HAL
open science

Design evaluation and optimisation in multiple response nonlinear mixed effect models: PFIM 3.0

Caroline Bazzoli, Sylvie Retout, France Mentré

To cite this version:

Caroline Bazzoli, Sylvie Retout, France Mentré. Design evaluation and optimisation in multiple response nonlinear mixed effect models: PFIM 3.0. Computer Methods and Programs in Biomedicine, 2010, 98 (1), pp.55-65. 10.1016/j.cmpb.2009.09.012 . inserm-00431457

HAL Id: inserm-00431457

https://inserm.hal.science/inserm-00431457

Submitted on 12 Nov 2009

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

*Manuscript

Design evaluation and optimisation in multiple response nonlinear mixed effect models: PFIM 3.0

Caroline Bazzoli*, Sylvie Retout, France Mentré

INSERM, U738, Paris, France; Université Paris Diderot, UFR de Médecine, Paris, France

*corresponding author: 16 rue Henri Huchard, 75018 Paris, France

Tel: 33 1 57 27 75 38, Fax: 33 1 57 27 75 21

Email: caroline.bazzoli@inserm.fr

Abstract

Nonlinear mixed effect models (NLMEM) with multiple responses are increasingly used in pharmacometrics, one of the main examples being the joint analysis of the pharmacokinetics (PK) and pharmacodynamics (PD) of a drug. Efficient tools for design evaluation and optimisation in NLMEM are necessary. The R functions PFIM 1.2 and PFIMOPT 1.0 were proposed for these purposes, but accommodate only single response models. The methodology used is based on the Fisher information matrix, developed using a linearisation of the model. In this paper, we present an extended version, PFIM 3.0, dedicated to both design evaluation and optimisation for multiple response models, using a similar method as for single response models. In addition to handling multiple response models, several features have been integrated into PFIM 3.0 for model specification and optimisation. The extension includes a library of classical analytical pharmacokinetics models and allows the user to describe more complex models using differential equations. Regarding the optimisation algorithm, an alternative to the Simplex algorithm has been implemented, the Fedorov-Wynn algorithm to optimise more practical D-optimal design. Indeed, this algorithm optimises design among a set of sampling times specified by the user. This R function is freely available at www.pfim.biostat.fr. The efficiency of this approach and the simplicity of use of PFIM 3.0 are illustrated with a real example of the joint PKPD analysis of warfarin, an oral anticoagulant, with a model defined by ordinary differential equations.

Key words: Fisher information matrix; nonlinear mixed effect models; multiple response models; optimal designs; D-optimality; PFIM.

1. Introduction

Nonlinear mixed effect models (NLMEMs) are increasingly used for the analysis of longitudinal data describing a biological process. They allow the estimation of the mean value of parameters in population studies and their inter-individual variability. They are also used for the joint modelling of several biological responses, such as the joint analysis of pharmacokinetic (PK) and pharmacodynamic (PD) data [1]. Pharmacokinetics deals with the time-course of drug concentration, whereas pharmacodynamics refers to the time-course of drug action in the body. In pharmacometrics, analysis through a NLMEM is called the population approach. To estimate parameters in NLMEMs, maximum likelihood estimation is used primarily, although the likelihood for these models has no analytical solution. Specific algorithms, implemented in several software packages, have therefore been proposed to perform this maximisation [2].

Before the estimation step, the investigator of a study is confronted with the choice of the design which is crucial for an efficient estimation of model parameters. A design in NLMEM, also called a population design, is composed of the number of elementary designs (or groups) and the specification of each elementary design and the associated number of subjects. In this setting, the term elementary design is used to describe a collection of subjects that have identical design characteristics defined by the number of sampling times and their allocation in time. To evaluate and compare population designs, a statistical approach based on the theory of optimum experimental design in classical nonlinear models and described for instance by Atkinson and Donev [3] or by Walter and Pronzato [4] has been extended to NLMEMs. This theory is based on the Fisher information matrix, whose inverse, according to the Cramer-Rao inequality, is the lower bound of the variance covariance matrix of any unbiased estimators of the parameters. Due to the lack of an analytical expression for the likelihood in NLMEM, an exact expression of the Fisher information matrix cannot be

defined. That is why an expression based on a linearisation of the model around the expectation of random effects has been proposed by Mentré *et al*. [5] and extended by Retout and Mentré [6] in the context of a single response model. The usefulness of this approach has been demonstrated, both by simulation [7, 8] and in real pharmacokinetic studies [9, 10]. To make the procedure more accessible to investigators, the approximate expression of the Fisher information matrix has been implemented in the R function PFIM 1.2, which can be used for design evaluation and comparison [11].

Regarding optimisation, two approaches can be used, either the optimisation of exact designs or of statistical designs. In the case of optimisation of exact designs, the group structure of the design is fixed: the number of elementary designs, the number of samples per design and the number of subjects per elementary design are given and the design variables used to optimise are only the sampling times. Optimisation of statistical designs consists in optimising both the allocation of the sampling times and the whole group structure, that is to say the number of elementary designs, the number of samples per elementary designs and the proportion of subjects in each elementary design. An exact design is then derived by rounding off the proportion of subjects in each elementary design. Optimisation based on the D-optimality criterion has been implemented in the R function PFIMOPT 1.0 [12], where the optimal design is the one that maximises the determinant of the Fisher information matrix. In PFIMOPT 1.0, both exact or statistical optimisation can be performed using the general Simplex algorithm [13]. It optimises the sampling times in given continuous intervals.

The main limitation of PFIM 1.2 and PFIMOPT 1.0 is that both aim at evaluating and optimising population designs only for single response models. Moreover, the model had to be written using an analytic expression and thus the need to use more complex models is limited. Regarding optimisation, the Simplex algorithm is a general optimisation algorithm. However, even if its applicability has been shown in pharmacokinetic examples [8], when

there are a large number of parameters to optimise, or when the model is complex, it could have difficulties in converging towards the optimal design and should sometimes be run again using the optimised design as a new initial design.

Recently, the expression for the Fisher information matrix has been extended in the context of a joint estimation of a set of parameters from multiple responses using the same linearisation as for single response [14-16]. In that context, some parameters can be included in several responses, as for instance in a classical PKPD model with the PD response depending on the PK parameters That joined estimation is then more informative, obtaining information on the PK parameters from both PK and PD responses. However, it increases the complexity of the computation of the Fisher information matrix compared to its computation for each response (single response). The relevance of the use of this approximation of the Fisher information matrix for that multiple response NLMEM context has been shown by Bazzoli *et al.* [14] through simulation of a PKPD model; results were very similar to those obtained from a more exact computation of the information matrix, without any linearization, using stochastic approximation through the SAEM algorithm of MONOLIX [17].

This expression has been implemented in PFIM 3.0, an extension of PFIM to handle both design evaluation and optimisation in NLMEMs with multiple responses. The extension includes a library of classical analytical pharmacokinetics models and allows more complex models to be described using a system of differential equations. Regarding the optimisation step, an alternative to the Simplex has been added in PFIM 3.0, the Fedorov-Wynn algorithm. It is a specific design optimisation algorithm implemented in PFIM 3.0 for statistical optimisation [5, 18] which has the property of converging towards the D-optimal design.

The aim of this paper is to present PFIM 3.0. In section 2, we present a nonlinear mixed effect multiple response design and model and the expression of the population Fisher information matrix for multiple responses. Then, the structure of PFIM 3.0 and the description

of its features and use are presented in Section 3. Lastly, an example of the use of PFIM 3.0 to design a new trial for the joint analysis of the pharmacokinetics and pharmacodynamics of warfarin, an oral anticoagulant, is provided in Section 4.

2. Statistical methods

a. Nonlinear mixed effect multiple response design and model

A design for NLMEM, i.e. a population design, is composed of N individuals to whom we allocate an "elementary" design ξ_i, $i = 1, \ldots, N$. Each elementary design is defined by a number n_i of sampling times and their allocation in time. A population design is therefore described by N elementary designs :

$$\Xi = \{\xi_1, \ldots, \xi_N\} \tag{1}$$

leading to a total number n of observations.

In the case of a multiple response model, an elementary design for one individual i is composed of several sub-designs, i.e $\xi_i = (\xi_{i1}, \xi_{i2}, \ldots, \xi_{iK})$, where ξ_{ik} is the design associated with the kth response, $k = 1, \ldots, K$. ξ_{ik} is defined by $(t_{ik1}, t_{ik2}, \ldots, t_{ikn_{ik}})$, the vector of the n_{ik} sampling times for the observations of the kth response, so that $n_i = \sum_{k=1}^{K} n_{ik}$.

Usually, population designs are composed of a limited number Q of groups of individuals. Each group is defined by an elementary design ξ_q, $q = 1, \ldots, Q$, which is composed, for the kth response, of n_{qk} sampling times $(t_{qk1}, t_{qk2}, \ldots, t_{qkn_{qk}})$ to be performed on a number N_q of individuals. The population design can then be noted as follows:

$$\Xi = \{[\xi_1, N_1]; [\xi_2, N_2]; \ldots; [\xi_Q, N_Q]\} \tag{2}$$

A nonlinear mixed effects multiple response model or a multiple response population model is defined as follows. The vector of observations Y_i for the ith individual is defined as the vector of the K different responses:

$$Y_i = \left[y_{i1}^T, y_{i1}^T, \ldots, y_{iK}^T \right]^T \tag{3}$$

where y_{ik}, $k = 1, \ldots, K$, is the vector of observations for the kth 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 models F, such as:

$$F(\theta_i, \xi_i) = \left[f_1\left(\theta_i, \xi_{i1}\right)^T, f_2\left(\theta_i, \xi_{i2}\right)^T, \ldots, f_K\left(\theta_i, \xi_{iK}\right)^T \right]^T \tag{4}$$

where θ_i is the vector of all the individual parameters needed for all the response models for 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, $\theta_i = g(\beta, b_i)$, which is usually additive, so that $\theta_i = \beta + b_i$, or exponential so that $\theta_i = \beta \exp(b_i)$. It is assumed that $b_i \sim N(0, \Omega)$ with Ω defined as a $p \times p$-diagonal matrix, each diagonal element ω_r^2, $r = 1, \ldots, p$, representing the variance of the rth component of the vector b_i. We consider here only the case of a diagonal Ω matrix with no correlation between the random effects.

The statistical model is thus given by:

$$Y_i = F\left(g\left(\beta, b_i\right), \xi_i\right) + \varepsilon_i \tag{5}$$

where ε_i is the vector composed of the K vectors of residual errors ε_{ik}, $k = 1, \ldots, K$, associated with the K responses. We also suppose $\varepsilon_{ik} \sim N\left(0, \Sigma_{ik}\right)$ with Σ_{ik} a $n_{ik} \times n_{ik}$-diagonal matrix such that

$$\Sigma_{ik}\left(\beta, b_i, \sigma_{\text{inter}k}, \sigma_{slopek}, \xi_{ik}\right) = diag\left(\sigma_{\text{inter}k} + \sigma_{slopek} f_k\left(g\left(\beta, b_i\right), \xi_{ik}\right)\right)^2 \qquad (6)$$

where $\sigma_{\text{inter}k}$ and σ_{slopek} qualify the model for the variance of the residual error of the kth response. The case $\sigma_{slopek} = 0$ returns a homoscedastic error model, whereas the case $\sigma_{\text{inter}k} = 0$ returns a constant coefficient of variation error model. The general case where the two parameters differ from 0 is called a combined error model. We then note $\Sigma_i\left(\beta, b_i, \sigma_{\text{inter}}, \sigma_{slope}, \xi_i\right)$ the variance of ε_i, over the K responses, such that Σ_i is a $n_i \times n_i$-diagonal matrix composed of each diagonal element of Σ_{ik} with $k = 1, \ldots, K$. σ_{slope} and σ_{inter} are two vectors of the K components $\sigma_{\text{inter}k}$ and σ_{slopek}, $k = 1, \ldots, K$, respectively. Finally, depending on the value of b_i, we assume that the errors ε_i are independently distributed.

Let Ψ be the vector of population parameters to be estimated, i.e. $\Psi^T = (\beta^T, \omega_1^2, \ldots, \omega_p^2, \sigma_{\text{inter}}{}^T, \sigma_{slope}{}^T)$ and let λ be the vector of variance terms $\lambda^T = (\omega_1^2, \ldots, \omega_p^2, \sigma_{\text{inter}}{}^T, \sigma_{slope}{}^T)$, so that $\Psi^T = \left(\beta^T, \lambda^T\right)$.

b. Fisher information matrix

The population Fisher information matrix $M_F\left(\Psi, \Xi\right)$ for multiple response models with the population design Ξ is given by:

$$M_F\left(\Psi, \Xi\right) = E\left(-\frac{\partial^2 L\left(\Psi, Y\right)}{\partial \Psi \partial \Psi^T}\right) \qquad (7)$$

where $L\left(\Psi, Y\right)$ is the log-likelihood of the vector of the whole observations Y for the population parameters Ψ.

Assuming independence across individuals, this log-likelihood can be defined as the sum of the log-likelihoods of the vectors of observations for each individual:

$L(\Psi,Y)=\sum_{i=1}^{N}L(\Psi,Y_i)$. Therefore, taking the second derivatives, the population Fisher information matrix for N individuals can also be defined as the sum of the N elementary information matrices $M_F(\Psi,\xi_i)$ computed for each individual *i:*

$$M_F(\Psi,\Xi)=\sum_{i=1}^{N}M_F(\Psi,\xi_i) \tag{8}$$

In the case of a limited number Q of groups, it is expressed as:

$$M_F(\Psi,\Xi)=\sum_{q=1}^{Q}N_q\,M_F(\Psi,\xi_q) \tag{9}$$

The expression of an elementary Fisher information matrix for multiple responses has been described in Bazzoli *et al.* [14] where further details are given. For the sake of simplicity, we have omitted the index i for the individual in the following. The Fisher information matrix is a block matrix depending on the approximated marginal expectation E and variance V of the observations.

$$M_F(\Psi,\xi)\cong\frac{1}{2}\begin{pmatrix} A(E,V) & C(E,V) \\ C^T(E,V) & B(E,V) \end{pmatrix} \tag{10}$$

where

$$(A(E,V))_{ml}=2\frac{\partial E^T}{\partial\beta_m}V^{-1}\frac{\partial E}{\partial\beta_l}+tr(\frac{\partial V}{\partial\beta_l}V^{-1}\frac{\partial V}{\partial\beta_m}V^{-1}) \text{ with } m \text{ and } l=1,\ldots,p$$

$$(B(E,V))_{ml}=tr(\frac{\partial V}{\partial\lambda_m}V^{-1}\frac{\partial V}{\partial\lambda_l}V^{-1}) \text{ with } m \text{ and } l=1,\ldots,\dim(\lambda)$$

$$(C(E,V))_{ml}=tr(\frac{\partial V}{\partial\lambda_l}V^{-1}\frac{\partial V}{\partial\beta_m}V^{-1}) \text{ with } l=1,\ldots,\dim(\lambda) \text{ and } m=1,\ldots,p$$

Note that in this work, we consider that the variance of the observations with respect to the mean parameters is constant, i.e. $C(E,V)_{ml} = 0$ and $A(E,V)_{ml} = 2\frac{\partial E^T}{\partial \beta_m} V^{-1} \frac{\partial E}{\partial \beta_l}$.

3. Implementation of PFIM 3.0 using R software

The development of the population Fisher information matrix described previously has been implemented in an extension of the R function PFIM: PFIM 3.0. It allows a design evaluation and optimisation of multiple response population models. PFIM 3.0 can be used for single response models instead of the previous version PFIM 1.2 or PFIMOPT 1.0. In addition to the extension for multiple response models, options have been added for model specification and for design optimisation. The free statistical R software (R 2.4.1 and higher versions) [19] (http://cran.r-project.org/) is required to use PFIM 3.0. This function is freely available on the PFIM website http://www.pfim.biostat.fr as well as an extensive documentation and some examples to use it.

a. Model specification

In PFIM 3.0, the model has to be entered in a model file called by default *model.r*. It must be specified by the user either in its analytical form or by using a system of differential equations. This extension to a system of differential equations requires the use of the *lsoda* function included in the library "odesolve" implemented by Setzer to solve the system [20] and the *fdHess* function included in the library "nlme" developed by Pinheiro and Bates [21] for numerical derivatives. The *lsoda* function uses a function of the same name written in Fortran by Petzold and Hindmarsh [22, 23]. This function solves a system of differential

equations using the Adams method, a predictor–corrector method for non-stiff systems; it uses the BDF (Backward Differentiation Formula) for stiff systems. Regarding the function *fdHess*, it evaluates an approximate gradient of a scalar function using finite differences.

Moreover, a library of standard PK models has been added. Twelve PK models are included: one or two compartment models, with first order oral, bolus or infusion administration and after a single dose, multiple doses or steady-state. Only models with first order elimination are presently available in the library of PFIM 3.0. Currently, there are no models with lag time. A list of the models and their characteristics included in the library is given in Table 1.

*b. Optimisation algorithm*s

An alternative to the Simplex algorithm has been added in PFIM 3.0, the Fedorov-Wynn algorithm. It is specifically dedicated to design optimisation problems and has the property of convergence towards the D-optimal design [5, 24, 25]. It optimises statistical designs (i.e the group structure, the number of samples per subject and the sampling times) for a given total number of samples. Minimum and maximum number of samples per subject are specified. To optimise the design, it considers only a set of possible sampling times defined by the user. This can be an advantage in clinical practice to avoid unfeasible sampling times. Design optimisation can be constrained to the same sampling times across responses or not. It is also possible to give each elementary design sampling times from a different set of finite sampling times. For instance, it is possible to specify that each elementary design should include two points among a first set of sampling times (for instance Day 1) and a third point among a second set of sampling times (for instance Day 2). The user therefore specifies a list of sampling windows, and for each window, the set of possible sampling times and the miminum and maximum number of sampling times within that window. The Fedorov-Wynn algorithm

is programmed in C code and is linked to PFIM 3.0 through a dynamic library. Moreover, PFIM 3.0 uses the function *combn* in the R package "combinat" to generate all possible elementary designs from the set of pre-specified sampling times. To start the algorithm, an initial population design is required.

c. *Input file*

The inputs to PFIM 3.0 are entered in R objects by filling in one input file called by default *stdin.r*. The same function PFIM 3.0 is used for the evaluation and optimisation of population designs, which is why the user has to notify it in the input file in a specific R object. Then, this input file is composed of two main sections of R objects. The first one is composed of general R objects required for evaluation and optimisation such as the model form (analytical or differential equation system), the parameterisation of the structural model, the type of inter-individual random effect model (additive or exponential), values of σ_{inter} and σ_{slope} for the variance error model and *a priori* values of the fixed effect parameters and variance parameters. The user also specifies the population design to evaluate or, for optimisation, an initial population design. They are defined as a group of elementary designs each one associated with a number of subjects. The second section is dedicated to design optimisation where the specific objects are algorithm options. First, the user specifies if the same sampling times are required for each response or not. Then, the algorithm is chosen (Simplex or Fedorov-Wynn). If the Fedorov-Wynn is required, the user must provide the following information: the number of sampling windows, the list of the allowed sampling times for each sampling window, the list of the number of allowed sampling times for each sampling window, the maximum and minimum total number of sampling times per subject.

Graphical representations are provided automatically if graph objects are completed. Once the model file and the input file are filled in, the user can run the function PFIM 3.0 by calling the function in the R Console window: PFIM ( ).

d. Output file

The results are written to an output file named by default *stdout.r* or with a name specified in the input file. PFIM 3.0 returns a summary of the input, the population design evaluated or optimised, the associated population Fisher information matrix, the standard error expected on each parameter, the corresponding coefficient of variation (%), its determinant and a criterion Φ defined as the determinant standardized by the dimension of the vector Ψ: $\Phi(\Xi)=\left[\det\left(M_F\left(\Psi,\Xi\right)\right)\right]^{\frac{1}{\dim(\Psi)}}$. When design optimisation is performed, the list of the algorithm options is also specified in the output file. For instance, for the Fedorov-Wynn algorithm, the set of allowed sampling times, the number of sampling times, the maximum and the minimum number of points for each response are returned. The optimised design is reported with the proportion of subjects optimised by the Fedorov-Wynn algorithm and their absolute number. If a graph has been supplied in the input file, the mean profile of each response and the associated sampling times of the evaluated or optimised design are represented on an R graph.

4. Illustration of design for PKPD of warfarin

We aim to illustrate the use of PFIM 3.0 for the joint PKPD modelling of the time course of total racemic warfarin plasma concentrations and effect on prothrombin complex activity (PCA), by designing a new trial for this joint population analysis.

To do this, we use an example extracted from a classical pharmacology study published 40 years ago, by O'Reilly *et al.* [26, 27] on the pharmacokinetics and pharmacodynamics of

warfarin. Previously, these data have been fitted by Holford for the analysis of the dose-effect relationship of warfarin [28] using nonlinear mixed effects models. This example was presented in [29] but using different constraints in the optimisation step.

a. Population design and model

Warfarin is administered by a single oral dose of 100 mg. A one compartment model with first order absorption and elimination adequately describes the concentration data (PK). The *PCA* effect is described by a turnover model with inhibition of the input (PD). The model can be written as a system of ordinary differential equations:

$$\frac{df_{PK}(\theta_{PK},t)}{dt} = k_a \cdot D \cdot e^{-k_a \cdot t} - \frac{CL}{V} \cdot f_{PK}(\theta_{PK},t) \tag{11}$$

$$\frac{df_{PD}(\theta_{PD},t)}{dt} = R_{in}\left(1 - \frac{f_{PK}(\theta_{PK},t)}{IC_{50} + f_{PK}(\theta_{PK},t)}\right) - k_{out} \cdot f_{PD}(\theta_{PD},t) \tag{12}$$

where f_{PK} and f_{PD} are respectively the warfarin plasma concentrations and effect model and θ_{PK} and θ_{PD} the parameters needed for each response. D is the dose of warfarin. The vector of the PK parameters is $\theta_{PK} = (k_a, CL, V)^T$ with k_a the absorption constant, CL the apparent clearance and V the apparent volume of distribution. PCA is controlled by the rate of input R_{in} and the output rate constant k_{out} of the response, and its baseline value is given by the ratio of those parameters R_{in}/k_{out}. The vector of PD parameters is thus defined by $\theta_{PD} = (R_{in}, IC_{50}, k_{out})^T$ where IC_{50} is the drug concentration which produces 50% of maximum inhibition achieved at the effect site.

The inter-individual random effects are assumed to be exponential for all parameters. We associate a proportional error for the PK model and an additive error for the PD model characterised by the parameters $\sigma_{slopePK}$ and $\sigma_{interPD}$. Thus, the vector of population

parameters Ψ is described by the vector of fixed effects $\beta^T = \left(\beta_{k_a}, \beta_{CL}, \beta_V, \beta_{R_{in}}, \beta_{IC_{50}}, \beta_{k_{out}}\right)$ and by λ^T the vector composed of the variance of the random effects and of the parameters for the error models such that $\lambda^T = \left(\omega^2_{k_a}, \omega^2_{CL}, \omega^2_V, \omega^2_{R_{in}}, \omega^2_{IC_{50}}, \omega^2_{k_{out}}, \sigma_{slopePK}, \sigma_{\text{int}erPD}\right)$.

Values of the parameters are those obtained by Holford performing the simultaneous PKPD population analysis of warfarin. The population parameters were estimated by maximum likelihood, with the NONMEM software [30]. They are given in Table 2.

b. Design optimisation for a new study

Using PFIM3.0, we first evaluate the design used in the previous study to determine the joint population PKPD model. We call this design the "empirical design" denoted Ξ_{emp}. It involves one group of 32 healthy volunteers, with 13 sampling times at 0.5, 1, 2, 3, 6, 9, 12, 24, 36, 48, 72, 96, 120 hours for warfarin concentration measurements and 8 sampling times at 0, 24, 36, 48, 72, 96, 120, 144 hours for PCA measurement. Ξ_{emp} thus has a total of 672 measurements.

We then optimise two population designs under several constraints using the Fedorov-Wynn algorithm implemented in PFIM 3.0. To do that, we first optimise a population design Ξ_{opt_iden} with only four sampling times per elementary design common to both responses. This optimisation step is performed on the same number of 32 volunteers and the set of admissible sampling times is the set of times used in the empirical design except times 1 and 3 for PK. We then optimise a population design Ξ_{opt_diff}, with four sampling times per elementary design, but now, the optimal sampling times can be different for each response.

c. Results

Fig. 1 reports the model file used for the design evaluation and optimisation of the joint PKPD model of warfarin. The input file of PFIM 3.0 for the evaluation of Ξ_{emp} is reported in Fig. 2. The output file of PFIM 3.0 for the optimal design Ξ_{opt_iden} and the associated R graph output are represented in Fig. 3 and Fig. 4, respectively. The optimal designs with their associated criterion are reported in Table 3. Ξ_{emp} and both optimal designs (Ξ_{opt_iden}, Ξ_{opt_diff}) are different by their group structure. Ξ_{opt_iden} and Ξ_{opt_diff} are composed of two elementary designs compared to only one for Ξ_{emp}. The Fedorov-Wynn algorithm optimises proportions of subjects, to be practical those numbers are then rounded to the nearest integers. Each elementary design involves 22 and 10 subjects for Ξ_{opt_iden} and 29 and 3 for Ξ_{opt_diff}. In order to get more practical designs, Ξ_{opt_diff} is simplified in a new design $\Xi_{opt_diff_simp}$, composed of one group of 32 subjects, meaning that the 3 subjects originally included in the second elementary design have been added to the first elementary design. The two optimised designs have a smaller number of samples than the empirical design and are therefore less efficient with lower criterion values (Table 3). These two designs include a sample at 144 hours for the PK measurement whereas this sample is not present in the empirical design. Indeed, in population PK studies, when a proportional residual error is associated with the model, the optimal design includes the latest time among those proposed. In our case, for optimisation, we allowed the sample at 144 hours both for PK and PD responses.

In order to get an efficiency similar to that obtained with Ξ_{emp}, we then derived two "enlarged" designs $\Xi_{opt_iden_enl}$ and $\Xi_{opt_diff_simp_enl}$ from the two optimal designs by increasing the number of subjects. 55 subjects and 52 subjects are involved in $\Xi_{opt_iden_enl}$ and $\Xi_{opt_diff_simp_enl}$, meaning a total of number of sampling times of 440 and 416, respectively. The expected relative standard errors (RSE) of Ξ_{emp}, $\Xi_{opt_iden_enl}$ and $\Xi_{opt_diff_simp_enl}$, i.e

standard error divided by the true value of the parameter, their total number of sampling times and the associated criterion are given in Table 4.

The evaluation of Ξ_{emp} returns expected RSEs smaller than 30%, except for the variance parameters $\omega^2_{IC_{50}}$ and $\omega^2_{k_{out}}$, where they are rather large (about 68% and 41%) due to the small inter-subject variability of these parameters. Comparison between both "enlarged" designs shows that only the coefficient of variation of the estimation of the variance parameter $\omega^2_{IC_{50}}$ is increased compared to Ξ_{emp}. The expected RSE on the terms for residual errors increase in both optimal designs, but they remain lower than 10%. For other parameters, the results from design optimisation, in terms of precision of estimation, are very close to those obtained with the empirical design, although "enlarged" designs involve only 440 and 416 measurements, respectively, 35% and 38% fewer than the empirical design. This sample reduction, together with a similar efficiency, leads to a more ethical and less costly study. Using PFIM 3.0, we find new efficient designs to study the joint PKPD of warfarin fitting to different clinical constraints.

5. Discussion

In this paper, we consider the R function PFIM 3.0, an extension of the R functions PFIM 1.2 and PFIMOPT 1.0, allowing evaluation and optimisation of designs for nonlinear mixed effect models with multiple responses. The methodology is based on the Fisher information matrix, developed using a linearisation of the model, as proposed for a single response by Mentré *et al.* [5] and extended for multiple responses [14-16]. In addition to handling multiple response models, several features have been integrated in PFIM 3.0. First, regarding the model specification, it can now be specified as a system of differential equations; this is an alternative to analytical model specification, particularly useful for complex models. For

"classical" pharmacokinetic models, a library of twelve pharmacokinetic models is available. Concerning the optimisation algorithm, the Fedorov-Wynn algorithm has been added as an alternative to the general Simplex algorithm to optimise more practical D-optimal designs [24, 25]. Indeed, this algorithm allows design optimisation with fixed sampling times specified by the user which can be a great advantage with respect to medical constraints. This algorithm optimises the sampling times, which can be different or imposed to be identical across responses, but also optimises the group structure, using the approach of statistical designs. In addition to convergence issues using the Simplex algorithm compared to the Fedorov-Wynn [18], optimisation within continuous intervals of times can be more questionable: the Simplex algorithm can, sometimes for a long time, pursue the optimisation by exchanging at each iteration one or several sampling times by some others which differ from the previous by only few seconds. This greatly slows down the whole optimisation process and has no relevance in clinical practice.

We illustrate the different options of this new extension of PFIM using a real example of the simultaneous population analysis of the time course of warfarin concentration and its effect on the prothrombin complex activity after single dose administration. We optimise and derive two population designs using the Fedorov-Wynn algorithm, either imposing the same sampling times across responses or not, and we compare them to an "empirical design" used in the previous study of warfarin [26, 27]. Globally, the expected precision of estimations are in the same range for the three designs, even if the number of samples per patient, and thus, the total number of samples are considerably reduced (38% fewer samples in the optimised designs compare to the empirical one). In this example, we show the interest of the optimisation procedure implemented in PFIM 3.0 from a medical, economic as well as ethical point of view. This approach can also be used for other complex modelling, such as the pharmacokinetics of a parent drug and its metabolite [31]. However, in some situations, due to

the different time scales of the pharmacokinetic profiles between the parent drug and the metabolite, different sampling times for the two responses may be required. It should be noted however, using the Fedorov-Wynn algorithm under this assumption, the number of possible elementary designs will be large and can considerably increase the computation time and the memory requirement. Limiting the number of allowed sampling times for each sampling window also helps to avoid computational issues.

In the example, the prothrombin complex activity is controlled by the rate of input R_{in} and the output rate constant k_{out} of the response, and the initial condition is given by the ratio of these parameters R_{in}/k_{out}. It would be possible by using a modification of the parameterisation of the model to estimate the initial condition and R_{in} or k_{out} or even to fix the initial condition (for instance to 100%) and to estimate only R_{in} or k_{out}.

The present development of the Fisher information matrix for multiple response models takes into account all the population parameters to be estimated. Another approach would have been to optimise separately one design for the PK parameters and one for the PD parameters, fixing the PK parameters to their mean in the population (sequential approach). However, this approach can be sub-optimal as no variability on the PK parameters is taken into account in the PD model. For instance, we compared this sequential approach to the one used in this paper, using the same example and similar constraints. We obtained the same optimal design for the PK model. Regarding the conditional D-optimal design for the PD, the design is quite different from $\Xi_{opt_diff_simp}$. Indeed, it involves two elementary designs with sampling times at (0, 24, 72, 96) and (0, 24, 36, 96) hours, and with 21 and 11 subjects, respectively. Comparison of the relative standard errors shows an increase by at least a factor 2 with this design compared to those obtained with $\Xi_{opt_diff_simp}$.

Following the first theoretical work on optimal design for NLMEM, this research theme has grown rapidly both in methodological and application developments. An email mailing

list server PopDesign is now available for questions and comments about the experimental design of any study for which nonlinear mixed effect modelling is proposed for the analysis. Since the first tool PFIM [11], there are now several different software tools that implement an evaluation of the Fisher information matrix for population PK and PD models and propose optimisation of the experimental designs (PFIM, PopDes, PopED, WinPOPT) [32]. All software packages have ongoing development and have specific features according to the needs and goals of the research teams that developed them. PFIM is the only one using the R software.

PFIM 3.0 uses an approximate Fisher information matrix to evaluate and optimise design. This approximation, using a linearisation of the model, may be a limitation. Indeed, even if the relevance of this approximation has been shown by comparison to an exact Fisher information matrix using a simulation study, the PKPD model used was very simple [14] and may not be generalised to any complexity of the models. On the other hand, use of more exact methods can be computationally intensive and thus time consuming. Their need is thus questionable for nonlinear models for which design evaluation and optimisation require some *a priori* values of the parameters to be estimated, which are often not known precisely.

In parallel to PFIM 3.0, PFIM Interface 2.1, a graphical user interface version of PFIM 1.2 and PFIMOPT 1.0 has been recently proposed, allowing both design evaluation and optimisation but only for single response models. PFIM Interface 2.1 has the same features for model specification and optimisation algorithm as in PFIM 3.0. PFIM Interface will soon be extended to accommodate multiple response models.

In addition to the new features added in PFIM 3.0, several other extensions are planned. First, the current library includes only pharmacokinetic models. Extensions to the usual pharmacokinetic-pharmacodynamic models are planned, even if model specification for

multiple responses is quite simple for the user as shown in the illustration using a system of differential equations.

Then, the next version could integrate the computation of the Fisher matrix for a non-diagonal variance of the random effects, as, in practice, one may want to allow correlation between the random effects. Expression of the Fisher information matrix has been proposed in this case for single response in Mentré *et al.* [5] and for multiple responses in Ogungbenro *et al.* [33] but has not been yet implemented in PFIM 3.0.

Afterwards, an extension of PFIM is envisaged to implement the expression of the Fisher information matrix for models including fixed effects for the influence of covariates on the parameters [6], and thus, to compute the predicted power of the Wald test to detect a covariate effect as well as the number of subjects needed to achieve a given power [18].

Moreover, Retout *et al.* [12] have recently used a modified version of PFIM for the problem of design optimisation using cost functions. They focus on the relative feasibility of the optimised designs, in terms of sampling times and of numbers of subjects. To do that the Fedorov-Wynn algorithm has been extended to include cost functions to penalize less feasible designs. An illustration of this extension of design optimisation to a joint population model of infliximab and methotrexate pharmacokinetics administered in rheumatoid arthritis was performed. The possibility of specifying user-defined cost-functions will be available in the next version of PFIM.

The implementation of the Fisher information matrix for multiple response models proposed in PFIM 3.0 is a relevant computing tool for the evaluation and comparison of designs in the spreading development of multiple response PK studies. PFIM 3.0 is distributed under the terms of the GNU GENERAL PUBLIC LICENSE (GNU GPL) Version 2, June 1991 and has been registered at the Agency for the Protection of Programs (APP) in 2008, at Paris, France.

PFIM functions, including their extensive documentation and examples to use them, are freely available from the PFIM website: http:/www.pfim.biostat.fr.

Conflict of Interest Statement

The authors have no conflicts of interest that are directly relevant to the content of this work.

References

[1] L. Sheiner and J. Wakefield, Population modelling in drug development. Stat Methods Med Res, 8 (1999) 183-193.

[2] G.C. Pillai, F. Mentré and J.L. Steimer, Non-linear mixed effects modeling - from methodology and software development to driving implementation in drug development science. J Pharmacokinet Pharmacodyn, 32 (2005) 161-183.

[3] A.C. Atkinson and A.N. Donev, Optimum Experimental Designs, Clarendon Press, Oxford, 1992.

[4] E. Walter and L. Pronzato, Identification of Parametric Models from experimental Data, Springer, New-York, 1997.

[5] F. Mentré, A. Mallet and D. Baccar, Optimal design in random effect regression models. Biometrika, 84 (1997) 429-442.

[6] S. Retout and F. Mentré, Further developments of the Fisher information matrix in nonlinear mixed effects models with evaluation in population pharmacokinetics. J Biopharm Stat, 13 (2003) 209-227.

[7] S. Retout and F. Mentré, Optimization of individual and population designs using Splus. J Pharmacokinet Pharmacodyn, 30 (2003) 417-443.

[8] S. Retout, F. Mentré and R. Bruno, Fisher information matrix for non-linear mixed-effects models: evaluation and application for optimal design of enoxaparin population pharmacokinetics. Stat Med, 21 (2002) 2623-2639.

[9] B. Green and S.B. Duffull, Prospective evaluation of a D-optimal designed population pharmacokinetic study. J Pharmacokinet Pharmacodyn, 30 (2003) 145-161.

[10] F. Mentré, C. Dubruc and J.P. Thenot, Population pharmacokinetic analysis and optimization of the experimental design for mizolastine solution in children. J Pharmacokinet Pharmacodyn, 28 (2001) 299-319.

[11] S. Retout, S. Duffull and F. Mentré, Development and implementation of the population Fisher information matrix for the evaluation of population pharmacokinetic designs. Comput Methods Programs Biomed, 65 (2001) 141-151.

[12] S. Retout, E. Comets and F. Mentré, Design optimisation in nonlinear mixed effects models using cost functions: application to a joint model of infliximab and methotrexate pharmacokinetics. Commun Stat A-Theor, (2009) In press.

[13] J.A. Nelder and R. Mead, A Simplex method for function minimization. Comput J, 1 (1965) 308-313.

[14] C. Bazzoli, S. Retout and F. Mentre, Fisher information matrix for nonlinear mixed effects multiple response models: Evaluation of the appropriateness of the first order linearization using a pharmacokinetic/pharmacodynamic model. Stat Med, 28 (2009) 1940-1956.

[15] I. Gueorguieva, L. Aarons, K. Ogungbenro, K.M. Jorga, T. Rodgers and M. Rowland, Optimal design for multivariate response pharmacokinetic models. J Pharmacokinet Pharmacodyn, 33 (2006) 97-124.

[16] A. Hooker and P. Vicini, Simultaneous population optimal design for pharmacokinetic-pharmacodynamic experiments. Aaps J, 7 (2005) E759-785.

[17] M. Lavielle, MONOLIX (MOdèles NOn LInéaires à effets miXtes), MONOLIX group, Orsay, France, 2005.

[18] S. Retout, E. Comets, A. Samson and F. Mentré, Design in nonlinear mixed effects models: optimization using the Fedorov-Wynn algorithm and power of the Wald test for binary covariates. Stat Med, 26 (2007) 5162-5179.

[19] R Development Core Team, R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, 2004.

[20] M.L. Lindstrom and D.M. Bates, Nonlinear mixed effects models for repeated measures data. Biometrics, 46 (1990) 673-687.

[21] J. Pinheiro and D.M. Bates, Mixed-effects models in S and Splus, New-York NY, 2000.

[22] A.C. Hindmarsh, ODEPACK, A systematized collection of ODE solvers, in Scientific Computing: Applications of Mathematics and Computing to the Physical Sciences, eds. R.S. Stepleman, pp. 55-64 (Elsevier Science Ltd, Amsterdam, 1983).

[23] L.R. Petzold, Automatic selection of methods for solving stiff and nonstiff systems of ordinary differential equations. Siam J Sci Stat Comput, 4 (1983) 136-148.

[24] V.V. Fedorov, Theory of Optimal Experiments, Academic Press, New York, 1972.

[25] H.P. Wynn, Results in the construction of D-optimum experimental designs. J R Statist Soc, 34 (1972) 133-147.

[26] R.A. O'Reilly and P.M. Aggeler, Studies on coumarin anticoagulant drugs. Initiation of warfarin therapy without a loading dose. Circulation, 38 (1968) 169-177.

[27] R.A. O'Reilly, P.M. Aggeler and L.S. Leong, Studies on the Coumarin Anticoagulant Drugs: the Pharmacodynamics of Warfarin in Man. J Clin Invest, 42 (1963) 1542-1551.

[28] N.H. Holford, Clinical pharmacokinetics and pharmacodynamics of warfarin. Understanding the dose-effect relationship. Clin Pharmacokinet, 11 (1986) 483-504.

[29] S. Retout, C. Bazzoli, E. Comets, H. Le Nagard and F. Mentré, Population designs evaluation and optimisation in R: the PFIM function and its new features, in: 16th Meeting of Population Group Approach in Europe (PAGE) (2007) Abstr 1164 [www.page-meeting.org/?abstract=1164].

[30] S.L. Beal and L.B. Sheiner, NONMEM users guide, University of California, San Francisco, 1992.

[31] C. Bazzoli, S. Retout, E. Rey, H. Benech, J.M. Tréluyer, D. Salmon, X. Duval, F. Mentré and the COPHAR2-ANRS 111 study group, Population pharmacokinetic of AZT and its active metabolite AZT-TP in HIV patients (COPHAR 2 – ANRS 111 trial) : joint modelling and design optimisation, in: 17th Meeting of Population Group Approach in Europe (PAGE) (2008) Abstr 1373 [www.page-meeting.org/?abstract=1373].

[32] F. Mentré, S. Duffull, I. Gueorguieva, A. Hooker, S. Leonov, K. Ogungbenro and S. Retout. Software for optimal design in population pharmacokinetics and pharmacodynamics: a comparison. in: Population Approach Group in Europe (PAGE), Copenhagen, Denmark, 2007.

[33] K. Ogungbenro, G. Graham, I. Gueorguieva and L. Aarons, Incorporating correlation in interindividual variability for the optimal design of multiresponse pharmacokinetic experiments. J Biopharm Stat, 18 (2008) 342-358.

Legends for Figures:

Fig. 1: Model file of PFIM 3.0 for the evaluation of the empirical design Ξ_{emp} and optimisation of both optimal designs Ξ_{opt_iden} and Ξ_{opt_diff} for the joint PKPD modelling of warfarin.

Fig. 2: Input file of PFIM 3.0 in the context of the evaluation of the empirical design Ξ_{emp} for the joint PKPD modelling of warfarin.

Fig. 3: Input file of PFIM 3.0 in the context of the optimisation of the optimal design Ξ_{opt_iden} using the Fedorov-Wynn algorithm for the joint PKPD modelling of warfarin.

Fig. 4: R graph output of the concentration and effect profiles versus time for the mean parameter values used in the joint PKPD modelling of warfarin using PFIM 3.0. PK sampling times and PD sampling times for the optimal design Ξ_{opt_iden} for each response are displayed using number values (i.e. 1 and 2 for samples from first and second elementary design, respectively).

Table 1. List of pharmacokinetic models included in the library of models of PFIM 3.0. The table includes all information needed in order to use the model function chosen: the name, the type of input, the number of compartments and the parameters used.

Name	Input	Number of compartment	Parametrisation
bolus_1cpt_Vk	IV-bolus	1	V, k
bolus_1cpt_VCl	IV-bolus	1	V, Cl
infusion_1cpt_Vk	IV-infusion	1	V, k
infusion_1cpt_VCl	IV-infusion	1	V, Cl
oral1_1cpt_kaVk	1st order	1	ka, V, k
oral1_1cpt_kaVCl	1st order	1	ka, V, Cl
bolus_2cpt_Vkk12k21	IV-bolus	2	V, k, k12, k21
bolus_2cpt_ClV1QV2	IV-bolus	2	Cl, V1, Q, V2
infusion_2cpt_Vkk12k21	IV-infusion	2	V, k, k12, k21
infusion_2cpt_ClV1QV2	IV-infusion	2	Cl, V1, Q, V2
oral1_2cpt_kaVkk12k21	1st order	2	ka, V, k, k12, k21
oral1_2cpt_kaClV1QV2	1st order	2	ka, Cl, V1, Q, V2

Table 2. Population parameter values estimated by the PKPD joint modelling of warfarin

Parameters	Fixed effects (β)	Inter-subject variability (ω^2)
k_a (h^{-1})	1.60	0.701
CL (L/h)	0.133	0.063
V (L)	7.95	0.020
R_{in} (%/h)	5.41	0.190
IC_{50} (mg/L)	1.20	0.013
k_{out} (h^{-1})	0.056	0.016
$\sigma_{slopePK}$	0.20	-
$\sigma_{interPD}$ (%)	3.88	-

Table

Table 3. Optimal population designs for the joint model of warfarin concentration (PK) and the effect on prothrombin complex activity (PD) according to different constraints. Ξ_{emp} is the empirical design. Ξ_{opt_iden} and Ξ_{opt_diff} are both optimal designs. $\Xi_{opt_diff_simp}$ is a design derived from Ξ_{opt_diff} to be simpler. For each design, the number of elementary designs, the rounded number of subjects with the elementary design, the sampling times for each response, the total number of sampling times and the associated criterion value are reported.

	Number of elementary designs	Sampling times for each response (hours)		Rounded number of subjects	Total number of sampling times	Criterion value Φ
		PK	*PD*			
Ξ_{emp}	1	0.5, 1, 2, 3, 6, 9, 12, 24, 36, 48, 72, 96,120	0, 24, 36, 48, 72, 92, 120, 144	32	672	984.20
Ξ_{opt_iden}	2	0.5, 12, 24, 144	0.5, 12, 24, 144	22	256	580.19
		0.5, 24, 120, 144	0.5, 24, 120, 144	10		
Ξ_{opt_diff}	2	0.5, 6, 9, 144	0, 12, 24, 120	3	256	607.86
		0.5, 6, 9, 144	0, 24, 96, 120	29		
$\Xi_{opt_diff_simp}$	1	0.5, 6, 9, 144	0, 24, 96, 120	32	256	607.32

Table

Table 4. Relative standard errors (RSE) (%) computed by the population Fisher information matrix for designs Ξ_{emp} and for the enlarged designs $\Xi_{opt_iden_enl}$ and $\Xi_{opt_diff_simp_enl}$ derived from the two optimal designs by increasing the number of subjects in order to get a similar efficiency obtained with Ξ_{emp}. The total number of sampling times and the criterion value associated with each design are also reported.

	Expected RSE (%)														Total number of sampling times	Criterion value Φ
	β_{k_a}	β_{CL}	β_V	$\beta_{R_{in}}$	$\beta_{IC_{50}}$	$\beta_{k_{out}}$	$\omega^2_{k_a}$	ω^2_{CL}	ω^2_V	$\omega^2_{R_{in}}$	$\omega^2_{IC_{50}}$	$\omega^2_{k_{out}}$	$\sigma_{slopePK}$	$\sigma_{interPD}$		
Ξ_{emp}	15.7	4.6	2.9	8.0	3.3	2.9	28.0	26.3	33.0	26.2	68.3	40.8	3.9	5.6	672	984.2
$\Xi_{opt_iden_enl}$	12.5	3.7	3.1	6.1	3.4	2.3	22.4	21.1	45.1	20.2	98.0	34.9	8.2	9.1	440	997.2
$\Xi_{opt_diff_simp_enl}$	12.7	3.8	2.8	6.3	3.2	2.6	22.9	21.8	38.9	20.8	83.6	41.7	8.8	9.4	416	987.5

Figure

Fig. 1

```
##########################################################################
                                    MODEL FILE
#########################################################################

formED<-function(t,y,p)
{
ka<-p[1]
cl<-p[2]
V<-p[3]
Rin<-p[4]
C50<-p[5]
kout<-p[6]
Imax<-1

  yd1<--ka*y[1]
  yd2<-ka*y[1]-(cl/V)*y[2]
  yd3<-Rin*(1-((Imax*y[2]/V)/((y[2]/V)+C50)))-kout*y[3]

list(c(yd1,yd2,yd3),c(y[2]/V,y[3]))
}
```

Figure

Fig.2

```
##################################################################
##                    INPUT FILE FOR PFIM 3.0                   ##
##################################################################

#Name of the project
#--------------------
project<-"PKPDWarfarine_indirect_model: empirical design evaluation"


#Name of the file containing the PK or PD model
#----------------------------------------------
file.model<-"model.r";


#Name of the output file for the results
#---------------------------------------
output<-"Stdout.r";

#RUN:  Evaluation (EVAL) or Optimisation (OPT)
#--------------------------------------------------------
run<-"EVAL"

#Number of responses
#-------------------------------------------------------------------

nr<-2

#Name of the fixed effects parameters
#------------------------------------
parameters<-c('ka','cl','V','Rin','C50','kout')


#Fixed effects parameters values
#-------------------------------
beta<-c(1.6,0.133,7.95,5.41,1.2,0.056)

#Diagonal Matrix of variance for the random effects:
#---------------------------------------------------
omega<-diag(c(0.701,0.0634,0.0206,0.19,0.0129,0.0167))


#Standard deviation of the residual error (sig.inter+sig.slope*f)^2:
#-------------------------------------------------------------------
sig.interA<-0
sig.slopeA<-0.2

sig.interB<-3.88
sig.slopeB<-0

#Between-subject variance model (1) = additive  (2) = exponential
#-----------------------------------------------------------------
Trand<-2;

#List of the vectors of sampling times for each elementary design
#you can specify any sampling times for a group by writing NULL
#ONLY if you have several responses
#-----------------------------------------------------------------
protA<-list(c(0.5,1,2,3,6,9,12,24,36,48,72,96,120))
protB<-list(c(0,24,36,48,72,96,120,144))

#Vector of initial proportions or numbers of subjects for each elementary
design
```

```
#-----------------------------------------------------------
subjects<-c(32)

#Subjects input: (1) for number of subjects (2) for proportions of sub
#---------------------------------------------------------------------
subjects.input<-1

#If 'proportions of subjects' give the total number of samples
#-------------------------------------------------------------
#Ntot<-1200

################### MODEL OPTION ##########################

#Model form: Differential equations (DE) or analytical form (AF)
#--------------------------------------------------------------
modelform<-"DE"

###### DIFFERENTIAL EQUATION OPTION ##################################
#######################################################################

#Initial time for which initial conditions are given
#--------------------------------------------------

time.condinit<-0

#Identical initial conditions in each elementary design (Yes=T, No=F)
#-------------------------------------------------------------------
condinit.identical<-T

# If 'Yes', enter once the expression of the initial values of the sys
the initial time
# else, enter the vectors of the initial conditions for each elementar
# If initial values depend on parameters to be estimated,
# enter this parameter into the expression without any quotation marks
#-------------------------------------------------------------------
condinit<-expression(c(100,0,Rin/kout))

# Error tolerance for solving differential equations
#--------------------------------------------------

RtolEQ<-1e-08
AtolEQ<-1e-08
Hmax<-Inf # Default value
#Hmax<-Inf #<1.5/24
###### END DIFFERENTIAL EQUATION OPTION ##############################
```

Figure

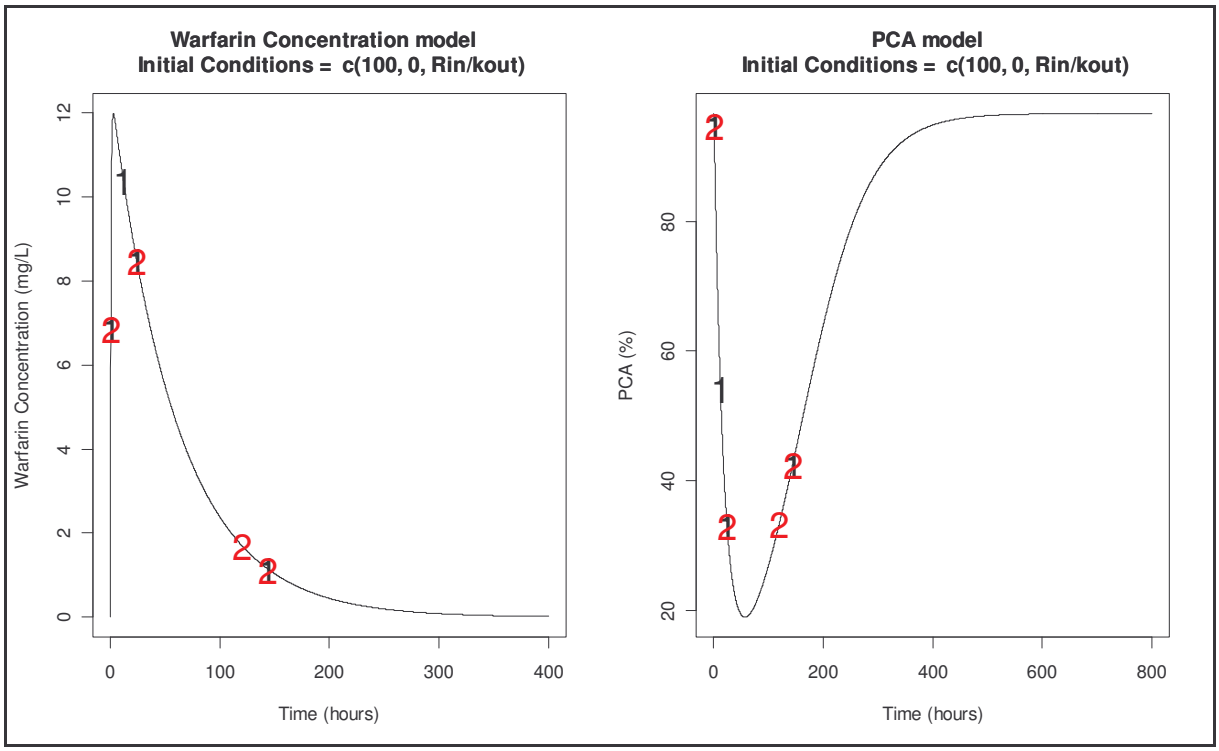
Fig.3

```
PFIM 3.0

Project: PKPDWarfarine_indirect_model : design optimisation

Date: Tue Mar 17 10:17:00 2009

*************************** INPUT SUMMARY ********************************
Initial Population design:

Sample times for response: A
            Protocol subjects            condinit
1 c=(0.5, 12, 48, 72)        32 c(100, 0, Rin/kout)


Sample times for response: B
            Protocol subjects            condinit
1 c=(0.5, 12, 48, 72)        32 c(100, 0, Rin/kout)


Initial Conditions at time 0 :

100 0 Rin/kout

Variance error model response A : ( 0 + 0.2 *f)^2

Variance error model response B : ( 3.88 + 0 *f)^2

Between-subject variance model: Trand =  2

Total number of samples: 256

Associated criterion value: 475.2673

Sampling windows for the response: A
Window 1 : t= 0 0.5 2 6 9 12 24 36 48 72 96 120 144
    Nb of sampling points to be taken in this window, n[ 1 ]= 4
Maximum total number of points in one elementary protocol : 4
Minimum total number of points in one elementary protocol : 4


Sampling windows for the response: B
Window 1 : t= 0 0.5 2 6 9 12 24 36 48 72 96 120 144
    Nb of sampling points to be taken in this window, n[ 1 ]= 4
Maximum total number of points in one elementary protocol : 4
Minimum total number of points in one elementary protocol : 4

Now evaluating the Fisher Information Matrix for the 495 protocols generated

*************************** OPTIMISED DESIGN *****************************
Optimised population design:
Sample times for response: A
                times      freq Subjects            condinit
1  c(0.5, 12, 24, 144) 0.6768466 21.65509 c(100, 0, Rin/kout)
2 c(0.5, 24, 120, 144) 0.3231534 10.34091 c(100, 0, Rin/kout)


Sample times for response: B
                times      freq Subjects            condinit
1  c(0.5, 12, 24, 144) 0.6768466 21.65509 c(100, 0, Rin/kout)
2 c(0.5, 24, 120, 144) 0.3231534 10.34091 c(100, 0, Rin/kout)

Associated optimised criterion: 580.1989
```

```
****************** POPULATION FISHER INFORMATION matrix ***************

              [,1]          [,2]          [,3]                [,14]
 [1,]  15.21253614   -22.802803   -2.6408043  -------   0.000000e+00
 [2,] -22.80280262 25806.590634 -126.2903231  -------   0.000000e+00
 [3,]  -2.64080430  -126.290323   10.9380478  -------   0.000000e+00
 [4,]  -0.04465257     2.990496   -0.2038661  -------   0.000000e+00
 [5,]   0.22893075   518.663794   -0.2501373  -------   0.000000e+00
 [6,] -41.54738605  1256.530406 -236.0756153  -------   0.000000e+00
 [7,]   0.00000000     0.000000    0.0000000  -------   3.932712e-03
 [8,]   0.00000000     0.000000    0.0000000  -------   1.588251e+00
 [9,]   0.00000000     0.000000    0.0000000  -------   2.456581e+00
[10,]   0.00000000     0.000000    0.0000000  -------   1.251327e+00
[11,]   0.00000000     0.000000    0.0000000  -------   6.318756e+01
[12,]   0.00000000     0.000000    0.0000000  -------   1.022446e+02
[13,]   0.00000000     0.000000    0.0000000  -------   1.152456e+01
[14,]   0.00000000     0.000000    0.0000000  -------   5.982762e+00

************************* EXPECTED STANDARD ERRORS *******************

----------------------- Fixed Effects Parameters ----------------------

      Beta    StdError        CV .
ka   1.600 0.263353095 16.459568 %
cl   0.133 0.006533504  4.912409 %
V    7.950 0.322403263  4.055387 %
Rin  5.410 0.437881955  8.093936 %
C50  1.200 0.052867047  4.405587 %
kout 0.056 0.001737771  3.103163 %


------------------------ Variance of Random Effects -------------------

      Omega    StdError        CV .
ka   0.7010 0.206505767  29.45874 %
cl   0.0634 0.017561742  27.69991 %
V    0.0206 0.012226360  59.35126 %
Rin  0.1900 0.050298864  26.47309 %
C50  0.0129 0.016460059 127.59736 %
kout 0.0167 0.007665362  45.90037 %


------------------------ Variance of residual error -------------------

            SIG  StdError        CV .
sig.slopeA 0.20 0.0216894 10.84470 %
sig.interB 3.88 0.4677695 12.05591 %


****************************** DETERMINANT ***************************

4.898658e+38

****************************** CRITERION *****************************

580.199

Time difference of 13.959 secs
sys.self
    1.62
```

Figure

Fig.4.


Warfarin Concentration model
Initial Conditions = c(100, 0, Rin/kout)
Warfarin Concentration (mg/L)
Time (hours)
PCA model
Initial Conditions = c(100, 0, Rin/kout)
PCA (%)
Time (hours)

Conflict of Interest Statement

The authors have no conflicts of interest that are directly relevant to the content of this work.