Assessment of the nlmixr R-package for population pharmacokinetic modeling: A metformin case study

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Principal investigator statement

This is a secondary data analysis. There is no principal investigator.

Conflict of interest statement

We have read and understood *British Journal of Clinical Pharmacology's* policy on disclosing conflicts of interest and declare that we have none.

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What is already known about this subject (up to three short bullet points, less than 50 words)

- *nlmixr* offers parameter estimation algorithms including SAEM and FOCE with or without interaction.
- Transit compartment model can be implemented with Stirling or log-gamma approximation to $n!$ but the resultant difference in estimated bioavailability has not been explored.
- Influence of flip-flop pharmacokinetics over the performance of *nlmixr* estimation algorithms was unknown

What this study adds (up to three short bullet points, less than 50 words)

- Non-linear mixed-effect modeling of flip-flop systems without external software dependency is possible with *nlmixr*
- SAEM is marginally superior to FOCEi with lower bias and higher precision when flip-flop is present, and is more robust.
- The log-gamma function is preferred in the transit compartment model over Stirling's approximation for n!

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ABSTRACT

Aim: *nlmixr* offers first-order conditional estimation with or without interaction (FOCE or FOCEi) and stochastic approximation estimation-maximisation (SAEM) to fit nonlinear mixed-effect models (NLMEM). We modelled metformin's population pharmacokinetics with flip-flop characteristics within *nlmixr* framework and investigated FOCEi and SAEM's performance with respect to bias, precision, and robustness.

Method: Compartmental pharmacokinetic models were fitted. The final model was determined based on the lowest objective function value and visual inspection of goodness-of-fit plots. To examine flip-flop pharmacokinetics, k_a values of a typical concentration-time profile based on the final model were perturbed and changes in the steepness of the terminal elimination phase were evaluated. The bias and precision of parameter estimates were compared between FOCEi and SAEM using stochastic simulations and estimations. For robustness, parameters were re-estimated as the initial estimates were perturbed 100-times and resultant changes evaluated.

Results: A one-compartment model with transit compartment for absorption best described the data. At low n, Stirling's approximation of $n!$ over-approximated plasma concentration unlike the log-gamma function. Flip-flop pharmacokinetics were evident as the steepness of the terminal elimination phase changed with k_a. Mean rRMSE for fixed-effect parameters were 0.932. When initial estimates were perturbed, FOCEi estimates of k_a and *food* effect on k_a appeared bimodal and were upward biased.

Discussion: *nlmixr* is reliable for NLMEM even if flip-flop is present but caution should be exercised when using Stirling's approximation for $n!$ in the transit compartment model. SAEM were marginally superior to FOCEi in bias and precision, but SAEM was superior against initial estimate perturbations.

INTRODUCTION

Nonlinear mixed-effects models (NLMEMs) are important tools to characterise pharmacokinetics (PK) and pharmacodynamics (PD) of a drug in a patient population. The adoption of commercial software for nonlinear mixed effect (NLME) modelling, the most popular of which are NONMEM and Monolix, has created a paradigm shift in drug development process [1]. Population PK/PKPD models are increasingly used to support new drug applications globally. This is demonstrated by the 2019 release of a US Food and Drug Administration draft guidance on population PK to assist pharmaceutical companies in the application of population PK analysis as an essential component of drug development [2].

As NLMEM becomes established in the pharmaceutical industry, the needs to ensure transparency and reproducibility of the modelling process and to improve accessibility to these tools have become apparent. Fidler *et al* (2019) developed an open-source R package *nlmixr* as an alternative to commercial tools. It is freely available and does not have software dependencies beyond the R environment [3]. Currently, *nlmixr* supports three parameter estimation algorithms: *nlme* by Pineiro and Bates [4], stochastic approximation-estimation maximisation (SAEM) [5], and first-order conditional estimation with or without interaction (FOCEi and FOCE) [6].

These estimation algorithms may produce different degree of bias and precision. FOCE takes into consideration all possible individual parameter values when evaluating the joint density of observed data and random effects (ETAs). It utilises a first-order Taylor series approximation around the posterior mode of ETAs [1]. SAEM, however, is a type of stochastic algorithm that computes the maximum likelihood estimates under the general setting of incomplete data (unobserved individual parameters) [7]. It was first introduced by Delyon *et al* (1999) [5] and has the benefit of often converging to a solution while other conditional estimation algorithms fail. SAEM has been reported to be relatively insensitive to the choice of initial parameters [1].

Since the release of *nlmixr* on the Comprehensive R Archieve Network (CRAN), there have been few publications beyond those published by the *nlmixr* developers [3,8,9]. As such, the aim of our study was twofold: to develop and evaluate a population PK model exclusively within the *nlmixr*

framework, and to evaluate and compare the FOCEi and SAEM algorithms with respect to bias and precision of parameter estimates as well as robustness of estimation to perturbation in initial estimates.

To achieve this we chose a metformin dataset with potentially complex PK to challenge *nlmxir*. Metformin is a widely used anti-diabetic medication but it may exhibit flip-flop PK when administered orally. It is a highly ionised molecule and shows incomplete oral absorption with an estimated absolute bioavailability between 40%-60% [10,11]. The oral absorption rate of metformin demonstrates an inverse relationship with the dose ingested (ranging from 0.5g to 1.5g) [12]. Pentikäinen *et al* (1979) first described the flip-flop PK characteristics of metformin [13], the finding of which was further supported by subsequent studies [14,15].

METHOD

Clinical dataset

Plasma concentration and subject demographic data from the reference formulation (250mg immediate release tablet) of two metformin bioequivalence (BE) studies were used [16,17]. The BE study design was identical apart from the condition under which metformin was administered: fed or fasted. All subjects received a single dose at baseline and had 15 blood samples taken within 24-hours.

Handling observations below the limit of quantification

Observations below the quantification limit (BQL) of the analytical assay were set to one-half of the limit of quantification (LoQ) such that $DV = \frac{LoQ}{2}$, where DV was the dependent variable [18,19], and the subsequent BQL observations were omitted from the analysis as these observations could be assumed to reflect a decresing true concentration after single dose administration.

Model development and diagnostic tools

A population PK analysis of metformin was conducted with *nlmixr* (version 2.0.4). Pre- and post-processing of data was conducted in R (version 4.0.5) using the R package xpose.nlmixr (version 0.2.0).

Pharmacokinetic model

The classical one-, two-, and three-compartment model with first-order oral absorption and first-order elimination kinetics were considered to described metformin's PK characteristics. Several studies had reported delays in metformin absorption especially when co-administered with food [11,20,21]. As such, we also considered the transit compartment model first described by Savic *et al* (2007) to model such delays in absorption [22]. The authors originally estimated the optimal number of transit compartments using the function $a_n(t) = F \cdot Dose \cdot \frac{(k_{tr} \cdot t)^n}{n!} \cdot e^{-k_{tr} \cdot t}$ where $a_n(t)$ denotes the drug amount in the nth compartment at time t, F denotes bioavailability, k_{tr} denotes a first-order transit rate constant from (nth-1) compartment to the nth compartment, and n denotes the number of transit compartment.

Stirling's approximation to $n!$ was used in the paper where $n! \approx \sqrt{(2\pi)} \cdot n^{n+0.5} \cdot e^{-n}$ [22]. However, it was mentioned that if the n value was smaller than 2, the approximation error may be substantial. It should be noted that Stirling's approximation always under-approximate $n!$, hence any further deviations from such under-approximations (when n is small) would be undesirable. The original equation of Stirling's approximation used by Savic *et al* (2007) is written as follow:

$$\frac{d(depot)}{dt} = e^{\left(log(F \cdot Dose) + log(k_{tr}) + n \cdot log(k_{tr} \cdot t) - k_{tr} \cdot t - S(n)\right)} - k_a \cdot depot \qquad \text{(Eq. 1)}$$

where $S(n) = log(2.5066) + (n + 0.5) \cdot log(n) - n$

To mitigate potential errors when n was small, we opted to use the log-gamma function to approximate $n!$. This was coded using ordinary differential equation (ODE) as follows:

$$\frac{d(depot)}{dt} = e^{\left(log(F \cdot Dose) + log(k_{tr}) + n \cdot log(k_{tr} \cdot t) - k_{tr} \cdot t - log\Gamma(n+1)\right)} - k_a \cdot depot \qquad \text{(Eq. 2)}$$

where $log\Gamma(n+1)$ represented the log-gamma function $log\Gamma(n) = log \int_0^\infty x^{n-1} e^{-x} dx$, $\Re(n) > 0$.

The transit compartment model was preferred as we deemed it more physiologically plausible compared to the “all-or-none” lag-time model [1].

Covariance between clearance and volume of distribution parameters was considered. Absolute bioavailability was fixed to 1 but we estimated the relative bioavailability of *fed* versus *fasted* condition.

Statistical models were used to describe variability. We modelled the interindividual variability (IIV) on an exponential scale to ensure the population PK parameter values were greater than zero, and each IIV term was introduced sequentially to the base model. Additionally, to ensure comparability between estimation methods, we expressed the parameters in a manner that was analogous to “mu-referencing” in NONMEM such that the equations took the following general form: $P_i = e^{\left(log(P_{pop} + \eta_i)\right)}$ where η_i is the IIV for the ith individual assumed to be normally distributed with a mean of zero and variance of ω^2, P_i is the individual PK parameter, and P_{pop} is the typical value of the parameter.

Three error models, additive, proportional, and combined (additive and proportional), were used to describe residual unexplained variability at the jth time for the ith individual, ε_{ij} which is assumed to be independent and identically normally distributed with a mean of zero and variance of σ^2.

Covariate model and structure

Covariates considered included *weight* and *food status*. *Weight* was introduced into the model via allometric scaling with a fixed exponent of $\frac{3}{4}$ for clearance terms and 1 for volume terms [23]. *Food status* was tested separately on to the first-order absorption rate constant (k_a) and relative bioavailability (F).

Model selection

Model selection was generally based on four criteria: (i) a reduction in the objective function value (OFV) for nested models with one degree of freedom by more than 3.84 unit (Chi-square [χ^2], p<0.05), (ii) an improvement in the goodness-of-fit plots and visual predicitive checks (VPCs) as recommended by the International Society of Pharmacometrics Model Evaluation Group [24] (iii) a reduction in IIV, (iv) parameter estimates that were precise and biologically plausible.

Model evaluation

The following diagnostic plots were used for model evaluation: dependent variable versus population predictions, dependent variables versus individual predictions, normalised prediction distribution versus population predictions, individual weighted residual versus time, and conditional weighted residual versus conditional population prediction. The VPCs were produced by simulating 1000 datasets under the final model and the 5^{th}, 50^{th}, and 95^{th} percentiles were plotted against data of the same percentiles from the original dataset.

Estimation algorithm

The SAEM algorithm [5] was used for model building. Once the final model was determined, the parameters were re-estimated again with FOCEi [6].

Area-under-the-curve (AUC)

The $AUC_{0-\infty}$ for the subjects in the analysis data was calculated with three different approaches: (i) using non-compartmental analysis (termed the “NCA method”), (ii) using the algebraic equation, $AUC_{0-\infty} = \frac{F_{EBE} \cdot Dose}{CL_{EBE}}$, which was based on the empirical Bayes estimate (EBE) of F and CL (termed the “algebraic method”), (iii) solving the system of ODE with an additional ODE added to the final model where $\frac{dAUC}{dt} = c_p$, c_p denotes plasma concentration (termed the “model-based method”).

To account for and to quantify potential estimation error in Stirling’s approximation, we derived a bias factor for the Stirling’s approximation and log-gamma function versus the exact values of $n!$ for

different n. Three bias factors were derived based on the following equation: (i) $\frac{n!_{log.gamma}}{n!_{exact}}$; (ii) $\frac{n!_{Stirling}}{n!_{exact}}$; and (iii) $\frac{n!_{Stirling}}{n!_{log.gamma}}$. We then simulated a typical subject's concentration-time profile with the R package RxODE (version 1.0.8) using parameter estimates from the final model but with the value of n perturbed to 0.25, 0.5, 1, 2, 3, 4, and 5. $AUC_{0-\infty}$ was then derived from the typical concentration-time profile by the model-based method (which approximated $n!$ using Stirling's approximation that had the tendency to over-estimate $AUC_{0-\infty}$) and the algebraic-method (which was invariant to approximation error). We then applied the bias factor to correct model-based $AUC_{0-\infty}$ and compared the resultant values to the algebraic-method.

Flip-flop pharmacokinetic

To investigate the presence of flip-flop PK in the dataset, we used final parameter estimates from both SAEM and FOCEi to predict a typical subject's concentration-time profile but perturbed k_a by a factor of 0.5, 1, or 2 to observe if the steepnes of the terminal elimination phase changed with different k_a values. RxODE (version 1.0.8) was used for simulations. Resultant concentration-time profiles under each k_a scenario were plotted on a semi-logarithmic scale to evaluate any changes to the terminal slope.

Bias and precision

A stochastic simulation and estimation study (SSE) was performed to evaluate bias and precision of parameter estimates given by the SAEM and FOCEi algorithms using RxODE (version 1.0.8). One hundred and fifty data sets were simulated, each containing 20 subjects with *fasted* and *fed* status respectively (total of 40 subjects in each set). Each simulated subject received one dose of 250mg metformin with 15 blood samples over a 24-hour period. The simulation code is available on the corresponding author's Github page.

Parameters were re-estimated with SAEM and FOCEi. Initial estimates of the models were set to true values used for simulation that were slightly perturbed. Percentage relative estimation error (%REE), mean prediction error (MPE) and root mean square error (RMSE) were used to evaluate

bias and precision of parameter estimates given by the algorithms. Denoting θ_{est} as the estimated value and θ_{true} as the true value of the parameter, $\%REE$ was given by $\%REE = \frac{\theta_{est}-\theta_{true}}{\theta_{true}} \cdot 100\%$; MPE was given by $MPE = N^{-1} \cdot \sum(\theta_{est} - \theta_{true})$; $RMSE$ was given by $RMSE = \sqrt{N^{-1} \sum_{i=1}^{N}(\theta_{est} - \theta_{true})^2}$; relative RMSE (rRMSE) of SAEM was calculated by dividing each RMSE value calculated by SAEM by RMSE calculated for the same parameter but with FOCEi ($rRMSE = \frac{RMSE_{SAEM}}{RMSE_{FOCEi}}$). When the value of rRMSE was less than 1, it indicated that SAEM was more precise than FOCEi and vice versa.

Robustness towards perturbation of initial estimates

The original set of initial estimates used in the final model (see Table 1S in Supplement I) were perturbed 100-times by up to 2-fold randomly (either by multiplying or dividing the initial estimates). These 100 sets of perturbed initial estimates were sequentially fed into the *ini* block of *nlmixr* to specify new starting points for subsequent estimations by SAEM and FOCEi. The *model* block remained unchanged. We then evaluated the robustness of each algorithm against perturbation in initial estimates by comparing the parameter estimates produced in each perturbation run with that of the final model. The distribution of each parameter estimates and $\%REE$ were plotted.

RESULTS

Clinical dataset

A total of 660 samples of plasma concentration from 44 subjects were available for analysis. The demographic characteristics are illustrated in Table 1. No aberrant plasma concentration was detected, but there were 37 (5.61%) post-dose samples with BQL concentration. These BQL observations were transformed according to M6 method and included in the analysis. The semi-logarithmic concentration-time profile of the raw data is shown in Figure 1S in Supplement I.

Population pharmacokinetic model of metformin

A one-compartment model with first-order absorption and elimination kinetics as well as with the delay in absorption described by a transit compartment model fitted the data best. The time course of drug concentration was described by the following equation:

$$\frac{dA(1)}{dt} = e^{\left(log(F \cdot Dose) + log(k_{tr}) + n \cdot log(k_{tr} \cdot t) - k_{tr} \cdot t - log\Gamma(n+1)\right)} - k_a * A(1)$$

$$\frac{dA(2)}{dt} = k_a \cdot A(1) - \frac{CL}{V} \cdot A(2)$$

$$C_p = \frac{A(2)}{V} \qquad \text{(Eq. 3)}$$

The model has seven parameters: k_{tr}, n, k_a, CL, V, θ_{fed,k_a}, and $\theta_{fed,F}$. The inclusion of the interindividual variability (IIV) component on k_a, CL, and V on the final model produced significant improvements in model fit. The combined error model was selected as it best described the data ($\Delta OFV = -130$). Final parameter estimates are given in Table 2 and the full model parameterised by ODE is shown in Table 1S in Supplement I. *Food status* was found to be a significant covariate on k_a (θ_{fed,k_a}) abd bioavailability, F ($\theta_{fed,F}$) respectively. The addition of *food status* as a covariate on k_a reduced BSV from 8.6% to 1.9% and improved overall global fit ($\Delta(OFV) = -18.4$). *Food status* as a covariate on F also improved model fit as seen in the VPC (not shown).

Diagnostic plots of the final model are shown in Figure 1. They suggested no apparent mispecification of model components. Two VPCs for the final model are presented in Figure 2 for SAEM and FOCEi respectively. The 5^{th}, 50^{th} and 95^{th} percentiles of the population PK model predicted plasma metformin concentration (represented by the blue colour bands) matched the percentiles of observed concentration values well. The final PK models provided an adequate description of the observed data. Individual plots are available in Figure 2S of Supplement I.

Area-under-the-concentration-curve (AUC)

The values of $AUC_{0-\infty}$ calculated from three approaches were in agreement with each other as illustrated in Figure 3. The NCA method was considered as the gold standard with which the other two methods were compared. Plots in the top row of Figure 3 (model-based method) suggested that $AUC_{0-\infty}$ derived from the final model ODE (which utilised log-gamma function instead of Stirling's approximation of $n!$) corresponded closely to the algebraic method, which relied on the values of CL_{EBE}.

However, when the $n!$ was approximated using Stirling's approximation, $AUC_{0-\infty}$ derived from the model-based method became consistently larger than values derived from the algebraic method regardless of the values of n (see Table 3). When the value of n decreased further (e.g. 0.5 or 0.25), the values of AUC_{model} diverged greater from $AUC_{algebraic}$ (see Figure 3S in Supplement I). Such a divergence was not observed when the log-gamma function was used to approximate $n!$. When the bias factor ($\frac{n!_{stirling}}{n!_{log.gamma}}$) was applied to $AUC_{model-based}$, the resultant values were corrected to match $AUC_{algebraic}$ (see Table 3).

Flip-flop pharmacokinetic

Two typical concentration-time profiles with perturbation of k_a by a factor of 0.5, 1, and 2, estimated with SAEM and FOCEi respectively are shown in Figure 4. The figures clearly showed doubling the k_a value led to a faster rate of elimination while halving the k_a led to a slower rate of elimination. When all else remained unchanged, fed subjects who tend to have lower k_a also appeared to have slightly more significant flip-flop in PK as compared to the fasted subjects, and the difference between *fasted* and *fed* was more prominent when final estimates given by FOCEi was used.

Bias and precision

Simulations were performed using the final parameter estimates from the SAEM algorithm (see Table 2) as they were more closely aligned with the figures reported in the literature.

The distribution of parameter estimates and the respective %REE were illustrated in Figure 5. Estimates for k_a, CL, V, θ_{fed,k_a} and $\theta_{fed,F}$ were equally precise and unbiased for both FOCEi and SAEM. Parameter estimates for k_{tr} and n were more imprecise for both algorithms, judging from the greater spread of the parameter point estimates. Additionally, estimates of k_{tr} and n given by FOCEi were more biased compared to other parameters and against SAEM.

Values of MPE and RMSE for fixed-effect parameters are tabulated in Table 4. The results further suggested that no apparent difference existed between two algorithms in estimating population PK of a flip-flop system.

Robustness against perturbation in initial estimates

One hundred runs of parameter re-estimation with perturbed initial estimates were performed with SAEM and FOCEi respectively. The distribution of parameter point estimates and %REE were illustrated in Figure 6.

Both algorithms demonstrated different degrees of robustness towards changes in initial estimates, with FOCEi being less robust towards such changes. In particular, parameter estimates of k_a and *food* effect on k_a (i.e. θ_{fed,k_a}) were significantly biased and imprecise when FOCEi was compared to SAEM. k_a, V, and θ_{fed,k_a} estimated by FOCEi appeared to be bimodal, while no such bimodality was observed with SAEM.

DISCUSSION

In this study, we have assessed the R package *nlmixr* for fitting compartmental PK models. The performance of its two main estimation algorithms in terms of bias, precision, and robustness against perturbations in initial estimates were challenged with metformin PK data that demonstrated flip-flop pharmacokinetics. In general, *nlmixr* was a reliable open-source tool for NLMEM, where a complete model-building, qualification and evaluation procedure could be completed entirely within the R environment. This package provided the much-needed tools to

researchers, particularly those working under a resource-limited setting, to analyse population PK/PKPD data as well as to promote better reproducibility and transparency in pharmacometrics.

Our study showed that a one-compartment model with first-order absorption and elimination kinetics as well as with the delay in absorption described by a transit compartment model best described the population PK of metfomin in healthy volunteers when the compound was administered with or without food. The transit compartment model was particularly important to model the absorption phase as first-order absorption model had the tendency to underestimate maximum concentration (C_{max}). Parameter estimates from SAEM were closer to values reported in the literature, and more plausible biologically. Despite the differences in parameter estimates, both algorithms appeared to give similar fit to the data as shown in the VPCs (Figure 2). This could be attributed to flip-flop where two sets of parameter estimates could give rise to the same concentration-time profile for a one-compartment model.

Several earlier studies used either the first-order absorption or lag-time model to describe metformin absorption [25–27]. Nonetheless, Cvijic *et al* (2014) summarised that metformin absorption appears to be quite complex, of which is characterised by saturable paracellular and transcellular pathway [20]. In fact, recent population PK studies on metformin showed that metformin absorption is better described by complex models such as first-order absorption followed by zero-order absorption with lag time [28] or two separate absorption pathways to account for different absorption sites in the gastrointestinal tract [29].

When using Stirling's approximation to $n!$, caution should be exercised when n is small. In our study, population estimate for n was 0.791 (relative standard error=0.0138). Stirling's approximation to $n!$ would underestimate its value. Since in transit compartment, dose was normalised by $n!$, an under-approximated $n!$ would lead to an over-approximated $\frac{Dose}{n!}$ in the equation $a_n(t) = F \cdot Dose \cdot \frac{(k_{tr} \cdot t)^n}{n!} \cdot e^{-k_{tr} \cdot t}$, thereby resulting in an over-approximated $a_n(t)$, and subsequently distort other parameter estimates. This was exemplified in the calculation of $AUC_{0-\infty}$ by model-based method which relied on Stirling's approximation rather than log-gamma function

where the values were consistently over-estimated. This over-estimation persisted even as bioavailability and clearance remained unchanged, and the magnitude of such over-estimation was invariant to the magnitude of k_a, thus confirming that the presence of flip-flop pharmacokinetic did not influence this over-estimation (see Supplement II). Additionally, when the bias factor was included in the $AUC_{0-\infty}$ calculation, the values derived from the corrected model-based method matched that of the algebraic method, which confirmed that the log-gamma function should be the preferred method to approximate $n!$ in the transit compartment equation.

Our metformin dataset also demonstrated the hallmark feature of flip-flop pharmacokinetics: the rate of absorption being slower than the rate of elimination. If the absorption process is not rate-limiting, the terminal elimination phase will be invariant to changes in k_a, i.e. the terminal slopes would run in parallel to one another. SAEM and FOCEi produced parameter estimates that were different although the model inputs were identical and model outputs were largely similar. This could be explained by local structural non-identifiability in the parameter estimates, i.e. flip-flop, where there were more than one set of parameter values that could give the same input-output relationship. In essence, flip-flop pharmacokinetics is a permutation of the rank order of parameter values where the two sets of parameter estimates (produced by SAEM and FOCEi respectively) would give the same input-output relationship in a one-compartment model (see Table 5). Nonetheless, clearance (CL) should remain invariant to flip-flip pharmacokinetics as shown in the parameter estimates of both algorithms ($CL_{FOCEi} = 53.6L \cdot h^{-1}$; $CL_{SAEM} = 54.8L \cdot h^{-1}$). This was demonstrated in the SSE study too where CL was invariant to flip-flop but k_a and V were not. The CL_{EBE} based on SAEM and FOCEi were also largely similar (not shown). As a result, the $AUC_{algebraic}$ calculated based on CL_{EBE} were also largely similar and matched that determined based on the NCA method and the model-based method (Figure 3).

Low bias and high precision in parameter estimates are two most important properties for an estimation algorithm [30]. Based on our SSE results, the SAEM algorithm was marginally superior to FOCEi in estimating fixed-effects parameters (mean rRMSE=0.932). The distribution of all fixed-

effect parameter estimates appeared to be normal and centred around the true value used for simulation (Figure 5).

Robustness against changes in initial estimates is another highly desirable trait of an algorithm. Johansson *et al* (2014) states that robustness depends strongly on the shape of the likelihood surface [30]. A likelihood surface with many local minima will results in a stronger dependence of a good starting point (i.e. initial estimates) compared to simple likelihood surface. As these initial estimates were perturbed, the SAEM algorithm was superior against FOCEi, as shown in Figure 6, where the algorithm was able to produce parameter estimates that were close to the final model.

Overall, user experience with *nlmixr* was good as we received great support from the developers who were responsive and helpful, and the algorithms functioned as intended to arrive at models that were stable in our study. Although the documentation for certain aspects of NLMEM with *nlmixr* were limited at the times of this work, we acknowledged the efforts from developers to update these documentations frequently and consistently throughout our study.

Limitation

The final model had slightly under-estimated plasma concentration at times between 2.5-hour to 5-hour. This was consistent with the goodness-of-fit plots. Regardless, we decided to retain the transit compartment model to describe the delay in absorption as it provided a better fit compared to models without the transit compartment such as the conventional first-order absorption model or the lag-time model (data not shown). Additionally, at the population level, we noticed that FOCEi arrived at much imprecise parameter estimates compared to SAEM despite using the same model and identical inputs (Table 2). For example, the $\%RSE$ for the estimates of k_{tr}, n, and θ_{fed,k_a} was 650%, 82.6% and 103% for FOCEi but were all under 15% for SAEM.

CONCLUSION

In this study, a population PK model for metformin was developed, evaluated, and used for simulations with *nlmixr* and related R packages, all of which are open-source tools developed for

NLME modeling entirely within the R environment. Both the SAEM and FOCEi algorithms performed well for population PK modelling but SAEM offer some advantage when flip-flop pharmacokinetics are present, as well as being more robust with respect to initial estimates changes. Overall, *nlmixr* appears well suited for NLME modelling.

Table legends

Table 1: Patient characteristics.

Table 2: Parameter estimates for the final PK model, estimated by SAEM and FOCEi. Abbreviations: k_{tr}, transfer rate constant; n, number of transit compartment; k_a, absorption rate constant; CL, clearance; V, volume of distribution; $\theta_{fed,F}$, food effect on bioavailability; θ_{fed,k_a}, food effect on absorption rate constant; $\%RSE$, percentage of relative standard error; CV, coefficient of variation; σ, standard deviation of IIV; RUV, random unexplained variability; add, additive; prop, proportional

Table 3: $AUC_{0-\infty}$ derived from the model-based and algebraic method, and corrected $AUC_{model-based}$ by the bias factors.

Table 4: Mean prediction error (MPE) and root mean square error (RMSE) of fixed and random parameters obtained with FOCEi and SAEM algorithms.

Table 5: Possible permutations for a one-compartment model using CL, V,and k_a.

Figure legends

Figure 1: Goodness-of-fit plots for final metformin PK model with FOCEi (top) and SAEM (bottom) algorithm. Black dots are observed values. Blue lines are loess smooths through the data. Grey lines are lines of identity. DV, observations; CPRED, conditional population prediction; IPRED, individual prediction; NPD, normalised prediction distribution; WRES, weighted residuals; IWRES, individual weighted residuals.

Figure 2: Visual predictive check of the final metformin PK model with FOCEi (top) and SAEM (bottom) algorithm. Black dots are observed values. Solid black lines are the observed median, while the dash black lines are the observed 5th and 95th percentiles. Blue shaded areas represent 90% confidence intervals around simulated 5th, 50th, and 95th percentiles. 12 bins are used. *N*=1000.

Figure 3: Estimates of area-under-the-curve derived from model-based approach (top) or algebraic approach (bottom) against non-compartmental analysis. Black dots are estimated values. Blue lines are linear regression line. Black dashed lines are lines of unity.

Figure 4: Simulated plasma concentration of metformin of a typical subject based on parameter estimates of the final model.

Figure 5: Distribution of parameter estimates obtained from SAEM and FOCEi algorithm (top) and the respective relative estimation error (bottom) from the SSE study.

Figure 6: Distribution of parameter estimates obtained from SAEM and FOCEi algorithm (top) and the respective relative estimation error (bottom) when the initial estimates were perturbed.

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