A.24}$$

$$\begin{aligned} Z_8 = {} & (\theta_c e^{\eta_c})^2 \theta_{ED50}^2 \theta_{12} e^{\eta_{12}} + \\ & (\theta_c e^{\eta_c})^2 (\theta_{ED50} e^{\eta_{ED50}})^2 \theta_e e^{\eta_e} + \\ & (\theta_c e^{\eta_c})^2 \theta_{ED50} e^{\eta_{ED50}} \theta_{Vmax} e^{\eta_{Vmax}} + \\ & (\theta_c e^{\eta_c})^2 (\theta_{ED50} e^{\eta_{ED50}})^2 \theta_{21} e^{\eta_{21}} \end{aligned} \tag{A.25}$$

$$Z_9 = \theta_c e^{\eta_c} D \tag{A.26}$$

$$Z_{10} = -\theta_c e^{\eta_c} \left(\frac{\theta_{Vmax} e^{\eta_{Vmax}}}{\theta_{ED50} e^{\eta_{ED50}} + D} + \theta_e e^{\eta_e} + \theta_{12} e^{\eta_{12}} \right) D. \tag{A.27}$$

The first and second moments of Z_9 are given by

$$\mathbb{E}[Z_9(\boldsymbol{\theta}, \boldsymbol{\eta})] = D\theta_c e^{\frac{\omega_c}{2}} \tag{A.28}$$

$$\mathbb{E}[Z_9^2(\boldsymbol{\theta}, \boldsymbol{\eta})] = D^2 \theta_c^2 e^{\omega_c} \left(e^{\omega_c} - 1 \right). \tag{A.29}$$

Solving the equation system

$$D\theta_c e^{\frac{\omega_c}{2}} = D\bar{\theta}_c e^{\frac{\bar{\omega}_c}{2}} \tag{A.30}$$

$$D^2 \theta_c^2 e^{\omega_c} \left(e^{\omega_c} - 1 \right) = D^2 \bar{\theta}_c^2 e^{\bar{\omega}_c} \left(e^{\bar{\omega}_c} - 1 \right) \tag{A.31}$$

yields only one solution, namely

$$\theta_c = \bar{\theta}_c \tag{A.32}$$

$$\omega_c = \bar{\omega}_c. \tag{A.33}$$

The first and second moments of Z_3 are given by

$$\mathbb{E}[Z_3(\boldsymbol{\theta}, \boldsymbol{\eta})] = 2\theta_c \theta_{\text{ED50}} e^{\frac{1}{2}(\omega_c + \omega_{\text{ED50}})} \tag{A.34}$$

$$\mathbb{E}[Z_3^2(\boldsymbol{\theta}, \boldsymbol{\eta})] = 4\theta_c^2 \theta_{\text{ED50}}^2 e^{\omega_c + \omega_{\text{ED50}}} \left(e^{\omega_c + \omega_{\text{ED50}}} - 1\right). \tag{A.35}$$

Using (A.32)–(A.33) and solving the first and second moments of Z_3 yields

$$2\theta_c \theta_{\text{ED50}} e^{\frac{1}{2}(\omega_c + \omega_{\text{ED50}})} = 2\theta_c \bar{\theta}_{\text{ED50}} e^{\frac{1}{2}(\omega_c + \bar{\omega}_{\text{ED50}})} \tag{A.36}$$

$$4\theta_c^2 \theta_{\text{ED50}}^2 e^{\omega_c + \omega_{\text{ED50}}} \left(e^{\omega_c + \omega_{\text{ED50}}} - 1\right) = \tag{A.37}$$

$$4\theta_c^2 \bar{\theta}_{\text{ED50}}^2 e^{\omega_c + \bar{\omega}_{\text{ED50}}} \left(e^{\omega_c + \bar{\omega}_{\text{ED50}}} - 1\right) \tag{A.38}$$

which gives only one solution

$$\theta_{ED50} = \bar{\theta}_{ED50} \tag{A.39}$$

$$\omega_{ED50} = \bar{\omega}_{ED50}. \tag{A.40}$$

The first and second moments of Z_8 are given by

$$\mathbb{E}[Z_8(\boldsymbol{\theta},\boldsymbol{\eta})] = \\ \theta_c^2\theta_{\mathrm{ED50}}e^{2\omega_c+\frac{\omega_{\mathrm{ED50}}}{2}}(\theta_{\mathrm{ED50}}e^{\frac{3\omega_{\mathrm{ED50}}}{2}}(\theta_{12}e^{\frac{\omega_{12}}{2}}+ \\ \theta_{21}e^{\frac{\omega_{21}}{2}}+\theta_{\mathrm{e}}e^{\frac{\omega_{\mathrm{e}}}{2}})+\theta_{\mathrm{Vmax}}e^{\frac{\omega_{\mathrm{Vmax}}}{2}}) \tag{A.41}$$

$$\mathbb{E}[Z_8^2(\boldsymbol{\theta},\boldsymbol{\eta})] = \\ \theta_c^4\theta_{\mathrm{ED50}}^2e^{4\omega_c+\omega_{\mathrm{ED50}}}(\theta_{\mathrm{ED50}}^2e^{3\omega_{\mathrm{ED50}}}(\theta_{12}^2e^{\omega_{12}}(e^{4\omega_c+4\omega_{\mathrm{ED50}}+\omega_{12}} \\ -1)+2\theta_{12}e^{\frac{\omega_{12}}{2}}\left(e^{4(\omega_c+\omega_{\mathrm{ED50}})}-1\right)\left(\theta_{21}e^{\frac{\omega_{21}}{2}}+\theta_{\mathrm{e}}e^{\frac{\omega_{\mathrm{e}}}{2}}\right)+ \\ \theta_{21}^2e^{\omega_{21}}\left(e^{4\omega_c+4\omega_{\mathrm{ED50}}+\omega_{21}}-1\right)+ \\ 2\theta_{21}\theta_{\mathrm{e}}\left(e^{4(\omega_c+\omega_{\mathrm{ED50}})}-1\right)e^{\frac{1}{2}(\omega_{21}+\omega_{\mathrm{e}})}+ \\ \theta_{\mathrm{e}}^2e^{\omega_{\mathrm{e}}}\left(e^{4\omega_c+4\omega_{\mathrm{ED50}}+\omega_{\mathrm{e}}}-1\right))+ \\ 2\theta_{\mathrm{ED50}}\theta_{\mathrm{Vmax}}\left(e^{4\omega_c+2\omega_{\mathrm{ED50}}}-1\right)e^{\frac{1}{2}(3\omega_{\mathrm{ED50}}+\omega_{\mathrm{Vmax}})} \\ \left(\theta_{12}e^{\frac{\omega_{12}}{2}}+\theta_{21}e^{\frac{\omega_{21}}{2}}+\theta_{\mathrm{e}}e^{\frac{\omega_{\mathrm{e}}}{2}}\right)+ \\ \theta_{\mathrm{Vmax}}^2e^{\omega_{\mathrm{Vmax}}}\left(e^{4\omega_c+\omega_{\mathrm{ED50}}+\omega_{\mathrm{Vmax}}}-1\right)). \tag{A.42}$$

The first and second moments of Z_6 are given by

$$\mathbb{E}[Z_6(\boldsymbol{\theta},\boldsymbol{\eta})] = \theta_{12}e^{\frac{\omega_{12}}{2}}+\theta_{21}e^{\frac{\omega_{21}}{2}}+\theta_{\mathrm{e}}e^{\frac{\omega_{\mathrm{e}}}{2}} \tag{A.43}$$

$$\mathbb{E}[Z_6^2(\boldsymbol{\theta},\boldsymbol{\eta})] = \theta_{12}^2e^{\omega_{12}}\left(e^{\omega_{12}}-1\right)+ \\ \theta_{21}^2e^{\omega_{21}}\left(e^{\omega_{21}}-1\right)+\theta_{\mathrm{e}}^2e^{\omega_{\mathrm{e}}}\left(e^{\omega_{\mathrm{e}}}-1\right). \tag{A.44}$$

Using the previous results (A.32)–(A.33) and (A.39)–(A.40) the following equation can be generated from (A.41)

$$\theta_c^2\theta_{\mathrm{ED50}}e^{2\omega_c+\frac{\omega_{\mathrm{ED50}}}{2}}(\theta_{\mathrm{ED50}}e^{\frac{3\omega_{\mathrm{ED50}}}{2}}(\theta_{12}e^{\frac{\omega_{12}}{2}}+ \\ \theta_{21}e^{\frac{\omega_{21}}{2}}+\theta_{\mathrm{e}}e^{\frac{\omega_{\mathrm{e}}}{2}})+\theta_{\mathrm{Vmax}}e^{\frac{\omega_{\mathrm{Vmax}}}{2}}) = \\ \bar{\theta}_c^2\bar{\theta}_{\mathrm{ED50}}e^{2\bar{\omega}_c+\frac{\bar{\omega}_{\mathrm{ED50}}}{2}}(\bar{\theta}_{\mathrm{ED50}}e^{\frac{3\bar{\omega}_{\mathrm{ED50}}}{2}}(\bar{\theta}_{12}e^{\frac{\bar{\omega}_{12}}{2}}+ \\ \bar{\theta}_{21}e^{\frac{\bar{\omega}_{21}}{2}}+\bar{\theta}_{\mathrm{e}}e^{\frac{\bar{\omega}_{\mathrm{e}}}{2}})+\bar{\theta}_{\mathrm{Vmax}}e^{\frac{\bar{\omega}_{\mathrm{Vmax}}}{2}}) \tag{A.45}$$

which can be simplified to given

$$\left(\theta_{12}e^{\frac{\omega_{12}}{2}} + \theta_{21}e^{\frac{\omega_{21}}{2}} + \theta_{\mathrm{e}}e^{\frac{\omega_{\mathrm{e}}}{2}}\right) + \frac{\theta_{\mathrm{Vmax}}e^{\frac{\omega_{\mathrm{Vmax}}}{2}}}{\theta_{\mathrm{ED50}}e^{\frac{3\omega_{\mathrm{ED50}}}{2}}} =$$
$$\left(\bar{\theta}_{12}e^{\frac{\bar{\omega}_{12}}{2}} + \bar{\theta}_{21}e^{\frac{\bar{\omega}_{21}}{2}} + \bar{\theta}_{\mathrm{e}}e^{\frac{\bar{\omega}_{\mathrm{e}}}{2}}\right) + \frac{\bar{\theta}_{\mathrm{Vmax}}e^{\frac{\bar{\omega}_{\mathrm{Vmax}}}{2}}}{\theta_{\mathrm{ED50}}e^{\frac{3\omega_{\mathrm{ED50}}}{2}}}. \tag{A.46}$$

From the equation generated from (A.43) we use $\mathbb{E}[Z_6(\boldsymbol{\theta},\boldsymbol{\eta})] = \mathbb{E}[Z_6(\bar{\boldsymbol{\theta}},\bar{\boldsymbol{\eta}})]$ to simplify (A.46) further to give:

$$\theta_{Vmax}e^{\frac{\omega_{Vmax}}{2}} = \bar{\theta}_{Vmax}e^{\frac{\bar{\omega}_{Vmax}}{2}}. \tag{A.47}$$

Using (A.32)–(A.33), (A.39)–(A.40) and (A.43), the following equation system, where $\mathbb{E}[Z_8^2(\boldsymbol{\theta},\boldsymbol{\eta})] = \mathbb{E}[Z_8^2(\bar{\boldsymbol{\theta}},\bar{\boldsymbol{\eta}})]$ has been omitted since the expression is very large,

$$\theta_{Vmax}e^{\frac{\omega_{Vmax}}{2}} = \bar{\theta}_{Vmax}e^{\frac{\bar{\omega}_{Vmax}}{2}} \tag{A.48}$$
$$\mathbb{E}[Z_8^2(\boldsymbol{\theta},\boldsymbol{\eta})] = \mathbb{E}[Z_8^2(\bar{\boldsymbol{\theta}},\bar{\boldsymbol{\eta}})] \tag{A.49}$$

which has only one solution

$$\theta_{Vmax} = \bar{\theta}_{Vmax} \tag{A.50}$$
$$\omega_{Vmax} = \bar{\omega}_{Vmax}. \tag{A.51}$$

The first and second moments of Z_5 are given by

$$\mathbb{E}[Z_5(\boldsymbol{\theta},\boldsymbol{\eta})] = \theta_c^2\theta_{\mathrm{ED50}}\theta_{21}e^{\frac{1}{2}(4\omega_{\mathrm{c}}+\omega_{\mathrm{ED50}}+\omega_{21})}$$
$$(\theta_{\mathrm{ED50}}\theta_{\mathrm{e}}e^{\frac{1}{2}(3\omega_{\mathrm{ED50}}+\omega_{\mathrm{e}})} + \theta_{\mathrm{Vmax}}e^{\frac{\omega_{\mathrm{Vmax}}}{2}}) \tag{A.52}$$
$$\mathbb{E}[Z_5^2(\boldsymbol{\theta},\boldsymbol{\eta})] = \theta_c^4\theta_{\mathrm{ED50}}^2\theta_{21}^2e^{4\omega_{\mathrm{c}}+\omega_{\mathrm{ED50}}+\omega_{21}}$$
$$(\theta_{\mathrm{ED50}}^2\theta_{\mathrm{e}}^2e^{3\omega_{\mathrm{ED50}}+\omega_{\mathrm{e}}}\left(e^{4\omega_{\mathrm{c}}+4\omega_{\mathrm{ED50}}+\omega_{21}+\omega_{\mathrm{e}}} - 1\right) +$$
$$2\theta_{\mathrm{ED50}}\theta_{\mathrm{e}}\theta_{\mathrm{Vmax}}\left(e^{4\omega_{\mathrm{c}}+2\omega_{\mathrm{ED50}}+\omega_{21}} - 1\right)$$
$$e^{\frac{1}{2}(3\omega_{\mathrm{ED50}}+\omega_{\mathrm{e}}+\omega_{\mathrm{Vmax}})}+$$
$$\theta_{\mathrm{Vmax}}^2e^{\omega_{\mathrm{Vmax}}}\left(e^{4\omega_{\mathrm{c}}+\omega_{\mathrm{ED50}}+\omega_{21}+\omega_{\mathrm{Vmax}}} - 1\right)). \tag{A.53}$$

The first and second moments of Z_2 are

$$\mathbb{E}[Z_2(\boldsymbol{\theta},\boldsymbol{\eta})] = \theta_{21}\theta_{\mathrm{e}}e^{\frac{1}{2}(\omega_{21}+\omega_{\mathrm{e}})} \tag{A.54}$$
$$\mathbb{E}[Z_2^2(\boldsymbol{\theta},\boldsymbol{\eta})] = \theta_{21}^2\theta_{\mathrm{e}}^2e^{\omega_{21}+\omega_{\mathrm{e}}}\left(e^{\omega_{21}+\omega_{\mathrm{e}}} - 1\right). \tag{A.55}$$

Using (A.32)–(A.33),(A.39)–(A.40), (A.50)–(A.51) and (A.54) the equation system

$$\mathbb{E}[Z_5(\boldsymbol{\theta}, \boldsymbol{\eta})] = \mathbb{E}[Z_5(\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\eta}})] \tag{A.56}$$

$$\mathbb{E}[Z_5^2(\boldsymbol{\theta}, \boldsymbol{\eta})] = \mathbb{E}[Z_5^2(\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\eta}})] \tag{A.57}$$

has the unique solution

$$\theta_{21} = \bar{\theta}_{21} \tag{A.58}$$

$$\omega_{21} = \bar{\omega}_{21}. \tag{A.59}$$

Using (A.58)–(A.59) the following equation system

$$\mathbb{E}[Z_2(\boldsymbol{\theta}, \boldsymbol{\eta})] = \mathbb{E}[Z_2(\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\eta}})] \tag{A.60}$$

$$\mathbb{E}[Z_2^2(\boldsymbol{\theta}, \boldsymbol{\eta})] = \mathbb{E}[Z_2^2(\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\eta}})] \tag{A.61}$$

has the unique solution

$$\theta_e = \bar{\theta}_e \tag{A.62}$$

$$\omega_e = \bar{\omega}_e. \tag{A.63}$$

Finally, we make use of (A.58)–(A.59) and (A.62)–(A.63) and solve

$$\mathbb{E}[Z_6(\boldsymbol{\theta}, \boldsymbol{\eta})] = \mathbb{E}[Z_6(\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\eta}})] \tag{A.64}$$

$$\mathbb{E}[Z_6^2(\boldsymbol{\theta}, \boldsymbol{\eta})] = \mathbb{E}[Z_6^2(\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\eta}})] \tag{A.65}$$

which has the unique solution

$$\theta_{12} = \bar{\theta}_{12} \tag{A.66}$$

$$\omega_{12} = \bar{\omega}_{12}. \tag{A.67}$$

From (A.32)–(A.33),(A.39)–(A.40), (A.50)–(A.51), (A.58)–(A.59), (A.62)–(A.63) and (A.66)–(A.67) we can conclude that $\boldsymbol{\theta} = \bar{\boldsymbol{\theta}}$ and $\boldsymbol{\Omega} = \bar{\boldsymbol{\Omega}}$ which means that the mixed-effects model (A.1)–(A.5) is structurally globally identifiable.

with(*LinearAlgebra*) : *with*(*Groebner*) :

Forsman Code

```
lieDer :=proc(H, F, vars)
  local V :
  V := map((a, b)→diff(b, a), vars, H) :
  DotProduct(Vector(F), Vector(V), conjugate= false)
 end:

listLieDer := proc(H, F, k)
  local L, i, tmp, N, vars :
  L := [y[0] − H] : tmp := H :
  N := nops(F) ;
  vars := [seq(x[t], t = 1 .. N)] ;
  for i to k do
    tmp := lieDer(tmp, F, vars) :
    L := [op(L), y[i]-tmp]
  od;
 end:

xlistLieDer := proc(H, F, k, uvars)
  local L, i, f, h, tmp, N, var, vars, duvars ;
  N := nops(F) ;
  f := F ;
  h := H ;
  for var in uvars do
    f := subs(var = var[0], f) ;
    h := subs(var = var[0], h) ;
  od;
  duvars := [] ;
  vars := [seq(x[t], t = 1..N)] ;
  for var in uvars do
    vars := [op(vars), seq(var[i], i = 0..10)] ;
    duvars := [op(duvars), seq(var[i], i = 1..11)] ;
  od;
  f := [op(f), op(duvars)] ;
  L := [y[0]-h]; tmp := h ;
  i := 1 ;
  for i to k do
    tmp := lieDer(tmp, f, vars) ;
    L := [op(L), y[i]-tmp] ;
   od;
 end:

iorel := proc(f, h, uvars)
  local n, L :
  n := nops(f) :
  if _params['uvars'] = NULL then
```

```
    L := listLieDer(h, f, n) :
  else
    L := xlistLieDer(h, f, n, uvars) :
  fi;
  L := map(expand, numer(L)) :
  UnivariatePolynomial(y[n], L, [seq(x[t], t = 1..n), y[n]]) ;
end:
```

2 compartments, 2 elimination from same compartment, MM and linear

Defining the system as:

$F := \left[-\left(\frac{V[maxx]}{ED50+x[1]}+k[e]+k[12]\right)\cdot x[1]+k[21]\cdot x[2], k[12]\cdot x[1]-k[21]\cdot x[2]\right]$

$$\left[-\left(\frac{V_{maxx}}{ED50+x_1}+k_e+k_{12}\right)x_1+k_{21}x_2, k_{12}x_1-k_{21}x_2\right] \quad (1)$$

$H := c\cdot x[1];$

$outptEqn := iorel(F, H)$

$$c\,x_1 \quad (2)$$

Collecting the coefficients and setting up expression with alternative parameter vector

$uA := \{coeffs(collect(outptEqn, [y[0], y[1], y[2]], 'distributed'), [y[0], y[1], y[2]])\}$

$$\{1, ED50^2c^2, k_{21}k_e, 2\,ED50\,c, c\,k_{21}V_{maxx}+2\,c\,k_{21}k_e ED50, c^2k_{21}k_e ED50^2+c^2k_{21}V_{maxx}ED50, k_{12}+k_e+k_{21}, 2\,c\,ED50\,k_{21}+2\,c\,ED50\,k_e+2\,c\,ED50\,k_{12}, c^2ED50^2k_{12}+c^2ED50^2k_e+c^2ED50\,V_{maxx}+c^2ED50^2k_{21}\} \quad (3)$$

$uB := eval(uA, [k[e]=kb[e], k[12]=kb[12], k[21]=kb[21], V[maxx]=Vb[maxx], ED50=ED50b, c=cb])$

$$\{1, ED50b^2cb^2, kb_{21}kb_e, 2\,ED50b\,cb, cb\,kb_{21}Vb_{maxx}+2\,cb\,kb_{21}kb_e ED50b, cb^2kb_{21}kb_e ED50b^2+cb^2kb_{21}Vb_{maxx}ED50b, kb_{12}+kb_e+kb_{21}, 2\,cb\,ED50b\,kb_{21}+2\,cb\,ED50b\,kb_e+2\,cb\,ED50b\,kb_{12}, cb^2ED50b^2kb_{12}+cb^2ED50b^2kb_e+cb^2ED50b\,Vb_{maxx}+cb^2ED50b^2kb_{21}\} \quad (4)$$

$eqns := convert(uA, list) - convert(uB, list)$

$$[0, -ED50b^2cb^2+ED50^2c^2, -kb_{21}kb_e+k_{21}k_e, -2\,ED50b\,cb+2\,ED50\,c, -cb\,kb_{21}Vb_{maxx}-2\,cb\,kb_{21}kb_e ED50b+c\,k_{21}V_{maxx}+2\,c\,k_{21}k_e ED50, -cb^2kb_{21}kb_e ED50b^2-cb^2kb_{21}Vb_{maxx}ED50b+c^2k_{21}k_e ED50^2+c^2k_{21}V_{maxx}ED50, -kb_{12}-kb_e-kb_{21}+k_{12}+k_e+k_{21}, -2\,cb\,ED50b\,kb_{21}-2\,cb\,ED50b\,kb_e-2\,cb\,ED50b\,kb_{12}+2\,c\,ED50\,k_{21}+2\,c\,ED50\,k_e+2\,c\,ED50\,k_{12}, -cb^2ED50b^2kb_{12}-cb^2ED50b^2kb_e-cb^2ED50b\,Vb_{maxx}-cb^2ED50b^2kb_{21}+c^2ED50^2k_{12}+c^2ED50^2k_e+c^2ED50\,V_{maxx}+c^2ED50^2k_{21}] \quad (5)$$

Setting up the equations for the inital conditions

$n := 2:$

Calculating the derivative of output function y, then evaluating the expressions for the initial conditions

$icsnoteval \overset{340}{:=} eval(\ -1 * listLieDer(H, F, n-1), [seq(y[i]=0, i=0..n-1)]\ )$

$$\left[c\, x_1, \left(-\left(\frac{V_{maxx}}{ED50 + x_1} + k_e + k_{12}\right) x_1 + k_{21}\, x_2\right) c\right] \qquad (6)$$

$icseval := eval(icsnoteval, \{x[1] = Dose, x[2] = 0\})$

$$\left[c\, Dose, -\left(\frac{V_{maxx}}{ED50 + Dose} + k_e + k_{12}\right) Dose\, c\right] \qquad (7)$$

Introducing alternative parameter vector and finalizing the expression for the initial conditions

$icsA := Vector(icseval);$

$icsB := eval(icsA, [k[e] = kb[e], k[12] = kb[12], k[21] = kb[21], V[maxx] = Vb[maxx], ED50 = ED50b, c = cb]);$

$$\left[\begin{array}{c} c\, Dose \\ -\left(\frac{V_{maxx}}{ED50 + Dose} + k_e + k_{12}\right) Dose\, c \end{array}\right] \qquad (8)$$

$ics := [icsA(1) - icsB(1), icsA(2) - icsB(2)]:$

$solve([op(ics), op(eqns)], [k[e], k[12], k[21], V[maxx], ED50, c])$

$$[[k_e = kb_e, k_{12} = kb_{12}, k_{21} = kb_{21}, V_{maxx} = Vb_{maxx}, ED50 = ED50b, c = cb]] \qquad (9)$$

Summary: The model is structurally globally identifiable.