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INFLUENCE OF COVARIANCE BETWEEN RANDOM EFFECTS IN DESIGN FOR NONLINEAR MIXED EFFECT MODELS WITH AN ILLUSTRATION IN PAEDIATRIC PHARMACOKINETICS

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Nonlinear mixed-effect models are used increasingly during drug development. For design, an alternative to simulations is based on the Fisher information matrix. Its expression was derived using a first-order approach, was then extended to include covariance and implemented into the R function PFIM. The impact of covariance on standard errors, amount of information and optimal designs was studied. It was also shown how standard errors can be predicted analytically within the framework of rich individual data without the model. The results were illustrated by applying this extension to the design of a pharmacokinetic study of a drug in paediatric development.

Key words: D-optimality; Fisher information matrix; Nonlinear mixed effect models; Optimal design; Paediatric studies; PFIM; Population pharmacokinetics.

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1 INTRODUCTION

Nonlinear mixed-effect models (NLMEM) are used increasingly during drug development for the analysis of dose-concentration-response data. They allow for a sparse sampling design with few data points per individual in a set of individuals and estimation of mean parameters, their inter-individual variability and covariate effects. This can be particularly useful in clinical trials with specific populations, such as children (Tod *et al.*, 2008), where classical studies with a large number of samples are often limited for ethical or physiological reasons. An appropriate choice of experimental design for estimating parameters in NLMEM is required. NLMEM are also now commonly used for the joint modelling of several biological responses, such as the joint analysis of the pharmacokinetics (PK) and pharmacodynamics (PD) of a drug, or the PK of parent drugs and their active metabolites.

Designs in NLMEM are called population designs. Population designs consist of a set of elementary designs to be carried out in a set of individuals (Mentré *et al.*, 1997). Elementary designs are composed of the design variables to be performed for each individual. Design variables are for example the number of samples by individual and the location of sampling times but can be more general. The choice of the design variables and the balance between the number of individuals and the amount of information by individual have a large impact on the precision of population parameter estimates. In this work, the sampling times were considered as the only design variables. Approaches to evaluating and optimizing the designs are required. To avoid simulations, which are time consuming, designs can be evaluated using the Fisher information matrix (M_F) and the optimization of its determinant. The expression of M_F for the single-response model was proposed by Mentré *et al.* (1997) and Retout *et al.* (2001), using a first-order Taylor expansion of the model around the expectation of random effects. The expression of M_F for multiple responses was first extended by Hooker and Vicini (2005), using the same development as for a single-response model. The expression of M_F for nonlinear mixed-effect multiple-response models was also implemented in PFIM, an R function dedicated to design evaluation and optimization, and was evaluated by simulations (Bazzoli *et al.*, 2009). Several software packages, which implement an evaluation of the population M_F and optimization of the experimental designs in the context of NLMEM, are

available, notably PFIM in R (Bazzoli *et al.*, 2010).

For M_F evaluation, it is often assumed that the variance-covariance matrix of random effects is diagonal, which means that individual random effects are assumed to be independent. The consequence is that covariance terms between parameters are ignored at the design stage. Ogungbenro *et al.* (2008) developed an expression of M_F that accounts for covariance between random effects for multiple-response population PK and for population PK/pharmacodynamics (PD). The objectives of Ogungbenro *et al.* were to investigate the implications of including and excluding correlation between random effects and to investigate the effect of correlation between responses on the optimal sampling times. They performed a simulation and presented the empirical and expected coefficients of variation of the parameter estimates for a PK/PD example as well as the mean relative error and root mean square error for the same example, with and without correlation, to assess the level of bias and precision of the estimates. The aim of the present work, is to investigate the impact of the size of covariance between random effects on the optimal sampling-time design for a pharmacokinetic example and in addition to study its effect on predicted standard errors (SE), predicted relative standard errors (RSE) and the amount of information, taking into consideration covariance between random effects. Moreover and conversely to the work of Ogungbenro *et al.*, in the present work, design optimization allowed several groups of elementary designs. In addition, it is shown how the SEs in the framework of rich individual data can be predicted analytically, without using the model. Assuming rich individual data, it can be assumed that individual parameters of each subject are known without error. Estimation of fixed effects and variance for these parameters is standard and predicted SEs can be derived analytically.

The results are illustrated by applying these developments to the design of a clinical PK study in children of a molecule in development. To illustrate the results, a molecule and its main metabolite were chosen. Indeed, as the parent drug is metabolised into an active metabolite, concentration-time data of both parent drug and its metabolite are of interest. Developments are applied to the single-response model (parent-drug model) and to the multiple-response model (joint parent- and metabolite-model). To optimize sampling times, it is necessary to have *a priori* information, and in this case, *a priori* information was model

and parameters obtained from data generated by the SIMCYP software using the adults-to-children extrapolation tool, the knowledge of the drug in adults, its physico-chemical properties and *in vitro* experimental data. SIMCYP is a software program of Physiologically-Based Pharmacokinetic (PBPK) modelling (Jamei *et al.*, 2009). PBPK models make it possible to describe the biodistribution of a substance (absorption, distribution, metabolism, excretion) and are based on a physiological reality. SIMCYP can predict the evolution of concentration *versus* time for a given molecule and in a target population (healthy volunteers, Caucasians, children, etc.). More specifically for the present study, the paediatric module of SIMCYP took into account all the changes occurring during childhood (Johnson *et al.*, 2006). Concentration-time profiles for parent and metabolite in children from SIMCYP simulations have already been analysed by NLMEM with the NONMEM software (Brendel *et al.*, 2010).

The present article is divided into four sections including the introduction. Section 2 presents the methodological development with notation definitions in 2.1. The calculation of the SEs of correlation and covariance terms is presented in subsection 2.2, methodology to approximate M_F and the SEs in the framework of individual-rich data is presented in subsection 2.3. In the fourth subsection (2.4), the focus is on the implementation and optimization of M_F considering covariance between random effects in PFIM. Section 3 first presents the PK example in paediatrics and the models (3.1). Then, the methods and results concerning the evaluation of the extension of M_F in PFIM are shown (3.2). The two last subsections present the methods and the results of the approximation of the M_F calculation (3.3) and the impact of covariance on SEs, the amount of information and optimal designs (3.4).

2 METHODOLOGICAL DEVELOPMENT

2.1. Model and notations

A nonlinear mixed-effect multiple-response model, or a multiple response population model, is defined as follows. The vector of observations Y_i for the i^{th} individual is defined as the vector of the K different responses: $Y_i = [y_{i1}^T, y_{i2}^T, \dots, y_{iK}^T]^T$, where y_{ik} , $k = 1, \dots, K$,

is the vector of n_{ik} observations for the k^{th} response. Each of these responses is associated with a known function f_k , which defines the nonlinear structural model. The K functions f_k can be grouped in a vector of multiple-response model F , such as $F(\theta_i, \xi_i) = [f_1(\theta_i, \xi_{i1})^T, f_2(\theta_i, \xi_{i2})^T, \dots, f_K(\theta_i, \xi_{iK})^T]^T$, where ξ_i is an elementary design for one individual i , composed of K sub-designs such that $\xi_i = (\xi_{i1}, \xi_{i2}, \dots, \xi_{iK})$. ξ_i is defined by n_i sampling times and its sub-design ξ_{ik} is defined by $(t_{ik1}, t_{ik2}, \dots, t_{ikn_{ik}})$, with n_{ik} sampling times for the observations of the k^{th} response, so that $n_i = \sum_{k=1}^K n_{ik}$.

θ_i is the vector of all the individual parameters for all the response models in individual i . The vector of individual parameters θ_i depends on β , the p-vector of the fixed effects parameters and on b_i , the vector of the p random effects for individual i . The relation between θ_i and (β, b_i) is modelled by a function g , that is $\theta_i = g(\beta, b_i)$, which is usually additive, so that $\theta_i = \beta + b_i$, or exponential so that $\theta_i = \exp(\beta + b_i)$, where $\exp(\beta) = \beta$. It is assumed that $b_i \sim N(0, \Omega)$ with Ω defined as a $p \times p$ -positive definite matrix, for which, each diagonal element ω_{rr}^2 , $r = 1, \dots, p$, represents the variance of the r^{th} component of the vector b_i and ω_{rs} , $r = 1, \dots, p$ and $s = 1, \dots, p$, with $r \neq s$, represents the covariance between the r^{th} and the s^{th} components of the vector b_i .

The statistical model is then given by $Y_i = F(g(\beta, b_i), \xi_i) + \varepsilon_i$, where ε_i is the vector composed of the K vectors of residual errors ε_{ik} , $k = 1, \dots, K$, associated with the K responses. It is also supposed that $\varepsilon_{ik} \sim N(0, \Sigma_{ik})$ with Σ_{ik} a $n_{ik} \times n_{ik}$ -diagonal matrix such that: $\Sigma_{ik}(\beta, b_i, \sigma_{inter_k}, \sigma_{slope_k}, \xi_{ik}) = \text{diag}(\sigma_{inter_k} + \sigma_{slope_k} \times f_k(g(\beta, b_i), \xi_{ik}))^2$ where σ_{inter_k} and σ_{slope_k} are two parameters of the model for the variance of the residual error of the k^{th} response. The case $\sigma_{slope_k} = 0$ returns a homoscedastic error model, whereas the case $\sigma_{inter_k} = 0$ returns a constant coefficient of variation error model. The case where the two parameters differ from zero is called a combined error model. The variance of ε_i over the K responses is then noted $\Sigma_i(\beta, b_i, \sigma_{inter}, \sigma_{slope}, \xi_i)$, where Σ_i is a $n_i \times n_i$ -diagonal matrix composed of each diagonal element of Σ_{ik} with $k = 1, \dots, K$ and σ_{inter} and σ_{slope} are two vectors of the K components σ_{inter_k} and σ_{slope_k} , $k = 1, \dots, K$, respectively. Finally, conditionally on the value of b_i , it is assumed that the ε_i errors are independently distributed.

Let λ be the vector of the unknown variance-covariance terms, corresponding to the vector of the lower triangular of Ω , which contains $p(p+1)/2$ variance-covariance terms and all

the parameters of the error model.

Then, λ is expressed as $\lambda^T = (\omega_{11}^2, \dots, \omega_{pp}^2, \omega_{21}, \omega_{31}, \omega_{32}, \dots, \omega_{p,p-1}, \sigma_{inter}^T, \sigma_{slope}^T)$. Let Ψ be the vector of the population parameters to be estimated such as $\Psi^T = (\beta^T, \lambda^T)$.

For N individuals, a population design is composed of the N elementary designs ξ_i , $i = 1, \dots, N$. A population design is therefore described by the N elementary designs for a total number n_{tot} of observations such as $n_{tot} = \sum_{i=1}^N n_i$ and $\Xi = \{\xi_1, \dots, \xi_N\}$. Usually, population designs are composed of a limited number Q of groups of individuals with identical designs and identical covariates within each group. Each of these groups is defined by an elementary design ξ_q , $q = 1, \dots, Q$, which is composed, for the k^{th} response, especially of n_{qk} sampling times $(t_{qk1}, t_{qk2}, \dots, t_{qkn_{qk}})$ to be performed in a number N_q of individuals. The population design can then be written as follows: $\Xi = \{[\xi_1, N_1]; [\xi_2, N_2]; \dots; [\xi_Q, N_Q]\}$.

2.2. Calculation of the standard errors of correlation and covariance

The population Fisher information matrix for a population design Ξ is computed as the sum of the N elementary Fisher information matrices $M_F(\Psi, \xi_i)$ for each individual i :

$$M_F(\Psi, \Xi) = \sum_{i=1}^N M_F(\Psi, \xi_i). \text{ In the case of a limited number } Q \text{ of groups, it is expressed by}$$

$$M_F(\Psi, \Xi) = \sum_{q=1}^Q N_q M_F(\Psi, \xi_q).$$

Ogungbenro *et al.* (2008) have developed the expression of M_F in NLMEM that accounts for covariance between random effects for multiple responses, i.e. with a non-diagonal Ω matrix. This expression is implemented in a working version of PFIM and the corresponding development is detailed in the appendix.

From the square roots of the diagonal elements of M_F^{-1} , the predicted SE for estimated parameters can be calculated. The aim is to predict the SE for estimated correlation, the correlation being the ratio of the covariance and the product of the standard deviations. It should be noted that, if $\varphi(\theta)$ is a function of θ , then $M_F(\theta) = JM_F(\varphi(\theta))J^T$, with $J = \frac{\partial \varphi(\theta)^T}{\partial \theta}$. In the following, B_{11} , B_{22} , B_{33} , B_{12} , B_{13} and B_{23} are terms of the 3×3 sub-matrix of B , formed of lines and columns corresponding to ω_{mm} , ω_{ll} and ω_{ml} , respectively. Thus:

$$SE^2(\widehat{\rho_{ml}}) = \rho_{ml}^2 \left(\frac{1}{4\omega_{mm}^4} B_{11}^{-1} + \frac{1}{4\omega_{ll}^4} B_{22}^{-1} + \frac{1}{\omega_{mm}^2 \omega_{ll}^2 \rho_{ml}^2} B_{33}^{-1} + \frac{1}{2\omega_{mm}^2 \omega_{ll}^2} B_{12}^{-1} - \frac{1}{\omega_{mm}^3 \omega_{ll} \rho_{ml}} B_{13}^{-1} - \frac{1}{\omega_{mm} \omega_{ll}^3 \rho_{ml}} B_{23}^{-1} \right)$$

And vice versa, if B is now computed as a function of ω_{mm} , ω_{ll} and ρ_{ml} , it becomes

$$SE^2(\widehat{\omega_{ml}}) = \omega_{ml}^2 \left(\frac{1}{4\omega_{mm}^4} B_{11}^{-1} + \frac{1}{4\omega_{ll}^4} B_{22}^{-1} + \frac{\omega_{mm}^2 \omega_{ll}^2}{\omega_{ml}^2} B_{33}^{-1} + \frac{1}{2\omega_{mm}^2 \omega_{ll}^2} B_{12}^{-1} + \frac{\omega_{ll}}{\omega_{mm} \omega_{ml}} B_{13}^{-1} + \frac{\omega_{mm}}{\omega_{ll} \omega_{ml}} B_{23}^{-1} \right)$$

where estimators are designated with $\hat{\cdot}$.

2.3. Approximation of the Fisher information matrix for rich design

In NLMEM, the parameters themselves are not estimated, but their mean and variance are. Let's consider that the vector of individual parameters θ_i of each subject is known without error, and estimation of β and Ω is performed from this set of N individual parameters. The SEs for $\hat{\beta}$ and $\hat{\Omega}$ can be predicted analytically without using the model and will depend on the design only through N, the number of subjects, therefore $\Xi = N$. To derive the expression of the M_F in that case, we assume that we have N vectors of observation θ_i with $\theta \sim N(\beta, \Omega)$. In that case, $\Psi^{*T} = (\beta^T, \lambda^{*T})$, where $\lambda^{*T} = \text{vec}(\Omega)$. The general term of the m^{th} line and l^{th} column of the elementary Fisher information matrix for β and Ω is given by expression:

$$M_F^*(\Psi^*, N)_{m,l} = \frac{\partial \beta^T}{\partial \Psi_m^*} \Omega^{-1} \frac{\partial \beta}{\partial \Psi_l^*} + \frac{1}{2} \text{tr} \left(\Omega^{-1} \frac{\partial \Omega}{\partial \Psi_m^*} \Omega^{-1} \frac{\partial \Omega}{\partial \Psi_l^*} \right)$$

where tr denotes the trace of a matrix.

The derivative of β with respect to fixed effects is equal to 1, and with respect to variance terms is equal to 0. The derivative of Ω with respect to fixed effects is 0 while the derivative of Ω with respect to variance terms is equal to a matrix composed of 1 for the corresponding term and 0 elsewhere. Therefore, the information matrix is block diagonal.

The block of M_F^* for the fixed effects, called block A, is given by $M_F^*(\beta, N) = \Omega^{-1}$. The block for λ^* , called block B, has the following components: $M_F^*(\lambda^*, N)_{m,l} = \frac{1}{2} \text{tr} \left(\Omega^{-1} \frac{\partial \Omega}{\partial \lambda_m^*} \Omega^{-1} \frac{\partial \Omega}{\partial \lambda_l^*} \right)$, $m, l = 1, \dots, \text{dim}(\lambda^*)$.

The population Fisher information matrix for N individuals is obtained with the above formula multiplied by N. It is then inverted to obtain SEs.

From these expressions, it can be shown that for β , the diagonal terms of block A^{-1} are then equal to $\frac{1}{N} \times \omega_{mm}^2$ in the case of additive random effect and to $\frac{1}{N} \times \omega_{mm}^2 \times \beta_m^2$ in the case of exponential random effect, $m = 1, \dots, p$. The standard errors for the fixed effects are

therefore given by

$$SE(\widehat{\beta}_m) = \frac{1}{\sqrt{N}} \times \omega_{mm}, \text{ for additive random effect,}$$

$$SE(\widehat{\beta}_m) = \frac{1}{\sqrt{N}} \times \omega_{mm} \times \beta_m, \text{ for exponential random effect, respectively.}$$

For Ω , the diagonal terms of the block B^{-1} are equal to $\frac{1}{N} \times 2 \times \omega_{mm}^4$ when the index corresponds to a variance term and to $\frac{1}{N} \times (\omega_{mm}^2 \omega_{ll}^2 + \omega_{ml}^2)$ when it corresponds to a covariance term. The SE of a variance parameter is equal to

$$SE(\widehat{\omega_{mm}^2}) = \frac{1}{\sqrt{N}} \times \sqrt{2} \times \omega_{mm}^2$$

and that of a covariance parameter is

$$SE(\widehat{\omega_{ml}}) = \frac{1}{\sqrt{N}} \times \sqrt{\omega_{mm}^2 \times \omega_{ll}^2 + \omega_{ml}^2}.$$

Similarly, the results for the correlation can be obtained:

$$SE(\widehat{\rho_{ml}}) = \frac{1}{\sqrt{N}} \times (1 - \rho_{ml}^2).$$

For variance components, the results are the same for additive and exponential random effect models.

Using this approach, the SEs can be viewed as lower limits of the expected SEs that we would get in the case of a rich design, where individual parameters are estimated rather precisely. They are the lower bound of any design Ξ with N subjects.

2.4. Implementation in PFIM and optimization

Having carried out the extension of the M_F for NLMEM with covariance between random effects, the calculation was implemented into a working version of PFIM, making it possible to evaluate and optimize designs with covariance between random effects for single- and multiple-response models. The impact of covariance size on SE and amount of information was examined. The total information was evaluated through the criterion:

$$criterion = \det(M_F)^{1/P}$$

with P being the total number of parameters in Ψ .

Optimization was performed using the Federov-Wynn algorithm implemented in PFIM. The

Federov-Wynn algorithm maximises the determinant of M_F using statistical design within a finite set of sampling times. It can be used to optimize both the structure (number of groups, number of subjects per group, number of samples per group) and the sampling times from a finite set of times (Retout *et al.*, 2007). Only one elementary design in all patients (i.e. one group, with a fixed number of samples) can be assumed.

3 EVALUATION VIA A PHARMACOKINETIC EXAMPLE IN PAEDIATRICS

3.1. Presentation of the example

In this section, the PK examples, to which the new developments will be applied, are presented. Data used to derive the models were "simulated" plasma concentration *versus* time in patients between 0 and 25 years old, obtained via the SIMCYP software using the adults-to-children extrapolation tool, the knowledge of the drug in adults, its physico-chemical properties and *in vitro* experimental data (Perdaems *et al.*, 2010). As the parent drug is metabolised into an active metabolite, concentration-time profiles of both the parent drug and its active metabolite were predicted in children. The plasma concentration-time profiles resulted from an intravenous bolus dose of 0.1 mg/kg in 400 children.

After intravenous injection in children, concentration-time data of the parent drug were described by a 3-compartment model (single-response model), which is written in ordinary differential equations as follows:

$$\begin{cases} \frac{dA_1(t)}{dt} = k_{21}A_2(t) + k_{31}A_3(t) - (k_{12} + k_{13} + k_{10})A_1(t) \\ \frac{dA_2(t)}{dt} = k_{12}A_1(t) - k_{21}A_2(t) \\ \frac{dA_3(t)}{dt} = k_{13}A_1(t) - k_{31}A_3(t) \end{cases}$$

A_1 , A_2 and A_3 being the amount of parent drug in the first, second, third compartment respectively, k_{ij} being the distribution rate constant from compartment i to compartment j . For each compartment, $C_i(t) = \frac{A_i(t)}{V_i}$ and C_1 is the concentration of the parent drug in

plasma. According to these notations, initial conditions are: $A_1(0) = Dose$, $A_2(0) = 0$, $A_3(0) = 0$.

The model is composed of 6 parameters: $CLP = k_{10} \times V_1$, which represents the elimination clearance, $Q_2 = k_{12} \times V_1 = k_{21} \times V_2$, the inter-compartmental clearance from compartment 1 to compartment 2, $Q_3 = k_{13} \times V_1 = k_{31} \times V_3$, the inter-compartmental clearance from compartment 1 to compartment 3, and V_1, V_2, V_3 the volumes of the central compartment and of the two peripheral compartments, respectively.

The distribution for the parameters was exponential. Inter-individual variabilities were found on the CLP clearance and on the volume of the central compartment V_1 and were significantly correlated. The residual error model was a combined error model. σ_{inter_P} corresponds to the additive error for the parent molecule and σ_{slope_P} corresponds to the proportional part. Table 1 shows the parameter values: as the dose was given per kilogram, all the parameters and dose are expressed by kilogram.

Regarding the multiple-response model, both parent and metabolite concentration-time profiles were described by a 4-compartment model. Figure 1 represents the model and the ordinary differential equations describing the model are presented below:

$$\begin{cases} \frac{dA_1(t)}{dt} = k_{21}A_2(t) + k_{31}A_3(t) - (k_{12} + k_{13} + k_{14} + k_{10})A_1(t) \\ \frac{dA_2(t)}{dt} = k_{12}A_1(t) - k_{21}A_2(t) \\ \frac{dA_3(t)}{dt} = k_{13}A_1(t) - k_{31}A_3(t) \\ \frac{dA_4(t)}{dt} = k_{14}A_1(t) - k_{40}A_4(t) \end{cases}$$

A_4 is the amount of metabolite and $C_4(t) = \frac{A_4(t)}{V_4}$ is the metabolite concentration in plasma. The initial conditions are the same as before, with $A_4(0) = 0$.

For reasons of model identifiability, metabolite volume (V_4) was fixed as being the sum of the three volumes of the parent, that is, $V_4 = V_1 + V_2 + V_3$. As before, CLP represents the elimination clearance for the parent, CLPM, defined as $k_{14} \times V_1$, represents the clearance from the parent to the metabolite, and CLM, defined as $k_{40} \times V_4$, represents the elimination clearance for the metabolite.

The parameter distribution was also exponential. Inter-individual variabilities were found on the CLP, CLPM and CLM clearances, and on the volume of the central compartment of the parent V_1 . As some of the corresponding random effects were significantly correlated, a full covariance matrix for those four parameters was retained. The residual error model was a combined error model. $\sigma_{inter_P}(\sigma_{inter_M})$ corresponds to the additive error for the parent molecule (metabolite, respectively) and $\sigma_{slope_P}(\sigma_{slope_M})$ its proportional part. The parameter values are presented in Table 1 and Figure 2 shows the mean PK profiles of the subjects; the parent on the left, and metabolite on the right.

3.2. Evaluation of the extension of the Fisher information matrix including covariance between random effects in PFIM

3.2.1. Methods

To evaluate the extension of M_F including covariance between random effects in PFIM for single- and multiple-responses, the results, with and without covariance, predicted by PFIM were compared with those obtained by both NONMEM and MONOLIX obtained from simulated data, using the models presented in the previous section. The parameter values used for the simulation are described in Table 1.

Data was simulated, using a rich design with 22 sampling times at 0.1, 0.2, 0.4, 0.6, 0.8, 1, 1.3, 1.6, 1.8, 2, 2.5, 3, 4, 5, 6, 7, 8, 9, 10, 12, 16 and 24 hours after dose injection, with 82 subjects and a dose equal to 0.1 mg/kg. The simulations with and without covariance were performed for both the single-response model (parent drug only) and for the multiple-response model (joint parent- and metabolite-model). In each case, only one simulation was performed because we put ourself in the framework of rich design. The model without covariance was the same as that with covariance, except for the covariance terms, which were equal to zero. Two estimation methods were used to estimate parameters: First Order Conditional Estimate with Interaction (FOCEI) method in NONMEM version 6 (Sheiner and Beal, 1998) and the Stochastic Approximation Expectation-Maximization (SAEM) algorithm in MONOLIX version 3.2 (Samson *et al.*, 2006). The estimation methods are different and so too were the calculations of M_F . NONMEM estimates the covariance and its SE,

whereas MONOLIX estimates the correlation and its SE. Estimating parameters with two different software programs provides a more accurate evaluation of the prediction using PFIM to observed SE. Parameter estimates obtained were called N and M for NONMEM and MONOLIX, respectively, whereas the parameters of the model are called the reference parameters (P) (Table 1).

The multiple-response model was implemented in PFIM, whereas the single-response model was available in the PFIM library (Dubois *et al.*, 2011). The SE of parameters predicted by PFIM for the 2 models using the reference parameter values (Table 1) were then compared to SEs obtained by NONMEM and MONOLIX. Given that MONOLIX gives the SE of correlation and not of covariance, the SE of covariance was then derived by the approach detailed in section 2.3.

Moreover, a comparison between results obtained by PopDes, the software for optimal design developed by Ogungbenro *et al.* (Gueorguieva *et al.*, 2007), and results obtained by PFIM, was performed for the multiple-response model with and without covariance.

3.2.2. Results

The objective was to compare the SEs predicted by PFIM to those obtained with both NONMEM and MONOLIX, in both cases, i.e., with and without covariance. Table 2 presents the reference parameters with and without covariance of the single-response model (parent molecule model), as well as the parameter estimates obtained with NONMEM (N) and MONOLIX (M). The SEs predicted by PFIM, and those obtained by NONMEM and MONOLIX with and without covariance for the PK model of the parent molecule, are also shown in Table 2. First of all, it can be seen that all parameters were accurately estimated and close to the reference parameter values. The SE values obtained in the case of the model without covariance with NONMEM and MONOLIX were very close to those predicted by PFIM for the single-response model. The largest difference between the SE of parameters was for Q_3 between MONOLIX and both PFIM and NONMEM, and was equal to 0.030. It corresponds to a relative standard error (RSE) equal to 12% for both PFIM and NONMEM and to 9% for MONOLIX. Similar differences were observed for the RSE of variance parameters (an absolute difference respectively equal to 3% and 4% between the RSEs for the variances of

CLP and V_1 obtained from PFIM and those obtained from MONOLIX). It can be observed that the differences between the SE of the other parameters were small (lower than 0.010). In the case of model with covariance, the SEs given by NONMEM and MONOLIX were also very close to the SE predicted by PFIM (the largest difference is still for Q_3 and is equal to 0.020 between NONMEM and MONOLIX and to 0.010 between PFIM and NONMEM). In terms of RSE, the differences between PFIM, NONMEM and MONOLIX for Q_3 were very small since the RSEs were equal to 12%, 12% and 11%, respectively. For the covariance term, the difference between the SE predicted by PFIM and the SEs obtained by both NONMEM and MONOLIX was equal to 0.0060, which is small. The main differences regarding the RSE of parameters were for the variance and covariance terms: the largest difference was equal to 10% between PFIM and NONMEM for the variance of V_1 . The SEs predicted by PFIM were similar to those obtained with NONMEM and MONOLIX, in both cases, with and without covariance, for the single-response model. Moreover, it was observed that the SEs of parameters were almost identical with and without covariance for PFIM, whether for fixed effects or variance components. The same results can be observed from the SEs obtained for both NONMEM and MONOLIX.

Table 3 displays the parameters and corresponding SE predicted by PFIM and obtained with NONMEM and MONOLIX, with and without covariance, for the PK model of parent and metabolite. The same conclusions can be drawn with the multiple-response model. As for the single-response model, it can be noted that PFIM accurately predicted the SE with and without covariance when compared to both NONMEM and MONOLIX. For some covariance terms corresponding to very small correlations, it was observed that the parameter values estimated by NONMEM and MONOLIX were different from the simulated values. However, the SEs of the parameters that were predicted by PFIM were in a similar range to those obtained with NONMEM and MONOLIX, with and without covariance, for the multiple-response model. Same conclusions are observed for the RSE. Moreover, the predictions were identical for the fixed effects and variance parameters, with and without covariance.

It has therefore been demonstrated that the development of M_F for covariance terms and its implementation in a working version of PFIM in R were satisfactory because the SEs of

parameters predicted by PFIM were similar to those obtained with NONMEM and MONOLIX, and that was also true for the SE of covariance terms. Table 4 also shows a comparison of RSEs obtained by PopDes and RSEs obtained by PFIM, for the multiple-response model with and without covariance. This evaluation shows the appropriateness of the extension of the population Fisher information matrix with covariance in PFIM. Moreover, it was observed that the results with covariance were similar to those without covariance for the SE and RSE of fixed effects and variance components.

3.3. Evaluation of approximation of the Fisher information matrix for rich design

To evaluate the results of the approximation of M_F in the framework of individual-rich data, the SEs obtained by analytical predictions, using the formula of section 2.3 assuming $N = 82$, were compared with those predicted by PFIM for both single- and multiple-response models for the parameters with inter-individual variability (Table 5). This approach can only be applied to parameters with non-null variance of random effects.

The analytical SEs were very close to the SE predicted by PFIM for both single- and multiple-response models. The results obtained by analytical predictions assuming rich data were similar to those given by PFIM using the model on a rich design and the same number of patients. They represent the lower limits of what could be observed with a sparse design.

3.4. Influence of covariance on designs

3.4.1 Methods

First, the impact of the size of covariance on the SEs, the RSEs and the amount of information for the single-response model (parent-drug model) was considered. Using PFIM, the SE and the RSE on fixed effects and variance components assuming different values of covariance between the two random effects, i.e. CLP and V_1 , were predicted. The influence of the covariance on SE, RSE and amount of information was tested on a range of covariance

from -0.12 to 0.12 (correlation from -0.9 to 0.9).

Second, the sampling time optimization for both the single- and multiple-response models with and without covariance is taken into consideration. The impact of covariance was studied on an optimal design with 6 sampling times out of 12 for the single-response model and with 4 out of 12 for the multiple-response model. For the latter, both response measurements (i.e. parent and metabolite concentrations) were carried out at each sampling time, i.e. a total of 8 measurements. The number of measurements was chosen depending on the number of fixed effects, 6 for the parent model and 8 for the joint parent and metabolite model. The design domain took place over a 24-hour period with allowed sampling times at 0.1, 0.2, 0.4, 0.8, 1, 2, 4, 6, 8, 12, 16 and 24 hours after dose injection.

Sampling time optimization was performed using the Federov-Wynn algorithm implemented in PFIM, which makes it possible to test for design with only one group (*one*) or with several groups (*several*). Optimal designs for the single-response model (*single*) and the multiple-response model (*multiple*) without covariance (*nocov*) were studied, as well as the covariance values of Table 1 (*cov*). For the single-response model, which had a covariance equal to 0.10, design was optimized for a covariance of 0.12 (*highcov*), corresponding to a correlation of 0.9. Optimizing design with the highest covariance value makes it possible to be in the furthest case and thus to have an idea of the impact of the covariance value when the latter is high. The corresponding designs were called $\Xi_{single_nocov_one}$, $\Xi_{single_cov_one}$, $\Xi_{single_highcov_one}$, $\Xi_{single_nocov_several}$, $\Xi_{single_cov_several}$, $\Xi_{single_highcov_several}$ for the single-response model and $\Xi_{multi_nocov_one}$, $\Xi_{multi_cov_one}$, $\Xi_{multi_nocov_several}$ and $\Xi_{multi_cov_several}$ for the multiple-response model. Then, the optimized designs obtained with and without covariance were evaluated considering a model with a covariance equal to 0.12 for the single-response model and with covariance terms of Table 1 for the multiple-response model. Therefore, the optimized designs obtained with and without covariance were then evaluated taking into consideration a model with covariance, i.e. the same model was used with these two different designs, concerning the parent in a first time and concerning both parent and metabolite in a second time. The focus was then on the relative standard error (RSE) of parameters and the criterion.

3.4.2 Results

As expected, regardless of the value of covariance between -0.12 and 0.12, the SEs and the RSEs for fixed effects, variances and error terms were similar (results not shown). Figure 3 shows how the covariance had an impact on the SE and the RSE of covariance and the amount of information. The SEs of covariance increased for large values of covariance, but were similar for covariance between -0.03 and 0.03. On the other hand, the RSE decreased and the amount of information increased for large value of covariance.

Focus on the influence of covariance on optimal designs: for the single-response model and one group, the optimized designs with 6 times were identical for a covariance equal to 0 and equal to 0.10 (covariance value of Table 1) and the optimal sampling times were 0.1, 0.4, 0.8, 2, 6 and 16 hours after dose injection ($\Xi_{single_nocov_one}$ and $\Xi_{single_cov_one}$). For the $\Xi_{single_highcov_one}$ design, where $cov = 0.12$, the optimal sampling times were the same as those of the $\Xi_{single_nocov_one}$ and $\Xi_{single_cov_one}$ designs, except for the second sampling time which was 0.2 instead of 0.4. These designs were then evaluated taking into consideration a model with covariance equal to 0.12. Figure 4 shows the RSE of parameters obtained for each design. It can be seen that the RSEs were very similar between these two evaluations. The criterion, was equal to 1955 for evaluation of the $\Xi_{single_nocov_one}$ and $\Xi_{single_cov_one}$ designs, and 1971 for evaluation of the $\Xi_{single_highcov_one}$ design. So, the efficiency loss was minor as it was below 1%.

Concerning the comparison of the optimal $\Xi_{single_nocov_several}$ and $\Xi_{single_highcov_several}$ designs: Table 6 shows the elementary design and the number of subjects for the two different designs. As previously demonstrated, several optimal sampling times obtained were not the same, whether taking into consideration the covariance or not. However, similar to the optimizations with one group, when the criteria were compared between the $\Xi_{single_nocov_several}$ and $\Xi_{single_highcov_several}$ designs, respectively, equal to 1996 and 2025, the efficiency loss was very low (1.5%). Therefore, even if the optimal designs were not exactly the same with or without covariance, the design efficiency was barely affected.

Focus on the influence of the covariance on optimal designs for the multiple-response model: one optimal sampling time differed between the $\Xi_{multi_nocov_one}$ and the $\Xi_{multi_cov_one}$

designs. The $\Xi_{multi_nocov_one}$ design was composed of optimal sampling times at 0.2, 2, 8 and 24 hours after dose injection and in the $\Xi_{multi_cov_one}$ design, the second sampling time was not at 0.2 but rather at 0.1. Then, these two optimal designs were evaluated with a covariance term model. It was observed that RSEs were very similar between the $\Xi_{multi_nocov_one}$ and $\Xi_{multi_cov_one}$ designs, be it for fixed effect parameters, variance parameters or covariance terms. Moreover, when the two criteria obtained for these two designs were compared, they were equal to 1612 ($\Xi_{multi_nocov_one}$ design) and 1622 ($\Xi_{multi_cov_one}$ design), respectively. Therefore, the efficiency loss here was still below 1%.

Considering several groups of subjects with 4 sampling times, Table 7 shows the different elementary designs with the corresponding number of subjects. The number of groups was different and some groups were present in the $\Xi_{multi_nocov_several}$ design and not in the $\Xi_{multi_cov_several}$ design and vice versa. Evaluating these designs with a covariance model, the RSEs of $\Xi_{multi_nocov_several}$ design and the RSEs of $\Xi_{multi_cov_several}$ design were very similar. Similarly, the criteria for these two designs were equal to 1747 and 1758, respectively. Therefore, the efficiency loss here is still minor (lower than 1%).

4 DISCUSSION

The first objective was to extend the Fisher information matrix in PFIM software for covariance between random effects and to evaluate it. For the evaluation, a comparison of the SEs of parameters predicted by PFIM and those given by two nonlinear mixed effect estimation software, NONMEM and MONOLIX, was carried out. To that end, data were simulated, using a rich design with 22 sampling times, a dose equal to 0.1 mg/kg in 82 individuals and with the single-response model and a more complex joint parent and metabolite model. Only one simulation was performed, it should not affect the results because it is a rich design with many subjects, so only very little changes are expected across replication. Also, the objective was the comparison between observed SE and predicted SE and not an evaluation of empirical SE. This comparison showed agreement between results predicted by PFIM and those obtained by both the NONMEM and MONOLIX software.

To carry out an optimal design, it is necessary to have *a priori* information, and in this

paediatric example, the *a priori* information was model and parameters obtained from data generated by the SIMCYP software. The population PK models were fitted to the predicted SIMCYP data, which were "simulated" plasma concentration *versus* time for a parent drug and its active metabolite.

Then, the impact of the size of covariance was studied. In their work, Ogungbenro *et al.* (2008) showed that RSE were very similar for fixed effect parameters, while for variance parameters, they are slightly higher for design without covariance compared to design with covariance, especially for pharmacodynamic parameters. Regarding the SEs for the present work, they did not differ for the fixed parameters or the variance parameters, no matter the size of the covariance. Similarly, regardless of the value of covariance, the RSEs did not differ for the fixed parameters or the variance parameters. Therefore, it is perhaps not necessarily useful to incorporate covariance in design stage for those parameters. But further comparisons would be needed. On the other hand, it was observed that the SE of covariance increased when covariance was large whereas the RSE of covariance decreased. Concerning the amount of information, it increased for very large values of covariance. The amount of information was lower without covariance, which means that the corresponding M_F was a lower limit of the information computed with covariance terms.

Concerning the approximation of the calculation of the SEs without using the model, which mimics the case of individual-rich data, it was possible to obtain an idea of the precision of estimates quickly, and to see if the number of subjects was large enough. Indeed, these analytical predictions were the lower limits of the SEs that could be obtained by the population approach, and could not be improved by increasing the number of samples per patient.

Focusing then on the influence of covariance on optimal designs, as Ogungbenro *et al.* (2008) demonstrated, it was shown that optimal design depends on the covariance between parameters. Indeed, the values of covariance terms can affect optimal sampling times, however it was rather slight in the case of a one-group design. When considering several groups of subjects, optimal sampling times and groups can be different with and without covariance, but the efficiency loss was very low for both one group and several groups. Further comparisons should be performed. Also, the design variables chosen in this work were the

location of sampling times but it would be possible to extend to other design variables, as for example the dose (Nyberg *et al.*, 2009).

In the present work, the calculation of M_F was proposed using a first-order linearization of the model by the Taylor expansion. The derivative of the variance of observations according to β was assumed to be zero. Therefore, M_F was expressed as a block diagonal matrix. An extension of M_F considering the dependence of the observations on the parameters of the model, that is, a complete M_F with an additional off-diagonal block, was also proposed (Retout and Mentré, 2003). However, Mentré *et al.* (2011) showed on two examples that if a linearization of the model was carried out, it is better to have a block diagonal expression for M_F . Mielke and Schwabe (2010) also showed that an approach with block diagonal M_F was more reliable than one with the full M_F . But it is not the case for all situations, as shown by Nyberg *et al.* (2008). In the MONOLIX software, the observed M_F calculated by linearization of the model also had a block diagonal expression. It is known that linearization can lead to problems when a very nonlinear model is used. An alternative to linearization, for example the Gaussian quadrature (Guedj *et al.*, 2007; Nguyen *et al.*, 2011), could also be developed for models with covariance.

In conclusion, the extension of M_F including covariance between random effects in PFIM provides a useful computing tool for design evaluation and optimization, and this extension will be available in the next version of PFIM. The size of covariance had no impact on the SE of fixed effects or variance parameters. Omitting the covariance at the design stage influenced the optimal sampling times, but barely had an influence on efficiency, and predicted SEs were only slightly changed.

5 APPENDIX

The Fisher information matrix $M_F(\Psi, \xi_i)$ for multiple-response models for the individual i with design ξ_i is given by $M_F(\Psi, \xi_i) = E(-\frac{\partial^2 L_i(\Psi; Y_i)}{\partial \Psi \partial \Psi^T})$, where $L_i(\Psi; Y_i)$ is the log-likelihood of the vector of observations Y_i of that individual for the population parameters Ψ . Because F is nonlinear, there is no analytical expression for the log-likelihood and a first-order Taylor expansion of the model $F(\theta_i, \xi_i) = F(g(\beta, b_i), \xi_i)$, around the expectation of b_i , that is to say

around 0, is used: $F(g(\beta, b_i), \xi_i) \cong F(g(\beta, 0), \xi_i) + (\frac{\partial F^T(g(\beta, b_i), \xi_i)}{\partial b_i})_{b_i=0} b_i$.

Therefore, $Y_i \cong F(g(\beta, 0), \xi_i) + (\frac{\partial F^T(g(\beta, b_i), \xi_i)}{\partial b_i})_{b_i=0} b_i + \varepsilon_i$.

Using this linearization, the approximated marginal expectation E_i and variance V_i of Y_i are given by:

$$E(Y_i) \cong E_i = F(g(\beta, 0), \xi_i)$$

$$Var(Y_i) \cong V_i = (\frac{\partial F^T(g(\beta, b_i), \xi_i)}{\partial b_i})_{b_i=0} \Omega (\frac{\partial F(g(\beta, b_i), \xi_i)}{\partial b_i^T})_{b_i=0} + \Sigma(\beta, 0, \sigma_{inter}, \sigma_{slope}, \xi_i)$$

The log-likelihood L_i is approximated by:

$$-2L_i(\Psi; Y_i) \cong n_i \ln(2\pi) + \ln(|V_i|) + (Y_i - E_i)^T V_i^{-1} (Y_i - E_i)$$

The elementary Fisher information matrix for a multiple-response model with non-diagonal variance matrix is derived from this expression of the log-likelihood. In the following, the i index for the individual is omitted for the sake of simplicity.

$$M_F(\Psi, \xi) = E_Y(-\frac{\partial^2(L(\Psi, Y))}{\partial \Psi \partial \Psi^T})$$

The elementary M_F depends on the approximated marginal expectation E and variance V of the observations. Assuming that the derivative of V does or does not depend on the fixed effects, the elementary M_F is a full matrix or a block diagonal matrix. It was suggested (Mentré *et al.*, 2011; Mielke and Schwabe, 2010) that for derivation of M_F using first-order linearization, the block diagonal expression might be better. This is not always the case, as presented by Nyberg *et al.* (2008). Here, the block diagonal expression for M_F was chosen and is expressed as:

$$M_F(\Psi, \xi) \cong \frac{1}{2} \begin{pmatrix} A(E, V) & 0 \\ 0 & B(E, V) \end{pmatrix}$$

where

$$(A(E, V))_{ml} = 2 \frac{\partial F^T}{\partial \beta_m} V^{-1} \frac{\partial F}{\partial \beta_l}$$

with m and $l = 1, \dots, p$ and

$$(B(E, V))_{ml} = tr(\frac{\partial V}{\partial \lambda_m} V^{-1} \frac{\partial V}{\partial \lambda_l} V^{-1})$$

with m and $l = 1, \dots, \dim(\lambda)$.

The calculations of the Fisher information matrix with all covariance terms in Ω were developed. For block A, it is the same calculation with and without covariance, except that the Ω matrix is full when covariance is considered. On the other hand, the calculations are different for block B. Indeed

$$\frac{\partial V}{\partial \lambda_m} = \left(\frac{\partial F^T(g(\beta, b), \xi)}{\partial b} \right)_{b=0} \frac{\partial \Omega}{\partial \lambda_m} \left(\frac{\partial F(g(\beta, b), \xi)}{\partial b^T} \right)_{b=0} + \left(\frac{\partial \Sigma(\beta, b, \sigma_{inter}, \sigma_{slope}, \xi)}{\partial \lambda_m} \right)_{b=0}$$

with $m = 1, \dots, \dim(\lambda)$.

And depending on whether m corresponds to a variance or covariance term, $\frac{\partial \Omega}{\partial \lambda_m}$ contains one or two terms different from zero, respectively. For a variance term, that is for λ_1 to λ_p (corresponding to ω_{11}^2 to ω_{pp}^2), the general term for the derivation of the matrix V according to λ_m , m varying from 1 to p , for one response, is expressed as:

$$\frac{\partial f_k(g(\beta, 0), t_j)}{\partial b_m} \frac{\partial f_k(g(\beta, 0), t_{j'})}{\partial b_m}$$

with $j = 1, \dots, n_k$ the line index, $j' = 1, \dots, n_k$ the column index and k corresponding to the k^{th} response.

For a covariance term from λ_{p+1} to $\lambda_{p(p+1)/2}$ (corresponding to ω_{21} to $\omega_{p,p-1}$), the derivation of the matrix V, for one response, according to λ_m is expressed as:

$$\frac{\partial f_k(g(\beta, 0), t_j)}{\partial b_r} \frac{\partial f_k(g(\beta, 0), t_{j'})}{\partial b_s} + \frac{\partial f_k(g(\beta, 0), t_j)}{\partial b_s} \frac{\partial f_k(g(\beta, 0), t_{j'})}{\partial b_r}$$

with $r, s = 1, \dots, p$, $r \neq s$, $j = 1, \dots, n_k$ the line index, $j' = 1, \dots, n_k$ the column index, and k corresponding to the k^{th} response.

Note that $\left(\frac{\partial \Sigma(\beta, b, \sigma_{inter}, \sigma_{slope}, t_j)}{\partial \lambda_m} \right)_{b=0}$ is equal to zero when m does not correspond to an item of an error parameter. Otherwise, the derivation of the matrix V according to λ_m , $\lambda_m = \sigma_{inter_k}$, is equal to $2 \times \text{diag}(\sigma_{inter} + \sigma_{slope} \times f_k(g(\beta, 0), t_j))$ and the derivation of the matrix V according to $\lambda_m = \sigma_{slope_k}$ is $2 \times \text{diag}(f_k(g(\beta, 0), t_j)(\sigma_{inter} + \sigma_{slope} \times f_k(g(\beta, 0), t_j)))$, with $j = 1, \dots, n_k$ and k corresponding to the k^{th} response.

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Table 1: Population pharmacokinetic parameter values for the parent drug and the joint model of the parent drug and its metabolite

Parameters (units)	Parameter values	
	Parent drug	Joint model
β_{CLP} ($L.h^{-1}.kg^{-1}$)	0.81	0.61
β_{CLPM} ($L.h^{-1}.kg^{-1}$)	-	0.11
β_{V_1} ($L.kg^{-1}$)	0.88	0.97
β_{Q_2} ($L.h^{-1}.kg^{-1}$)	0.58	0.12
β_{V_2} ($L.kg^{-1}$)	1.0	0.41
β_{Q_3} ($L.h^{-1}.kg^{-1}$)	1.3	0.69
β_{V_3} ($L.kg^{-1}$)	0.39	0.87
β_{CLM} ($L.h^{-1}.kg^{-1}$)	-	0.91
ω_{CLP}^2	0.23	0.25
ω_{CLPM}^2	-	1.4
$\omega_{V_1}^2$	0.075	0.070
ω_{CLM}^2	-	0.27
ω_{CLP,V_1}	0.10	0.045
$\omega_{CLP,CLPM}$	-	0.036
$\omega_{V_1,CLPM}$	-	0.024
$\omega_{CLP,CLM}$	-	0.091
$\omega_{V_1,CLM}$	-	0.083
$\omega_{CLPM,CLM}$	-	-0.045
σ_{inter_P} ($\mu g.L^{-1}$)	0.22	0.072
σ_{slope_P}	0.10	0.16
σ_{inter_M} ($\mu g.L^{-1}$)	-	0.011
σ_{slope_M}	-	0.053

Table 2: SE of the parameters with and without covariance predicted by PFIM (P), and obtained by NONMEM (N) and MONOLIX (M) on a simulated data set for the parent molecule and the rich design in 82 patients (22 observations per patient)

Parameters	Model without covariance						Model with covariance					
	Reference parameters	Estimated parameters		Predicted SE	Obtained SE		Reference parameters	Estimated parameters		Predicted SE	Obtained SE	
	P	N	M	P	N	M	P	N	M	P	N	M
β_{CLP}	0.81	0.87	0.87	0.043	0.052	0.051	0.81	0.88	0.87	0.043	0.053	0.053
β_{V_1}	0.88	0.88	0.88	0.037	0.037	0.037	0.88	0.91	0.89	0.037	0.042	0.040
β_{Q_2}	0.58	0.56	0.53	0.031	0.029	0.030	0.58	0.55	0.54	0.031	0.034	0.030
β_{V_2}	1.0	1.0	1.0	0.024	0.022	0.025	1.0	1.0	1.0	0.024	0.026	0.025
β_{Q_3}	1.3	1.3	1.3	0.15	0.15	0.12	1.3	1.4	1.4	0.15	0.16	0.14
β_{V_3}	0.39	0.39	0.41	0.027	0.021	0.026	0.39	0.40	0.41	0.027	0.025	0.027
ω_{CLP}^2	0.23	0.28	0.28	0.036	0.038	0.044	0.23	0.30	0.30	0.036	0.039	0.047
$\omega_{V_1}^2$	0.075	0.086	0.088	0.013	0.015	0.016	0.075	0.097	0.098	0.013	0.020	0.017
$\omega_{CLP, V_1}(\rho_{CLP, V_1})$	-	-	-	-	-	-	0.10(0.78)	0.14(0.84)	0.14(0.84)	0.019	0.025	0.025
σ_{interP}	0.22	0.28	0.22	0.011	0.011	0.011	0.22	0.28	0.22	0.011	0.011	0.011
σ_{slopeP}	0.10	0.13	0.11	0.0020	0.0030	0.0030	0.10	0.13	0.11	0.0025	0.0025	0.0028

Table 3: SE of the parameters with and without covariance predicted by PFIM (P), and obtained by NONMEM (N) and MONOLIX (M) on a simulated data set for parent and metabolite and the rich design in 82 patients (22 observations per patient)

Parameters	Model without covariance						Model with covariance											
	Reference parameters		Estimated parameters		Predicted SE		Obtained SE		Reference parameters		Estimated parameters		Predicted SE		Obtained SE			
	P	N	M	P	N	M	P	N	M	P	N	M	P	N	M			
β_{CLP}	0.61	0.67	0.67	0.034	0.041	0.040	0.61	0.68	0.67	0.034	0.041	0.041	0.61	0.68	0.67	0.034	0.041	0.041
β_{V_1}	0.97	0.97	0.95	0.030	0.028	0.027	0.97	0.98	0.98	0.030	0.026	0.026	0.97	0.98	0.98	0.030	0.026	0.026
β_{Q_2}	0.12	0.098	0.13	0.044	0.038	0.045	0.12	0.11	0.14	0.044	0.041	0.047	0.12	0.11	0.14	0.044	0.041	0.047
β_{V_2}	0.41	0.37	0.44	0.093	0.079	0.089	0.41	0.39	0.47	0.093	0.083	0.094	0.41	0.39	0.47	0.093	0.083	0.094
β_{Q_3}	0.69	0.69	0.65	0.037	0.031	0.038	0.69	0.68	0.65	0.037	0.034	0.041	0.69	0.68	0.65	0.037	0.034	0.041
β_{V_3}	0.87	0.89	0.80	0.097	0.085	0.094	0.87	0.86	0.78	0.097	0.089	0.099	0.87	0.86	0.78	0.097	0.089	0.099
β_{CLPM}	0.11	0.11	0.11	0.015	0.015	0.015	0.11	0.11	0.11	0.015	0.015	0.016	0.11	0.11	0.11	0.015	0.015	0.016
β_{CLM}	0.91	0.93	0.91	0.054	0.048	0.050	0.91	0.95	0.95	0.054	0.048	0.050	0.91	0.95	0.95	0.054	0.048	0.050
ω_{CLP}^2	0.25	0.30	0.29	0.040	0.039	0.046	0.25	0.30	0.30	0.040	0.037	0.046	0.25	0.30	0.30	0.040	0.037	0.046
$\omega_{V_1}^2$	0.070	0.063	0.056	0.012	0.012	0.010	0.070	0.053	0.048	0.012	0.011	0.0090	0.070	0.053	0.048	0.012	0.011	0.0090
ω_{CLPM}^2	1.4	1.6	1.6	0.21	0.22	0.25	1.4	1.6	1.6	0.21	0.22	0.25	1.4	1.6	1.6	0.21	0.22	0.25
ω_{CLM}^2	0.27	0.23	0.23	0.043	0.036	0.036	0.27	0.21	0.21	0.043	0.039	0.034	0.27	0.21	0.21	0.043	0.039	0.034
$\omega_{(CLP,V_1)}(\rho_{CLP,V_1})$	-	-	-	-	-	-	0.045(0.34)	0.044(0.35)	0.043(0.36)	0.016	0.012	0.014	0.045(0.34)	0.044(0.35)	0.043(0.36)	0.016	0.012	0.014
$\omega_{CLP,CLPM}(\rho_{CLP,CLPM})$	-	-	-	-	-	-	0.036(0.061)	0.13(0.19)	0.12(0.18)	0.065	0.073	0.077	0.036(0.061)	0.13(0.19)	0.12(0.18)	0.065	0.073	0.077
$\omega_{V_1,CLPM}(\rho_{V_1,CLPM})$	-	-	-	-	-	-	0.024(0.078)	-0.015(-0.052)	-0.0050(-0.018)	0.036	0.040	0.031	0.024(0.078)	-0.015(-0.052)	-0.0050(-0.018)	0.036	0.040	0.031
$\omega_{CLP,CLM}(\rho_{CLP,CLM})$	-	-	-	-	-	-	0.091(0.35)	0.082(0.33)	0.081(0.32)	0.031	0.028	0.029	0.091(0.35)	0.082(0.33)	0.081(0.32)	0.031	0.028	0.029
$\omega_{V_1,CLM}(\rho_{V_1,CLM})$	-	-	-	-	-	-	0.083(0.61)	0.052(0.49)	0.049(0.49)	0.019	0.017	0.012	0.083(0.61)	0.052(0.49)	0.049(0.49)	0.019	0.017	0.012
$\omega_{CLPM,CLM}(\rho_{CLPM,CLM})$	-	-	-	-	-	-	-0.045(-0.075)	-0.14(-0.23)	-0.13(-0.22)	0.068	0.073	0.066	-0.045(-0.075)	-0.14(-0.23)	-0.13(-0.22)	0.068	0.073	0.066
σ_{inter_P}	0.072	0.095	0.076	0.0061	0.0045	0.0052	0.072	0.094	0.076	0.0061	0.0047	0.0052	0.072	0.094	0.076	0.0061	0.0047	0.0052
σ_{slope_P}	0.16	0.19	0.17	0.0032	0.0031	0.0036	0.16	0.19	0.17	0.0032	0.0030	0.0036	0.16	0.19	0.17	0.0032	0.0030	0.0036
σ_{inter_M}	0.011	0.015	0.011	0.00082	0.00073	0.00062	0.011	0.014	0.012	0.00082	0.00064	0.00062	0.011	0.014	0.012	0.00082	0.00064	0.00062
σ_{slope_M}	0.053	0.057	0.049	0.0014	0.0012	0.0013	0.053	0.057	0.049	0.0014	0.0011	0.0013	0.053	0.057	0.049	0.0014	0.0011	0.0013

Table 4: RSE (%) of the parameters with and without covariance predicted by PopDes and PFIM for the parent and metabolite and the rich design in 82 patients (22 observations per patient)

Parameters	Parameter values	Model without covariance		Model with covariance		
		PopDes	PFIM	Parameter values	PopDes	PFIM
β_{CLP}	0.61	5.6	5.6	0.61	5.6	5.6
β_{V_1}	0.97	3.0	3.1	0.97	3.0	3.1
β_{Q_2}	0.12	32	38	0.12	32	38
β_{V_2}	0.41	20	23	0.41	20	23
β_{Q_3}	0.69	4.7	5.4	0.69	4.7	5.4
β_{V_3}	0.87	9.7	11	0.87	9.7	11
β_{CLPM}	0.11	13	13	0.11	13	13
β_{CLM}	0.91	5.8	5.8	0.91	5.8	5.8
ω_{CLP}^2	0.25	16	16	0.25	16	16
$\omega_{V_1}^2$	0.070	17	17	0.070	17	17
ω_{CLPM}^2	1.4	16	16	1.4	16	16
ω_{CLM}^2	0.27	16	16	0.27	16	16
$\omega_{(CLP,V_1)}$	-	-	-	0.045	35	36
$\omega_{CLP,CLPM}$	-	-	-	0.036	183	183
$\omega_{V_1,CLPM}$	-	-	-	0.024	147	148
$\omega_{CLP,CLM}$	-	-	-	0.091	34	34
$\omega_{V_1,CLM}$	-	-	-	0.083	22	22
$\omega_{CLPM,CLM}$	-	-	-	-0.045	151	151
σ_{inter_P}	0.072	7.7	8.3	0.072	7.7	8.3
σ_{slope_P}	0.16	1.8	2.0	0.16	1.8	2.0
σ_{inter_M}	0.011	5.1	5.3	0.011	5.1	5.3
σ_{slope_M}	0.053	2.0	2.4	0.053	2.0	2.4

Table 5: Comparison of SEs obtained by analytical predictions and SEs predicted by PFIM for both single- and multiple-response models for the parameters with inter-individual variability, in the framework of rich design in 82 patients (22 observations per patient)

Parameters	Single-response model			Multiple-response model		
	Parameter values	Analytical predictions	PFIM predictions	Parameter values	Analytical predictions	PFIM predictions
β_{CLP}	0.81	0.043	0.043	0.61	0.034	0.034
β_{V_1}	0.88	0.027	0.027	0.97	0.028	0.028
β_{CLPM}	-	-	-	0.11	0.014	0.014
β_{CLM}	-	-	-	0.91	0.052	0.052
ω_{CLP}^2	0.23	0.036	0.036	0.25	0.039	0.039
$\omega_{V_1}^2$	0.075	0.012	0.012	0.070	0.011	0.011
ω_{CLPM}^2	-	-	-	1.4	0.22	0.22
ω_{CLM}^2	-	-	-	0.27	0.042	0.042
$\omega_{(CLP,V_1)}$	0.10	0.018	0.018	0.045	0.015	0.015
$\omega_{CLP,CLPM}$	-	-	-	0.036	0.065	0.065
$\omega_{V_1,CLPM}$	-	-	-	0.024	0.035	0.035
$\omega_{CLP,CLM}$	-	-	-	0.091	0.030	0.030
$\omega_{V_1,CLM}$	-	-	-	0.083	0.018	0.018
$\omega_{CLPM,CLM}$	-	-	-	-0.045	0.068	0.068

Table 6: Optimal designs with 6 points with several groups for the single-response model: $\Xi_{single_nocov_several}$ design is for a model with covariance = 0 and $\Xi_{single_highcov_several}$ design for a model with covariance = 0.12

Time (in hour)	Number of subjects	
	$\Xi_{single_nocov_several}$	$\Xi_{single_highcov_several}$
0.1, 0.2, 0.4, 2, 6, 16	0	16
0.1, 0.2, 0.8, 2, 6, 16	0	28
0.1, 0.2, 0.8, 4, 12, 16	5	22
0.1, 0.4, 0.8, 2, 4, 12	5	14
0.1, 0.4, 0.8, 2, 6, 16	52	0
0.1, 0.4, 1, 4, 12, 16	18	0
0.1, 0.4, 2, 6, 16, 24	2	2

Table 7: Optimal designs with 4 points with several groups for the multiple-response model: $\Xi_{multi_nocov_several}$ design is for a model with every covariance = 0 and $\Xi_{multi_cov_several}$ design with at least one covariance $\neq 0$ (see Table 1)

Time (in hour)	Number of subjects	
	$\Xi_{multi_nocov_several}$	$\Xi_{multi_cov_several}$
0.1, 0.8, 4, 12	22	21
0.1, 0.8, 12, 24	3	2
0.1, 2, 6, 16	0	5
0.1, 2, 8, 24	37	42
0.1, 2, 12, 24	0	2
0.1, 6, 16, 24	14	10
0.2, 2, 8, 24	1	0
0.8, 4, 12, 24	4	0
2, 6, 16, 24	1	0

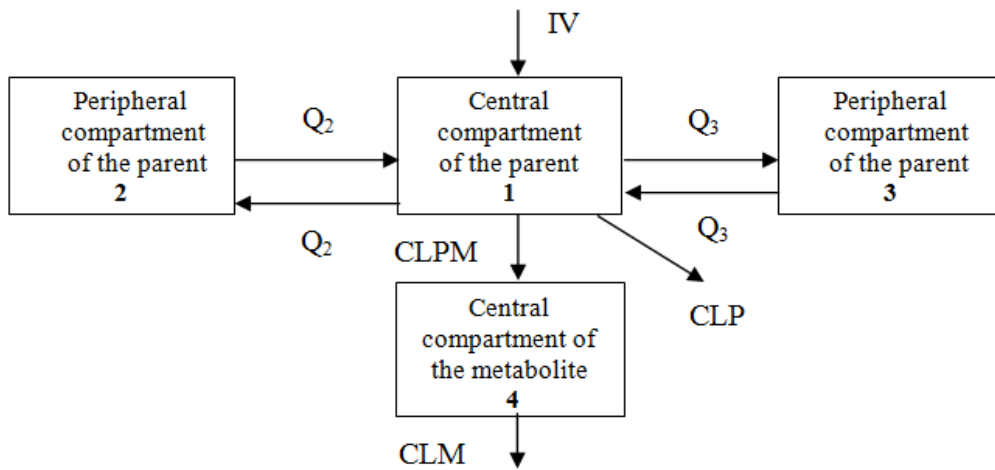


Figure 1: Structural PK model with 4 compartments: 3 compartments for the parent drug and one additional compartment for the metabolite.

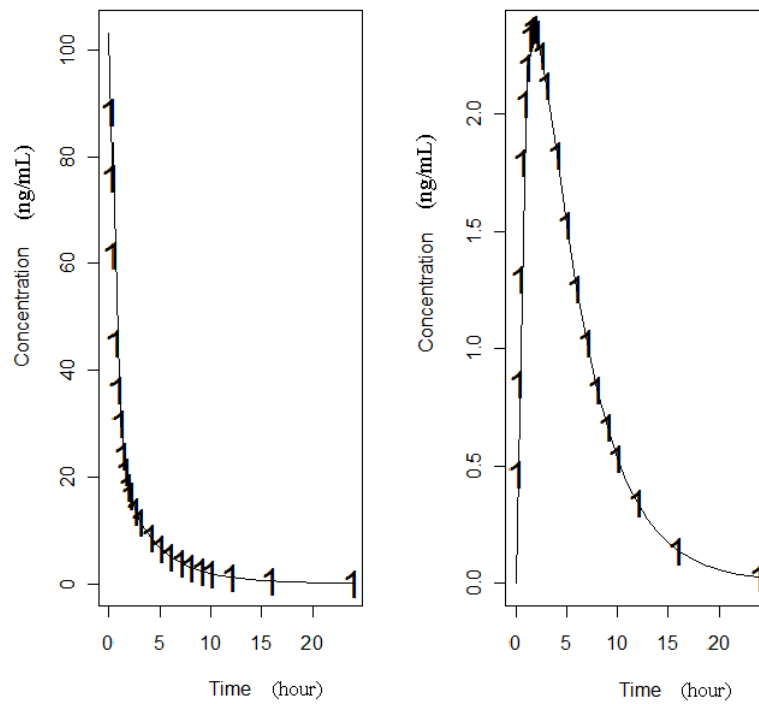


Figure 2: Mean concentration-time profiles for the parent (left) and for the metabolite (right) for 22 sampling times per subject.

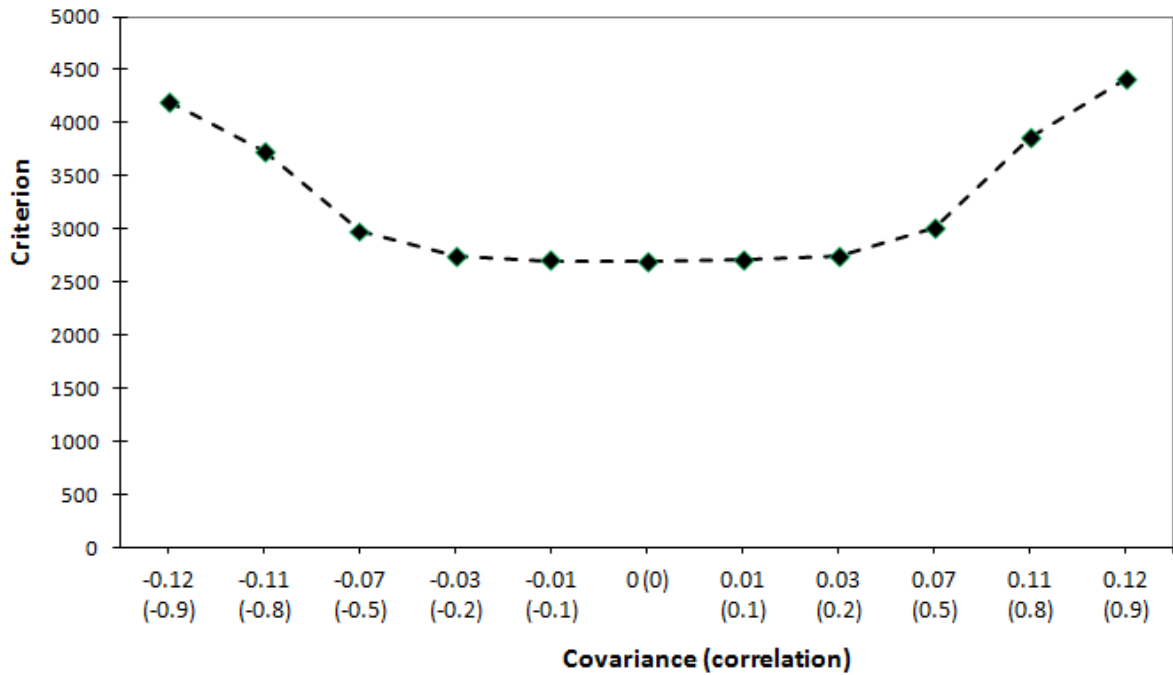
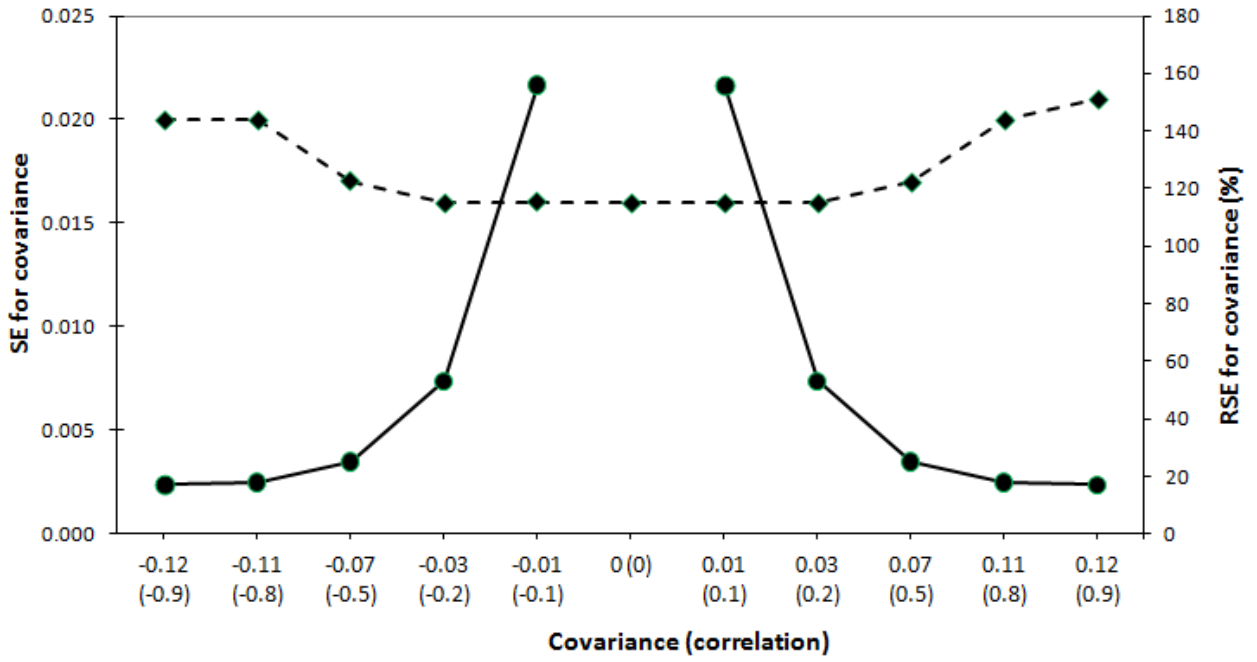


Figure 3: Influence of covariance on SE (left, \blacklozenge) and RSE (right, \bullet) of covariance for the single-response model (top), and influence of covariance on criterion for the single-response model (bottom).

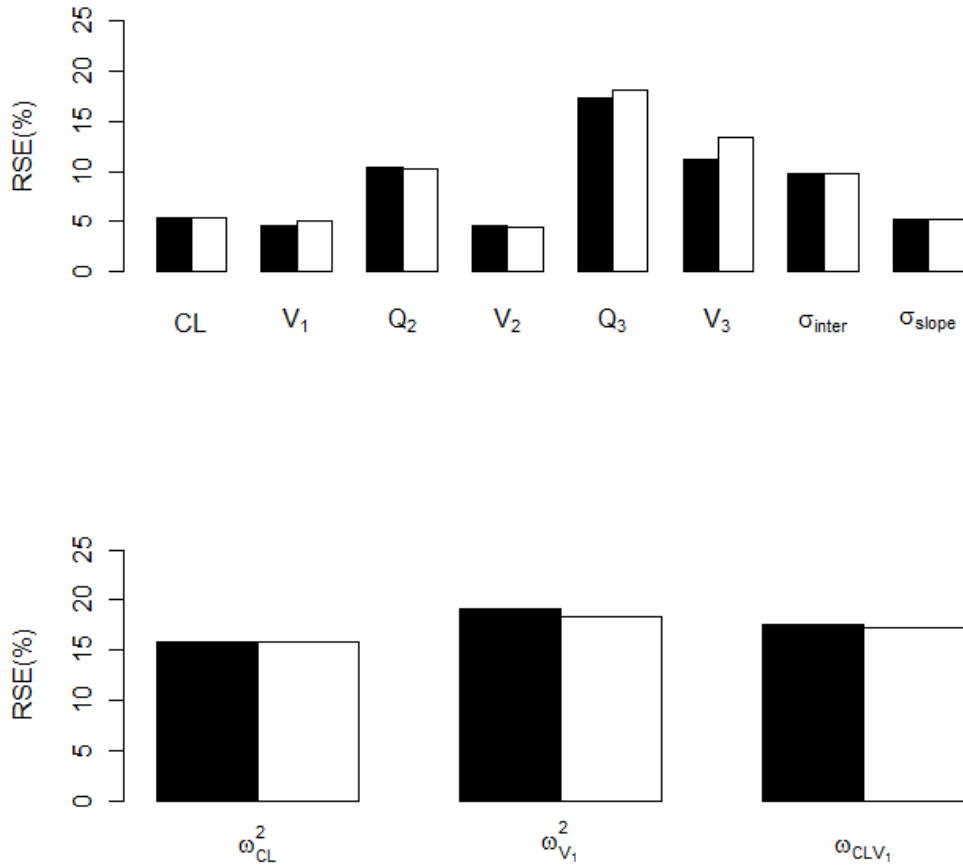


Figure 4: Predicted RSE (%) of fixed effect parameters (top) and variance components (bottom) for $\Xi_{single_nocov_one}$ and $\Xi_{single_cov_one}$ designs in black (optimized with a covariance = 0 and with a covariance = 0.10) and the $\Xi_{single_highcov_one}$ design in white (optimized with covariance = 0.12) for the single-response model with covariance = 0.12.