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ORIGINAL ARTICLE

An Approach for Identifiability of Population Pharmacokinetic–Pharmacodynamic Models

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Mathematical models are routinely used in clinical pharmacology to study the pharmacokinetic and pharmacodynamic properties of a drug in the body. Identifiability of these models is an important requirement for the success of these clinical studies. Identifiability is classified into two types, structural identifiability related to the structure of the mathematical model and deterministic identifiability which is related to the study design. There are existing approaches for assessment of structural identifiability of fixed-effects models, although their use appears uncommon in the literature. In this study, we develop an informal unified approach for simultaneous assessment of structural and deterministic identifiability for fixed and mixed-effects pharmacokinetic or pharmacokinetic–pharmacodynamic models. This approach uses an information theoretic framework. The method is applied both to simple examples to explore known identifiability properties and to a more complex example to illustrate its utility.

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Models are frequently used to study, understand, and quantify the relationship between the components of a biological system.[1] Mathematical models play an important role in systems biology to understand the underlying mechanisms such as physiological, pathological, and pharmacological responses. Examples of system models include the blood glucose model by Cobelli *et al.*,[2] the tumor growth model by Adam,[3] and the humoral coagulation network model by Wajima *et al.*[4] Identifiability of these models or reduced versions of these models is an important component in their application to modeling experimental results.

Application of mathematical models is routine in pharmacokinetics (PK) and in pharmacokinetic–pharmacodynamic (PKPD) studies. Estimation of unknown parameters in the model is a critical step in understanding the underlying exposure–response relationship and has an important role in decision making. A unique solution for the unknown parameters that links any set of inputs to a set of outputs is a critical requirement for any model-based analysis. Although this may be relaxed to a finite set of solutions in the special case of "flip–flop" PK.[5] The process of assessing for a unique solution for the parameters is encompassed within the framework of identifiability analysis. Parameters in the model that are not identifiable, i.e., not able to be estimated, pose challenges during the estimation step, leading to both imprecise parameter estimation and misleading conclusions or failure of the modeling process. Issues with identifiability are often intuitive for simple models (e.g., attempting to estimate the bioavailable fraction for an orally administered drug when data are only available after oral administration) but not so obvious in the case of more complex models (see the work on ivabradine by Evans *et al.*[6]). Recent developments in PK, PKPD, and systems pharmacology have centered on the development of more mechanistic (and hence complicated) models, and it is likely that identifiability of these models may not be intuitive.

Various methods are available in the literature for assessment of identifiability of linear and nonlinear PK models.[7–10] Assessment of identifiability based on evaluation of the Jacobian matrix was investigated by Jacquez[11] and Jacquez and Perry[12] for fixed-effects models. Although these are a very important aspect of model validation as illustrated by Cobelli and DiStefano[13] and Yates *et al.*,[14] identifiability analyses are often not practiced routinely, which may be due to the complexity of the mathematical computation involved in its execution or ease of availability and use of software. Bortz and Nelson[15] briefly mentioned the importance of identifiability analysis in their work on mixed-effects modeling of and model selection for HIV infection dynamics.

Clinical studies are now more often analyzed using a population analysis approach.[16] Population models are encompassed within the framework of nonlinear mixed-effects models that have natural hierarchies. It is desired in a population-based approach that all of the underlying parameters (fixed and random-effects parameters) are identifiable and should have an expected reasonable precision in their estimates. Currently, existing approaches to identifiability focus on identifiability of the fixed-effects parameters, and no specific approaches have been proposed to formally study the identifiability of random-effects parameters in population models. An exploration of this area in PK was recently proposed by Knutsson and Aarons.[17]

Identifiability of models is classified into two types: structural and deterministic identifiability. Structural identifiability, also termed *a priori* identifiability, is related to the structure of the underlying mathematical model and reflects whether the parameters in the assumed model have a unique solution given perfect input–output data. Structural identifiability classifies a model into any one of the three following subtypes:

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1. Structurally globally identifiable: All parameters in the model have unique solutions.
2. Structurally locally identifiable: One or more parameters in the model have a finite number of alternate solutions.
3. Structurally unidentifiable: One or more parameters in the model have an infinite number of alternate solutions.

Deterministic identifiability, also termed *a posteriori* identifiability or practical identifiability[18] or numerical identifiability,[19] relates to the informativeness of the data and is influenced by the study design and its execution. Deterministic identifiability deals with assessment of whether the parameters in a model can be estimated precisely given imperfect input–output data. In its most basic form, deterministic identifiability requires that the number of observations (n) is greater than or equal to the number of unknown parameters (p). Of note, all structurally unidentifiable models are deterministically unidentifiable, whereas a structurally identifiable model need not be deterministically identifiable. Although few software tools[20,21] were designed to assess the structural identifiability, no dedicated software is available to assess deterministic identifiability. However, any software that has been developed for an optimal design of experiments[22,23] can be used to explore the deterministic identifiability.

The purpose of this paper is to develop and evaluate a unified approach for identifiability analysis of both fixed and mixed-effects PKPD models that encompasses both structural and deterministic identifiability. Note here we only consider PK models, but the methods are also applicable to PKPD models. The proposed approach is described in the following theory section. Furthermore, we provide the results for three specific objectives and a general discussion. The specific objectives are (i) to evaluate the method for assessing identifiability for simple fixed-effects PK models; (ii) to explore the method for testing identifiability of random-effects parameters in simple population PK models; and (iii) to apply the method for identifiability analysis of a more complicated parent-metabolite PK model. A description of the notation for nonlinear fixed- and mixed-effect models in general, and the methods for evaluating the proposed approach based on the specific objectives is given in the methods section.

THEORY

Criteria for identifiability analysis

In this work, assessment of both structural and deterministic identifiability is based on an information theoretic approach (see Mentré *et al.*[22] for an introduction to this approach for nonlinear mixed-effects models) involving computation of the Fisher information matrix ($\mathbf{M_F}$). In this approach, the sensitivity of the model-predicted response to changes in the parameter values is evaluated at each design point. Parameters are defined by $\boldsymbol{\theta}$, a $p \times 1$ vector ($\boldsymbol{\theta} = [\theta_1,\ldots,\theta_p]^T$), and the design points representing the time points of observation by $\boldsymbol{\xi}$, a $n \times 1$ vector ($\boldsymbol{\xi} = [\xi_1,\ldots,\xi_n]^T$). We use ^{T} to indicate the matrix transpose. In this manuscript, we use bold face notation to represent a vector or a matrix.

$$\mathbf{J} = \begin{bmatrix} \frac{\partial f(D,\xi_1,\boldsymbol{\theta})}{\partial \theta_1} & \cdots & \frac{\partial f(D,\xi_1,\boldsymbol{\theta})}{\partial \theta_p} \\ \vdots & \ddots & \vdots \\ \frac{\partial f(D,\xi_n,\boldsymbol{\theta})}{\partial \theta_1} & \cdots & \frac{\partial f(D,\xi_n,\boldsymbol{\theta})}{\partial \theta_p} \end{bmatrix} \quad (1)$$

The sensitivity of the system is defined by the Jacobian matrix ($\mathbf{J}$), a $n \times p$ matrix of all first partial derivatives overall design points. D in the above expression represents the dose administered and f denotes the structural model.

Random noise in the observed response across the observation points is represented by the variance–covariance matrix ($\boldsymbol{\Sigma}$);

$$\boldsymbol{\Sigma} = \begin{bmatrix} \sigma_1^2 & \cdots & 0 \\ \vdots & \ddots & \vdots \\ 0 & \cdots & \sigma_n^2 \end{bmatrix} \quad (2)$$

$\boldsymbol{\Sigma}$ is a $n \times n$ square matrix and is computed from the product of the residual variance or random noise (σ^2) and a $n \times n$ identity matrix ($\mathbf{I}_n$). Equal variances across all observation points ($\sigma_1^2 = \sigma_2^2 = \ldots = \sigma_n^2$) are assumed. The assumption of equal variances can be relaxed without affecting the proposed identifiability method.

For a fixed-effects model, the $\mathbf{M_F}$ is constructed as

$$\mathbf{M_F}(D,\xi,\boldsymbol{\theta},\Sigma) = \mathbf{J}^T \cdot \Sigma^{-1} \cdot \mathbf{J} \quad (3)$$

$\mathbf{M_F}$ in this instance represents the fixed-effects parameters and does not include the residual variance term.

The determinant is represented as $\left|\mathbf{M_F}(D,\xi,\boldsymbol{\theta},\Sigma)\right|$.

Here, we propose a general and a revised criterion based on the $|\mathbf{M_F}|$ for the assessment of identifiability of models.

General criterion: Given a standard dose D, a specific design $\boldsymbol{\xi}$, a parameter vector $\boldsymbol{\theta}$, and a matrix showing an assumed random noise in the observed response $\boldsymbol{\Sigma}$, the $\mathbf{M_F}$ of an identifiable model is invertible and its determinant is greater than zero. This general criterion is given as:

$$\xi: \left|\mathbf{M_F}(D,\xi,\boldsymbol{\theta},\Sigma)\right| > 0 \quad (4)$$

A singularity of the $\mathbf{M_F}$ with a determinant value of zero indicates that the model has one or more underlying parameters that are not identifiable. The $\mathbf{M_F}$ of a model that is not identifiable will be rank deficient and may contain a column and row of zeros. Although theoretically this criterion holds well, it may fall down on practical utility due to two reasons: (i) accuracy issues with matrix algebra operations can result in determinants for unidentifiable models to be represented as very small positive or negative values rather than zero and (ii) it requires a search across a large multidimensional design space to assess whether there exists a set of design variables that may satisfy the above criterion. In order to simplify the application of the criterion, we propose a second, revised

criterion that is robust to the above shortfalls. It is based on the relation between the $|\mathbf{M_F}|$ and the random noise associated with the observed response.

Revised criterion: For all values of design variables ($\boldsymbol{\xi}$), where $n > p$, and all design variables have a unique value (i.e., $\xi_i \neq \xi_j$ for all $i \neq j$), the $|\mathbf{M_F}|$ approaches infinity as the associated noise approaches zero.

$$\forall \xi: \left|\mathbf{M_F}\left(D,\xi,\theta,\Sigma\right)\right| \underset{\lim \sigma^2 \to 0}{=} \infty\ ,\ \xi_i \neq \xi_j\ ;\ \text{for all}\ \ i \neq j \quad (5)$$

This criterion has greater practical utility than the general criterion as there are no limitations associated with its computation. As this criterion accounts for all designs, there is no necessity to search over the design space and an arbitrary set of values that fulfils the condition above in Eq. (5) can be chosen.

For fixed-effects models, the log of the determinant will be linearly related to the log of the random noise if the model is structurally identifiable. In the case of mixed-effects models, where $\mathbf{M_F}$ includes fixed-effects, random-effects, and the residual variance, we find

$$\forall \xi: \left|\mathbf{M_F}\left(D,\xi,\theta,\Omega,\Sigma\right)\right| \underset{\lim \sigma^2 \to 0}{=} \Psi\ ,\ \xi_i \neq \xi_j\ ;\ \text{for all}\ \ i \neq j,$$
$$\text{where}\ \ 0 < \Psi < \infty\ ,\ \Psi = f(\mathbf{V})\ ;\ \mathbf{V} \approx \mathbf{J}^{\mathrm{T}} \cdot \boldsymbol{\Omega} \cdot \mathbf{J} + \Sigma \quad (6)$$

In the above expression, $\boldsymbol{\Omega}$ is a vector of the variances of the random effects representing between the subject variability (BSV) in the population. $\mathbf{V}$ represents the first-order approximated likelihood of the variance. See Retout and Mentré for details relating to the expression for the population Fisher information matrix.[24] The asymptote of the log $|\mathbf{M_F}|$ in this case will not approach infinity. The numerical value to which Ψ asymptotes will have functional dependence on $\mathbf{V}$. The relationship between the log of the determinant and the log of the random noise will be smooth and will asymptote to a constant Ψ in the space $\Re^+$. We show this relationship graphically which for identifiable models shows the convergent nature, to the asymptote, of the relationship.

In the following sections, assessment of identifiability is based on the revised criterion across the models.

RESULTS

Evaluation of the method for assessing identifiability of simple fixed-effects PK models

Assessment of the structural identifiability of the simple fixed-effects PK models indicated that both Bateman and Dost models were unidentifiable when all parameters (including *F*) were considered to be estimable. Both of these models were rendered identifiable by fixing *F* to a constant parameter value (**Figure 1**, see **Supplementary Table S1** online). It is seen that unidentifiable models showed a discontinuous relationship for the log $|\mathbf{M_F}|$ vs. log random noise, whereas a

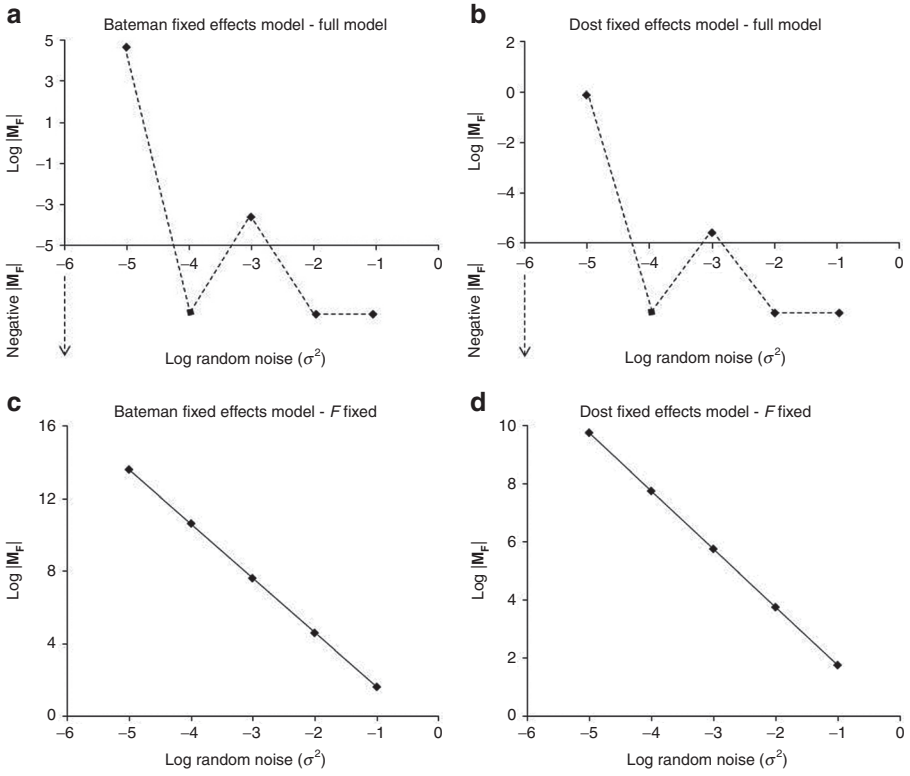

Figure 1 Graphical representation of log $|\mathbf{M_F}|$ vs. log random noise (σ^2) for simple fixed-effects pharmacokinetic models. In this graph, log $|\mathbf{M_F}|$ above the abscissa are as represented. Data below the abscissa represent the negative determinants that do not have log values and are shown for the purpose of displaying discontinuity of the line. (**a**,**c**) Bateman model, (**b**,**d**) Dost model; upper row: all parameters estimated, lower row: *F* fixed.

continuous linear relationship was observed for identifiable models.

Identifying unidentifiable parameter(s) in the model: The results of the case deletion assessment for identifying the unidentifiable parameters in the Bateman fixed-effects model indicate that F is the unidentifiable parameter in the model (**Supplementary Table S2** online).

Exploration of the method for testing the identifiability of random-effects parameters in simple population PK models

The criterion for assessment of identifiability of random-effects parameters was explored for the Bateman and Dost population PK models. The results for these mixed-effects models indicated that both the models were unidentifiable when all parameters were considered to be estimable. Fixing F alone rendered the Bateman model to become identifiable, whereas the Dost model was still unidentifiable. The Dost model was only identifiable when F and its BSV parameter ω_F were fixed (**Figure 2**, **Supplementary Table S1** online).

Application of the method for identifiability analysis of a parent-metabolite PK model

Application of the criterion for assessment of identifiability of the motivating PK model was performed separately for models describing intravenous and oral administration. Both the models were considered for a fixed-effects analysis (as per the previous identifiability analysis reported by Evans *et al*.[6]) and for a full mixed-effects analysis.

Assessment of the intravenous PK model: The intravenous fixed-effects model was unidentifiable when all parameters in the model were considered to be estimable. Fixing the value of V_3 (volume of distribution of metabolite in the central compartment) rendered the model structurally identifiable. The results of this analysis are in agreement with Evans *et al*.[6] Results of the analysis of mixed-effects model revealed that all random-effects parameters were identifiable in the model. Although the fixed-effect parameter V_3 was unidentifiable, its corresponding random-effect parameter ω_{V3} was identifiable (**Figure 3**, **Supplementary Table S3** online). Note here that in the full mixed-effects model, the typical value of V_3 remained unidentifiable.

Assessment of the oral PK model: The oral fixed-effects model was unidentifiable when assessed with all parameters in the model. The model was still unidentifiable when V_3 was fixed. This was not unexpected as the model considered two additional parameters, namely, the fractions of parent and metabolite absorbed (F_I and F_S, respectively) following the oral administration of parent. Fixing one of these fractions (e.g., F_I) rendered the model identifiable, whereas F_S was identifiable as its corresponding volume term V_3 was already fixed in the model. Therefore, the oral fixed-effects model has two unidentifiable parameters. Assessment of the

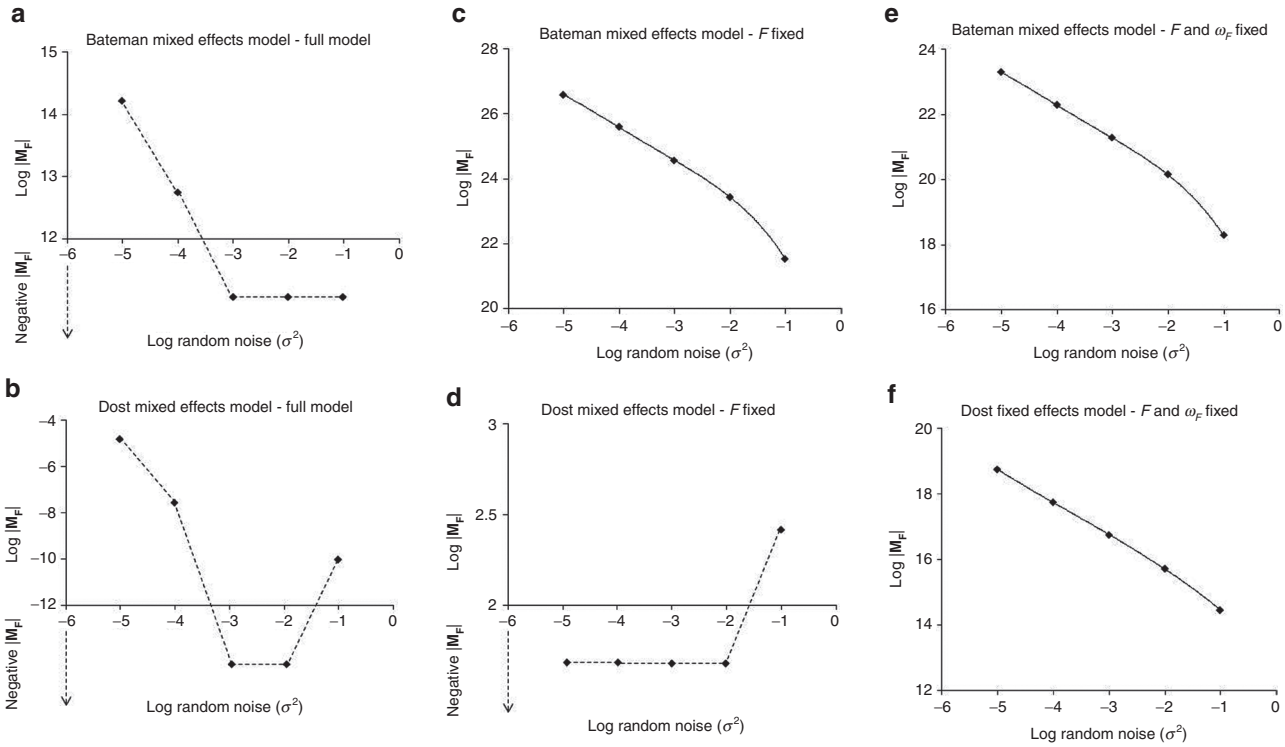

Figure 2 Graphical representation of log $|\mathbf{M_F}|$ vs. log random noise (σ^2) for simple mixed-effects PK models. In this graph, log $|\mathbf{M_F}|$ above the abscissa are as represented. Data below the abscissa represent the negative determinants that do not have log values and are shown for the purpose of displaying discontinuity of the line. (**a**,**c**,**e**) Bateman model, (**b**,**d**,**f**) Dost model; left column: all parameters estimated, middle column: F fixed, right column: F and ω_F fixed.

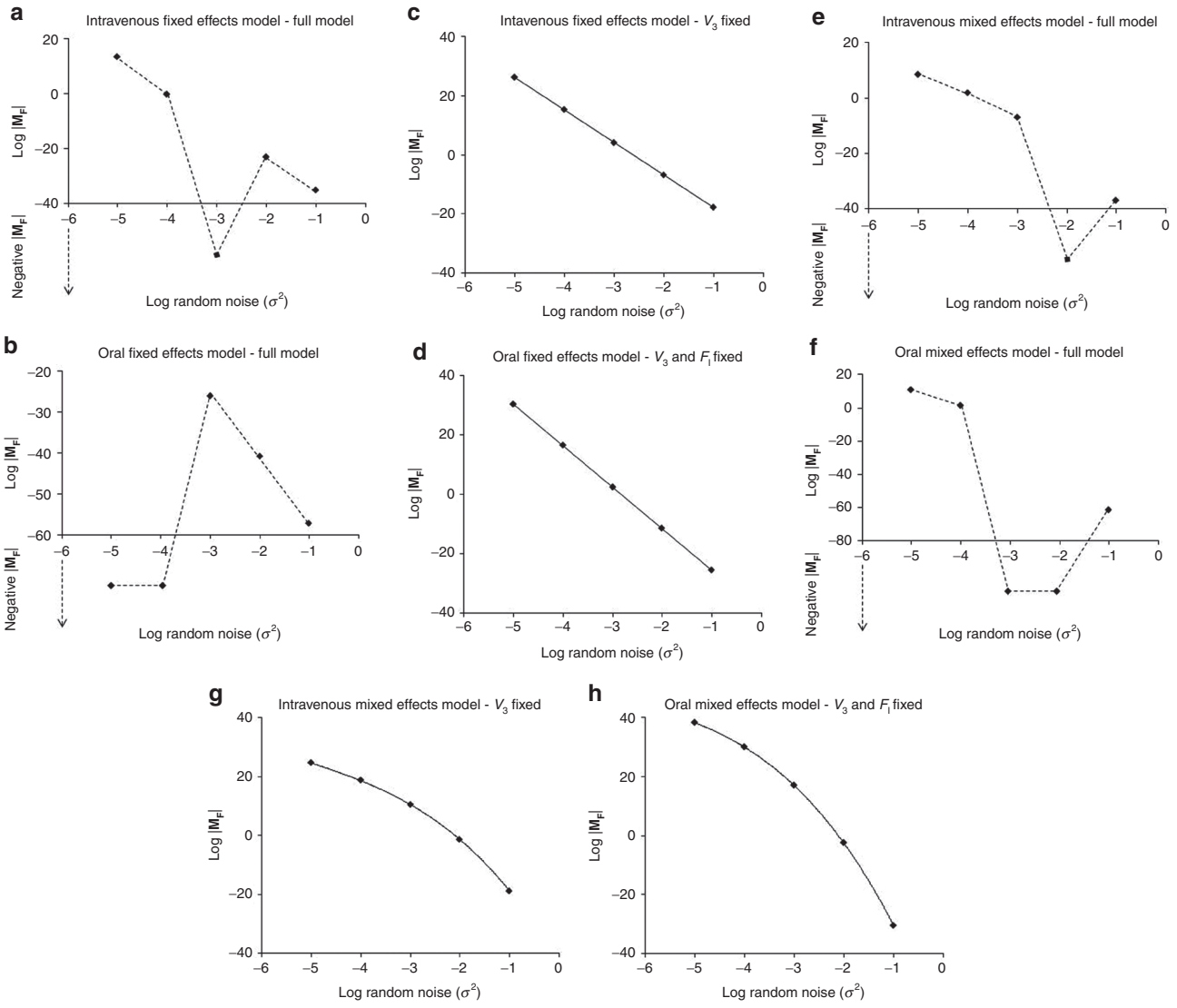

Figure 3 Graphical representation of log $|\mathbf{M}_F|$ vs. log random noise (σ^2) for parent-metabolite PK model of ivabradine. In this graph, log $|\mathbf{M}_F|$ above the abscissa are as represented. Data below the abscissa represent the negative determinants that do not have log values and are shown for the purpose of displaying discontinuity of the line. (**a**,**c**,**e**,**g**) Intravenous model, (**b**,**d**,**f**,**h**) oral model; (**a**,**b**) all parameters estimated (fixed-effects model), (**c**,**d**) $V_3 \pm F_1$ fixed (fixed-effects model), (**e**,**f**) all parameters estimated (mixed-effects model), (**g**,**h**) $V_3 \pm F_1$ fixed (mixed-effects model).

full mixed-effects model indicted that all of the random-effects parameters were identifiable. Random-effects parameters ω_{V3} and ω_{F1} were still identifiable, although their corresponding fixed-effects parameters (V_3 and F_1, respectively) were unidentifiable (**Figure 3**, see **Supplementary Table S3** online).

DISCUSSION

Analysis of the results from both simple and a more complicated PK models have shown that the $|\mathbf{M}_F|$ shows a continuous linear log–log relationship with the associated random noise for structurally identifiable fixed-effects models. Similarly for structurally identifiable mixed-effects models, we also see a continuous relationship between log $|\mathbf{M}_F|$ and log residual variance, although the relationship is no longer linear on this scale due to the noninfinite asymptote.

An important feature of this analysis is that all identifiable models considered here yielded positive values of $|\mathbf{M}_F|$ for all values of the residual variance. This, however, is not in itself sufficient to confirm identifiability, as nonidentifiable models also yielded positive $|\mathbf{M}_F|$ values for some values of the residual variance. This emphasizes the need to show two necessary conditions for claiming identifiability for a model: (i) log $|\mathbf{M}_F|$ should have a continuous relationship with log residual variance and (ii) $|\mathbf{M}_F|$ should approach infinity (or a noninfinite asymptote for mixed-effects models) as residual variance approaches zero. The requirement to satisfy these two conditions requires $|\mathbf{M}_F|$ to be positive at varying random noise.

Evaluation of the results for the Bateman and Dost fixed- and mixed-effects models indicate that this method is capable of evaluating the structural identifiability of fixed- and random-effects parameters in population models. Analysis of the population models reveal that random-effects parameters may or may not follow the same rule as their corresponding fixed-effects parameters in regard to the identifiability. At this point, there does not appear to be a standard rule that could be applied to assess the identifiability of a random-effects parameter given known identifiability of a fixed-effects parameter. However, in the limited examples explored here, we see that in cases where the fixed-effects parameter is identifiable, the corresponding random-effects parameter is also always identifiable. However, other than for the Dost model, there appear to be circumstances when the random-effects parameters are identifiable despite the corresponding fixed-effects parameters being unidentifiable. This emphasizes the need to assess the identifiability of random-effects parameters in population models. Results from the assessment of the parent-metabolite PK model strengthen the ability of the current approach in assessing the identifiability of models with greater complexity and where identifiability may not be obvious. However, further investigations are needed to evaluate more precisely the identifiability of the random-effects parameters by assessing the $\mathbf{M}_F$ and to understand how they differ from their corresponding fixed-effects parameters in regard to identifiability.

Assessment of deterministic identifiability was not explicitly performed in this work. However, due to the known relationship between the information in a design and the standard error of the estimator (via Cramér-Rao inequality[25,26]), it is straightforward from this analysis to choose a design that performs sufficiently well to meet the needs of deterministic identifiability. Once structural identifiability of the model is established, a formal assessment of the diagonal elements of the inverse of $\mathbf{M}_F$ can be made to assess the precision of parameter estimates for a candidate design at a value of residual variance of interest. It is noticed that highly constrained designs will impact the parameter estimation in the form of high standard error values. These are consequences of deterministic identifiability issues that can be studied easily by the current approach before the study execution. Unlike other traditional available approaches for structural identifiability analysis that involve significant mathematical computation, our proposed approach is simple and any optimal design software (e.g., PFIM,[27] PopED,[28] and PopDes[29]) can be used for assessing the identifiability of a model.

The proposed method, using an information theoretic approach, can be used to assess complete identifiability of population PK models. The criterion was able to evaluate the model in relation to indirectly assessing for a unique solution for individual parameters, a consequence of structural identifiability as shown in this study using simple and motivating PK models. The approach developed in this study can be used formally to assess the identifiability of proposed candidate models during study design. In the case of population models, the $\mathbf{M}_F$ can be studied separately, block wise to assess identifiability of the respective model parameters. The determinant of each submatrix can be studied to assess the identifiability of fixed effects, random effects, and error terms, respectively. We have not explored nonzero off-diagonal elements in the between subject variance–covariance matrix, however, we believe that the techniques described here are likely to be generalizable to this and other circumstances. We also believe that the identifiability of random-effects parameters, such as the random effect on bioavailable fraction in the Bateman model, would be affected in presence of covariances (non-zero off diagonals), such as between clearance and volume of distribution, in the variance–covariance matrix, and further exploration is warranted.

In conclusion, we have developed an informal unified approach for the assessment of both structural and deterministic identifiability for both fixed and random-effects parameters in population models. The approach was evaluated against both simple PK models with known identifiability issues and expanded to a more complicated parent-metabolite model. This approach is not limited to PK models and is extendable to identifiability analysis of population PKPD models.

METHODS

Notation for nonlinear fixed- and mixed-effect models. We consider nonlinear models where the observed response is nonlinear in the parameter values. The notation for a nonlinear fixed-effects model is

$$y_j = f\left(D, \xi_j, \boldsymbol{\theta}\right) + \varepsilon_j;\ \varepsilon_j \overset{iid}{\sim} N\left(0, \sigma^2\right) \tag{7}$$

y_j is the observed response at the j^{th} observation, and ε_j denotes the random error in the j^{th} observation. All other variables are defined as before, and we are using the same notation throughout the article.

Population models are encompassed within the framework of nonlinear mixed-effects models. Population models have two-stage hierarchy in describing the observed response across the individuals in a group of population.

Stage I constitutes the model for the data (structural model) representing the observed response in the population, which is given by

$$y_{ij} = f\left(D_i, \xi_{ij}, \boldsymbol{\theta}_i\right) + \varepsilon_{ij}\ ;\ \ \varepsilon_{ij} \overset{iid}{\sim} N\left(0, \sigma^2\right) \tag{8}$$

Here, y_{ij} represents j^{th} response in i^{th} individual.

Stage II constitutes the model for heterogeneity (covariate model) in the parameter values between individuals, which is given for any parameter θ in the model by

$$\theta_i = g\left(\mathbf{Z}_i, \bar{\theta}\right) \cdot \left(\exp\left(\eta_i\right)\right)\ ;\ \ \eta_i \overset{iid}{\sim} N\left(0, \omega\right) \tag{9}$$

θ_i represents the parameter value in i^{th} individual (we have dropped the index specifying the parameter for simplicity but note that parameters may have covariance), g is the functional form of the covariate model, $\mathbf{Z}_i$ is a vector of covariates in i^{th} individual, $\bar{\theta}$ is the population mean value of the parameter estimate, η_i is the random effect for the i^{th} individual, and

ω is the variance of the random effect across the population. For the entire model, the BSV in all parameters with random effects is given by a $q \times q$ matrix '$\boldsymbol{\Omega}$', where q is the number of fixed-effect parameters that have a random effect ($q \leq p$).

$$\boldsymbol{\Omega} = \begin{bmatrix} \omega_{11} & \cdots & \omega_{1q} \\ \vdots & \ddots & \vdots \\ \omega_{q1} & \cdots & \omega_{qq} \end{bmatrix} \tag{10}$$

Diagonal elements represent the variances, and off-diagonal elements represent the covariances of the BSV.

Objective 1: Evaluation of the method for assessing identifiability of simple fixed-effects PK models. The criterion was explored by evaluating two simple PK models with known identifiability properties.

The first model considered was a one-compartment first-order input–output model (also known as "Bateman model").[30,31] The model is given by:

$$f\left(D,\xi_j,\boldsymbol{\theta}\right) = \frac{D \cdot F \cdot k_a}{V \cdot (k_a - k)}\left(\exp\left(-k \cdot t_j\right) - \exp\left(-k_a \cdot t_j\right)\right); \quad k = \frac{CL}{V} \tag{11}$$

The estimated parameters in the model are clearance (CL), volume of distribution (V), absorption rate constant (k_a), and bioavailable fraction (F), while k denotes the derived elimination rate constant.

The second model considered is the so-called "Dost model,"[32] which is a simplification of the Bateman model.

$$f\left(D,\xi_j,\boldsymbol{\theta}\right) = \frac{D \cdot F \cdot k' \cdot t_j}{V}\left(\exp\left(-k' \cdot t_j\right)\right) \tag{12}$$

The parameters in this model are similar to the parameters in the Bateman model, except k' is a hybrid parameter that represents both the absorption and elimination rate constants (where $k' = k_a = k$).

Identifiability analyses were performed using MATLAB 7.12 (version R2011a).[33] A constant dose of 100 mg and a generic study design for the sampling times ($\boldsymbol{\xi}$ = [0, 0.25, 0.5, 0.75, 1, 2, 4, 8, 12, 18, 24]^T) was assumed for both models. An arbitrary set of the parameter values was used (**Table 1**). The identifiability was assessed for both models based on two scenarios: (i) F was considered to be an unknown and estimable parameter and (ii) F was assumed to be known and fixed. Values for the random noise were $\log(\sigma^2)$ = (–5, –4, –3, –2, and –1).

Table 1 Empirical set of parameter values used for the assessment of identifiability of simple PK models (fixed and mixed-effects models)

Parameter	Mean value (θ)	BSV (ω)†
CL	4	0.1
k_a	1	0.1
V‡	20	0.1
F‡	1	0.1
k'	0.5	0.1

PK, pharmacokinetics.
†Used for the mixed-effects models only. ‡Common parameters in the Bateman and Dost model.

Identifying unidentifiable parameter(s) in the model. For unidentifiable models, the unidentifiable parameters were identified by a case deletion methodology. A specific column corresponding to a specific parameter was removed from the Jacobian matrix, and the subsequent effect on the determinant was evaluated. Cases (parameters) for which the deletion provided a linear relationship in log $|\mathbf{M_F}|$ vs. log random noise were taken as unidentifiable. The Bateman model was chosen as an illustrative example to show this method with two sets of parameterization (V, CL k_a, F and V, k_a, k, F).

Objective 2: Exploration of the method for testing identifiability of random-effects parameters in simple population PK models.

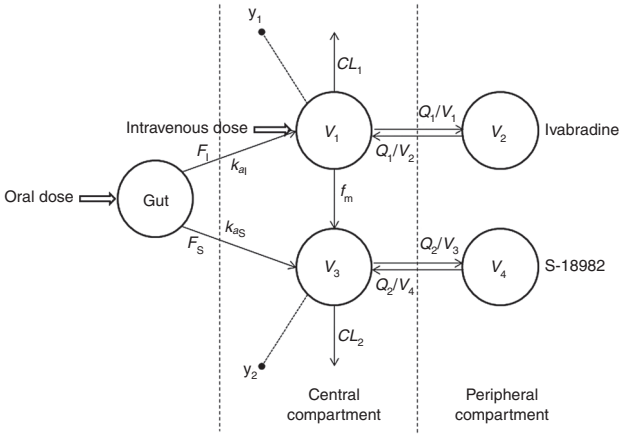

Figure 4 Schematic representation of the combined parent-metabolite pharmacokinetic model of ivabradine. y_1 represents the observations corresponding to the parent (ivabradine) and y_2 represents the observations corresponding to the metabolite (S-18982).

Table 2 Empirical set of parameter values used for the assessment of identifiability of the fixed and mixed-effects parent-metabolite PK model for ivabradine

Parameter	Mean value (θ)	BSV (ω)†
CL_1‡	25	0.2
V_1‡	200	1.4
Q_1‡	75	1.7
V_2‡	650	0.4
CL_2‡	150	0.2
V_3‡	100	0.3
Q_2‡	250	0.1
V_4‡	650	0.7
f_m‡	0.5	0.1
k_{a1}	1.5	0.2
F_1	0.8	0.1
k_{aS}	2	0.2
F_S	0.8	0.1

PK, pharmacokinetics.
†Used for the mixed-effects models only. ‡Common parameters in the intravenous and oral PK models.

The Bateman and Dost models were also used to explore the criterion for testing identifiability of random-effects parameters in a population analysis. The study design, dose, fixed-effects parameters and random noise in the observed response were the same as for Objective 1. The number of individuals in the population was set to 100. The parameter values, including the random-effects values, are presented in **Table 1**. Assessment of identifiability of the mixed-effects models was conducted using the POPT (Population OPTimal design) software tool,[34] a MATLAB application. Three scenarios were considered: (i) F and its corresponding random-effect BSV parameter ω_F were considered to be unknown and estimable parameters, (ii) F was assumed to be known and fixed, whereas ω_F was considered to be unknown and estimable, and (iii) F and ω_F were both assumed to be known and fixed.

Objective 3: Application of the method for identifiability analysis of a parent-metabolite PK model. A combined parent-metabolite model describing the PK of ivabradine and its *N*-desmethylated metabolite (S-18982) was used as the motivating model (a schematic is provided in **Figure 4**). Ivabradine is a bradycardiac agent used in the treatment of angina pectoris and ischemic heart disorders. Probable identifiability issues associated with this model were first proposed by Duffull *et al*.[35] and a structural identifiability analysis based on a similarity transformation approach was reported by Evans *et al*.[6] Ivabradine is administered either intravenously or as oral dose.

In the case of intravenous administration, the drug is known to follow two-compartment pharmacokinetic behavior with first-order elimination. Metabolism of the parent produces S-18982, an active metabolite. The metabolite is also known to undergo two-compartment disposition similar to the parent.

In the case of oral administration, a certain portion of the parent is known to undergo presystemic metabolism in the gut producing S-18982, which is then absorbed into the systemic circulation. There is also a possibility that a certain portion of the parent is neither absorbed into the systemic circulation nor undergoes presystemic metabolism. This portion of the parent can either convert into other, unknown metabolites, or may be eliminated from the gut. The parameters describing bioavailability for the parent and metabolite following oral administration of parent are denoted F_I and F_S, respectively. In the current study, identifiability analyses were performed separately for models describing intravenous and oral dose administration, each in two stages for fixed- and mixed-effects models, respectively, using POPT with an empirical set of parameter values (**Table 2**). All other study variables were similar as described for the simple example models. Algebraic equations used in the assessment of identifiability of intravenous and oral PK model are provided in the **Supplementary Data** online. The POPT code (**p_model.m** and **popt_ini.m**) of the oral mixed-effects PK model of ivabradine is available as **Supplementary Data** online.

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Conflict of Interest. The authors declared no conflict of interest.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

✓ Existing approaches and available software for the assessment of structural identifiability are mainly focused on fixed-effects models, while no approach has been proposed to assess both fixed-effects and random-effects parameters in population models.

WHAT QUESTION DID THIS STUDY ADDRESS?

✓ This study describes an approach for concurrent assessment of both structural and deterministic identifiability of fixed and random-effects parameters in population models.

WHAT THIS STUDY ADDS TO OUR KNOWLEDGE

✓ We propose a novel approach that can be used routinely for the assessment of structural and deterministic identifiability for population models.

HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY AND THERAPEUTICS

✓ Recent advances in clinical pharmacology have led to the design of complicated mechanistic models for which identifiability is not intuitive and are likely to be associated with significant identifiability issues. The approach presented in this study facilitates the evaluation of these quantitative models.

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