Université de Liège

Population pharmacokinetic modelling of an investigational prodrug.

Erasmus 2010 – 2011, Pharmacometrics research group, Uppsala University.

Crunenberg Robin, second master
Pharmaceutical sciences.

UPPSALA UNIVERSITET

Acknowledgements

I would like to thank the following persons who have all contributed to this work.
My supervisor MSc. Sebastian Ueckert for not only his time and quality of management, but also for his encouragement and guidance these past four months.
My project provider MSc. Martin Bergstrand for his availability and advice from the beginning to the final level of this research project.
My university supervisor Professor Vincent Seutin for supporting me in this internship project.
Professor Mats Karlsson for his cordial welcome into his department.
And finally all the members of the pharmacometrics research group for their help, advice and sympathy.

Table of contents

1. List of abbreviations 4
2. Abstract 5
3. Introduction 6
3.1. Overview of the field 6
3.2. Introduction to the prodrug 7
4. Materials and methods 8
4.1. Mathematical and statistical methods 8
4.1.1. Theory of nonlinear mixed effects models 8
4.1.2. General NMLE model formulation 8
4.2. Model selection and validation 9
4.2.1. Goodness of fit criteria 9
4.2.2. Likelihood ratio test 10
4.2.3. Visual predictive check 10
4.3. Study data 11
4.4. Software and computing tools. 13
5. Results 14
5.1. First model: active compound 14
5.2. Second model: Pro-drug 18
5.2.1. Prodrug data results 21
5.2.2. Metabolite data results 24
5.2.3. Active compound data results, prodrug treatment 27
5.2.4. Active compound data results, active compound treatment 30
6. Discussion 33
6.1. First model: active compound 33
6.2. Second model: Prodrug 34
6.2.1. Prodrug data results 34
6.2.2. Metabolite data results 34
6.2.3. Active compound data results, prodrug treatment 34
6.2.4. Active compound data results, active compound treatment 35
7. Conclusion 36
8. References 37
9. Appendix 38

1. List of abbreviations

Add	Additive
BQL	Below quantification limit
BGOF	Basic goodness of fit
CL	Clearance
CV	Coefficient of variation
CWRES	Conditional weighted residuals
DV	Dependent variable
EH	Hepatic extraction
FM	Metabolised fraction
FOCE	First order conditional estimate
IPRED	Individual prediction
IRES	Individual residuals
IWRES	Individual weighted residuals
LLOQ	Lower limit of quantification
LRT	Likelihood ratio test
NLMEM	Non-linear Mixed Effects Modelling
NONMEM	Software of Non-linear Mixed Effects Modelling
OFV	Objective function value
PD	Pharmacodynamic
PK	Pharmacokinetic
PRED	Population prediction
Prop	Proportional
PsN	Pearl speaks NONMEM
Q	Inter-compartmental clearance
Ruv	Residual error
SD	Standard deviation
SE	Standard error
V	Volume of distribution
VPC	Visual predictive check

2. Abstract

The aim of this study was to develop a population pharmacokinetic model for an investigational prodrug with the intention of linking this model to previous works on a modified release form of this prodrug. The data used to create this model were provided from a phase I study. Concentration-time measurements were available for three compounds; the prodrug, the metabolite and the active compound. The model developed is able to describe the pharmacokinetics of the prodrug and its metabolite. Perspectives for future investigations are presented.

3. Introduction

3.1. Overview of the field

Pharmacometrics is the science of developing and applying mathematical and statistical methods to characterize, understand and predict the pharmacokinetic, pharmacodynamic, and biomarker-outcome behaviour of drugs[1]. The goal of this emerging science is to influence drug development, regulatory and therapeutic decisions [2].

The *Journal of Clinical Pharmacology* gives a structural representation of this emerging science in constant evolution. The structure of this evolution focuses on three general inter-connected themes: integration, innovation and impact [2]. "The quantitative integration of multisource data and knowledge (a)", as this field is not solely focused on the pharmacological, statistical, mathematical, engineering or biological concepts, but instead takes them all together. This management will lead to the "continuous methodological and technological innovation enhancing scientific understanding and knowledge (b)", which in turn has an "impact on discovery, research, development, approval and utilization of new medicine (c)".

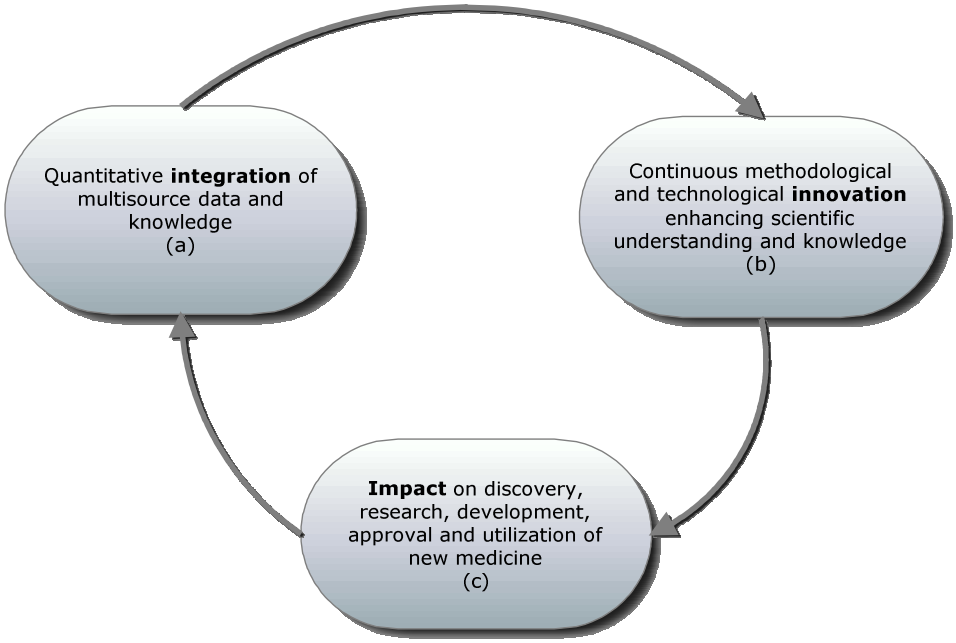

Fig. 1: Evolution structure of the pharmacometrics.

Pharmacometric models describe the relationship between dose, concentrations and time, *i.e.* the pharmacokinetics (PK) of the drug, and/or the change in the effects due to drug treatment over time as a function of drug exposure (dose, concentration or other summary measure), *i.e.* the pharmacodynamics (PD) of the drug.

This work concerns the modelling of pharmacokinetic data. Pharmacokinetics describes the dynamics of drug absorption, distribution, metabolism and elimination. PK models are defined with pharmacologically meaningful coefficients, *i.e.* clearance, volume of distribution and rate constant. A well characterized PK model can be used to predict, for example, the concentration variations when altering the doses or the time of dosing. PK model in

association with PD guides recommendations for optimal dosage. A clear dose-concentration-effect relationship should prevent marketing a drug at a dose later recognized to be unnecessarily high [3].

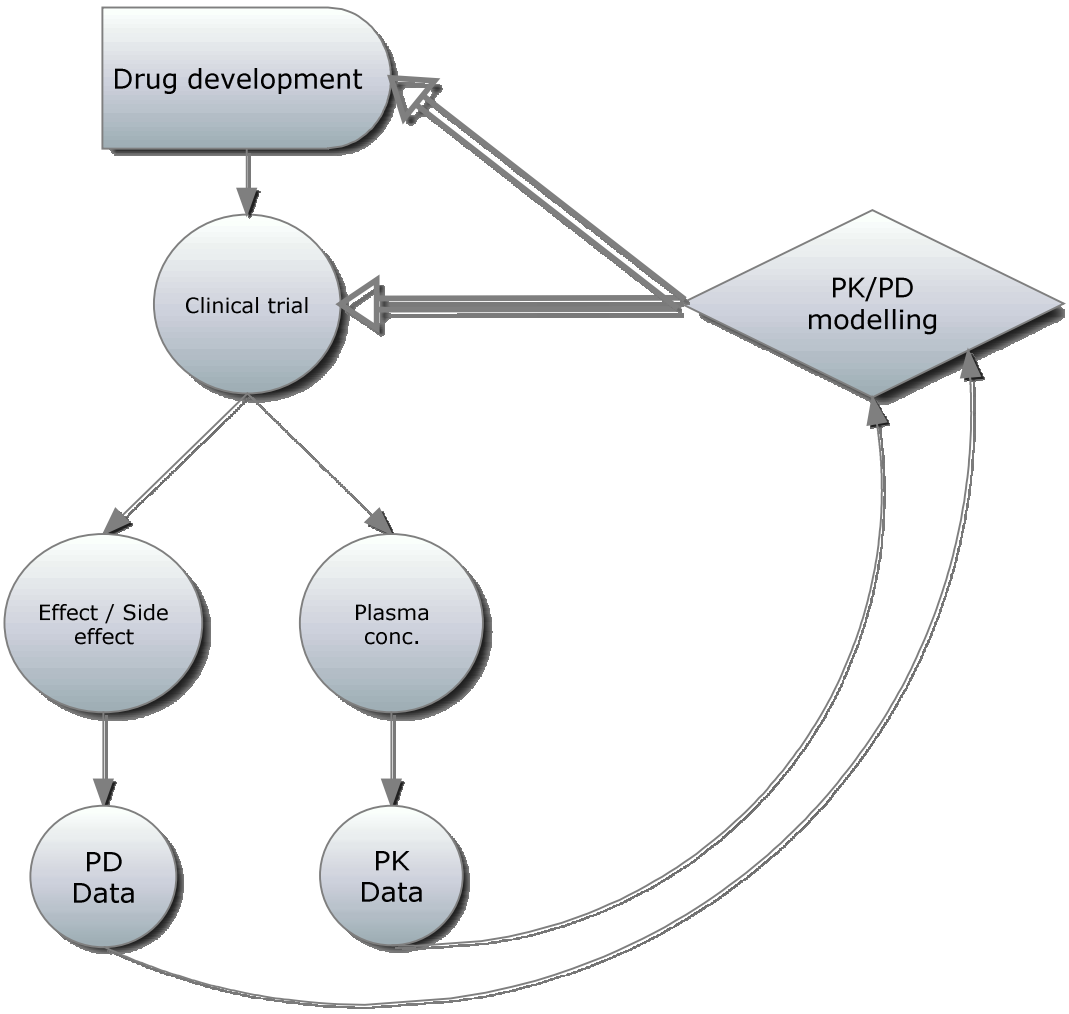

Fig. 2: Overview of the pharmacometric field and its impacts.

The aim of this work is to develop a PK model describing the pharmacokinetics of an investigational prodrug. In order to link this modelling with previous studies, the model will contain a hepatic compartment describing the inter-conversion process between the different compounds.

3.2. Introduction to the prodrug

The investigational prodrug is under development for the treatment/prevention of thrombosis. This project is part of a larger modelling initiative towards describing the population pharmacokinetics of an investigational drug following the administration of modified release formulations [4].

4. Materials and methods

This section is divided in four parts. Firstly a short introduction to the main statistic and mathematics of the population modelling is presented. Secondly the different tools used to evaluate and select the model are discussed. The third part constitutes a presentation of the data available to produce the model. Finally, the different software packages used to develop the model are listed.

4.1. Mathematical and statistical methods

Pharmacometric research focuses on population data. Population modelling involves analyzing data from all individuals simultaneously instead of data from each individual separately. To account for the different levels of variability in the population, nonlinear mixed effects models are used.

4.1.1. Theory of nonlinear mixed effects models

The nonlinear mixed effects (NMLE) modelling approach involves the simultaneous estimation of the typical and variance parameters using data from all patients, *i.e.* the population parameters. Many statistical processes can be described by models that incorporate fixed and random effects.

The term mixed refers to the combination of these fixed and random effects for the description of the data. The fixed effects are those not occurring at random or associated with an entire population e.g. the dose. They are described in the model as fixed effects parameters and they give a model prediction for the typical individual.

The random effects are those occurring at random in the population or associated with individual experimental units. They are described with a distribution function in the model and these random effects parameters are usually an estimate of the variability [5, 6].

Mixed effects models allow the analysis of different levels of variability. For pharmacometric models, the two most important levels are inter- and intra-individual variability. Inter-individual or between-subject variability is a result of considering multiple individuals with different physiological parameters. Intra-individual variability is associated with the measurement error and the limited ability of the model to describe the response; because of that it is sometimes called residual variability [7].

4.1.2. General NMLE model formulation

The NLME models are used in different areas and can be formulated by many mathematically identical ways. The following pharmaceutical terminologies reflect one of its applications in a pharmacological sense.

The observed response (e.g. concentration) in an individual i within the framework of population in NLME can be described as [1]

$$y_i = f(\phi_i, x_i) + \varepsilon_i$$

where y is the observed data (e.g. concentration). This value depends on the system output through a function f on the individual parameter ϕ_i and on all the experimental/design variables x_i (e.g. time) but also on the random vector εi or within-subject error terms, normally distributed with mean 0 and covariance matrix Σi.
At a second stage the variability of the individual parameters ϕ_i is modelled through a function g by

$$\phi_i = g(\theta, z_i, \eta_i)$$

where θ is a fixed effect population parameter vector, z_i is a vector of possibly time varying covariates, and η_i is a vector of subject specific random effect parameters. The η_i are restricted to be normally distributed with mean 0 and covariance matrix Ω, i.e. $\eta_i \sim \mathcal{N}(0,\Omega)$. Examples of covariates (z_i) are body mass, age and sex [7, 8].

Given a model function of the form described above and a vector of observed values y, the mathematical problem is to estimate the fixed effect parameters θ and the covariance matrix of the random effects Ω and error terms Σ. Different estimation methods have been proposed to solve this. Two of these methods have been investigated in the present work, the first one is the first order conditional estimate (FOCE) and the second one is the Laplace method.

The FOCE method [9] makes the linearization around the current conditional estimate of the random effect [10].
A higher order approximation method is the LAPLACE estimation method [9]. It uses second-order Taylor series linearization around the current conditional estimate of the random effect.
In this work the LAPLACE method was used to handle data below the limit of quantification. More specifically the M3 method as described by Ahn [11].
All the step wise model building was done firstly with the FOCE and secondly with the M3 method to decrease the risk of model misspecification.
Both methods belong to the class of maximum likelihood estimators, which allow drawing inference on the parameters of a distribution given a set of observed data. The general approach of a maximum likelihood estimator is to find an estimate for a parameter such that the likelihood of actually observing the data is maximal [7, 12]. Applied to the specific problem of a NLME model, the maximum likelihood approach is to maximize the likelihood function L over the set of possible values for θ, Ω and Σ.

4.2. Model selection and validation

The models selection is performed by comparison between new model and previous one. Various tools are used to evaluate if the change made led to an improvement. With the evaluation, the validation is investigated as presented below. In this work the following diagnostics were used:

4.2.1. Goodness of fit criteria

Graphical evaluations [10], where used to explore the model fits to the data. In this work, four kinds of plots were used (Fig. 8).

DV *versus* PRED – Dependent variable *versus* population predictions:
The plot is generated by plotting the population predictions of the model (i.e. without considering random effects) versus the observed data. The satisfactory model is expected to produce data points that scatter evenly around the line of identity. Since, random effects are ignored in this diagnostic, deviations from the expected appearance are usually due to misspecifications in the structural model.

DV *versus* IPRED – Dependent variable *versus* individual predictions:
The plot is generated by plotting the individual predictions (i.e with random effects) versus the observed data. It used to diagnose misspecifications in the random structure of the model.

|IWRES| *versus* IPRED – Absolute value of the individual weighted residual *versus* the individual predictions:
This plot is used for assessing the stochastic model, in particular the residual error model.
Ideally there should be no trend in magnitude of |IWRES|.

CWRES *versus* TIME – conditional weighted residual [13] *versus* the independent variable:
This is a plot is used to diagnose the structural model where ideally the residuals should be scattered evenly around the zero line.

4.2.2. Likelihood ratio test

The likelihood ratio test (LRT) [14] can be used to test which one of two competing models fits the data best. Usually this involves one full model and one reduced model.
The LRT is an approximate test of adding or deleting parts of a model and utilises the minimum objective function value (OFV). The OFV is a goodness-of-fit statistic, calculated by NONMEM (see "4.4. Software and computation tools"). This value is proportional to the likelihood of the data, when the value decreases the fit to the data is improved. The critical difference between the OFVs for a reduced and full model (ΔOFV) values for certain significance levels (α) and degrees of freedom (df = number of differing parameters between models) are shown below:

Table 1: If we take a full model and delete a parameter (df=1), then for a significance level of 0.05, the increment of OFV should be less than 3.84 to keep the reduced model.

	$\alpha = 0.05$	$\alpha = 0.01$	$\alpha = 0.001$
df = 1	3.84	6.63	10.83
df = 2	5.99	9.21	13.82

4.2.3. Visual predictive check

The visual predictive check (VPC) [15, 16] can be used to evaluate how the model can describe the data used for model development (Fig. 10). The main principle of this method is the simulation of a high number of data sets from the model, calculation of summary statistics

over all replicates and the comparison of those statistics with the corresponding statistics of the original data. In this work, the 95% confidence interval of the simulated median response is compared to the median of the original data. For a model that adequately describes the data, the observed median is expected to be entirely contained in the confidence interval of the simulation.[10]

4.3. Study data

Data from a phase I clinical study was available. This included nine different treatment approaches (pro-drug or drug, different routes of administration, doses and formulations), four analytes (pro-drug, intermediate, drug and non-release pro-drug in tablet) on a single occasion and covariates (weight, height, sex, age and fed status).
The initial modelling will use only a part of the data available, i.e. patients treated with one 10 mg i.v. dose of the investigational active compound and twenty concentration-time measurements per patient. In total, 200 active compound concentration-time measurements, with 12% data below the limit of quantification (BQL) were available from ten patients.

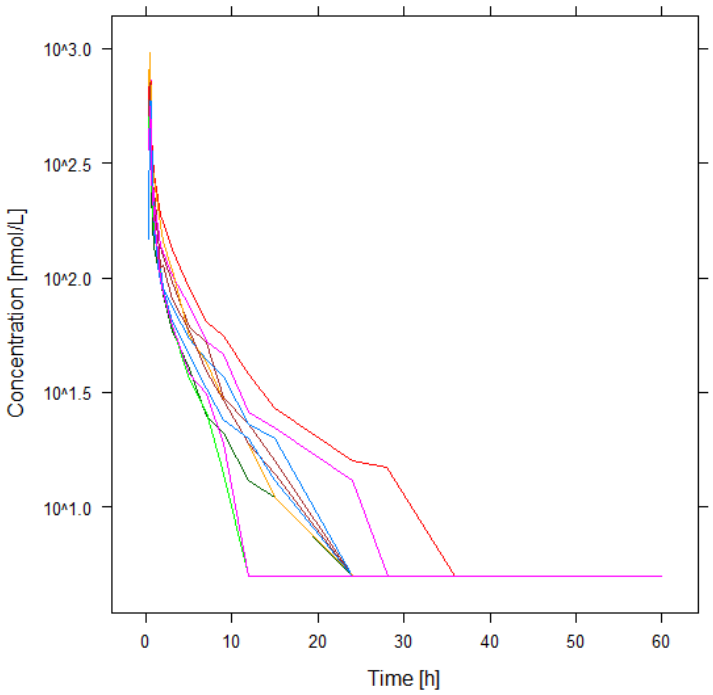

Fig. 3: Blood concentration versus time profiles for the investigational active compound, after 10 mg i.v. dose of the investigational active compound. A different color is used for each individual.

To build a prodrug model the data from the active compound treatment and the same patients treated with 30 mg i.v. dose of the prodrug (forty-eight concentration-time measurements per patient) were used. For the prodrug treatment, 480 concentration-time measurements were available: 151 prodrug measurements, with 11.25% BQL data, 140 metabolite compound measurements, with 20.71% of BQL data and 189 active compound measurements, with 11.64% BQL data.

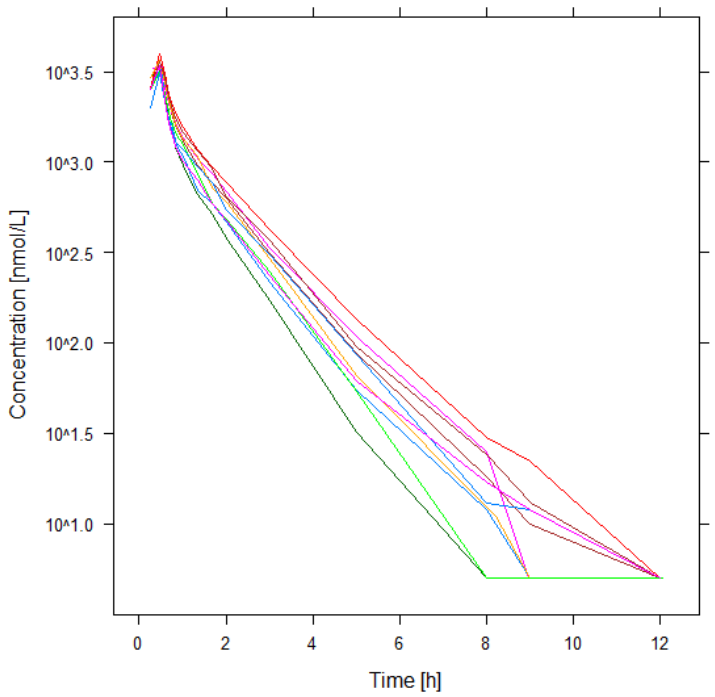

Fig. 4: Blood concentration versus time profiles for the investigational prodrug, after 30 mg i.v. dose of the investigational prodrug. A different color is used for each idividual.

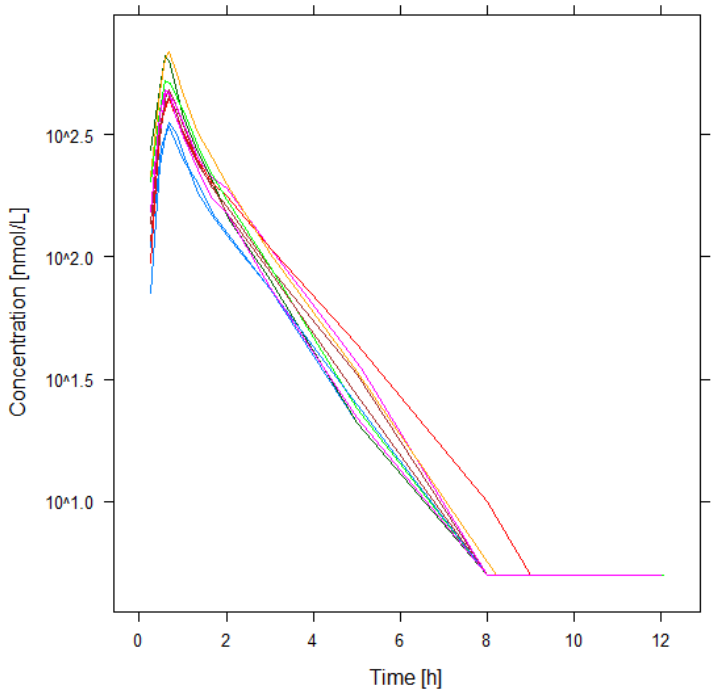

Fig. 5: Blood concentration versus time profiles for the investigational intermediate compound, after 30 mg i.v. dose of the investigational prodrug. A different color is used for each idividual.

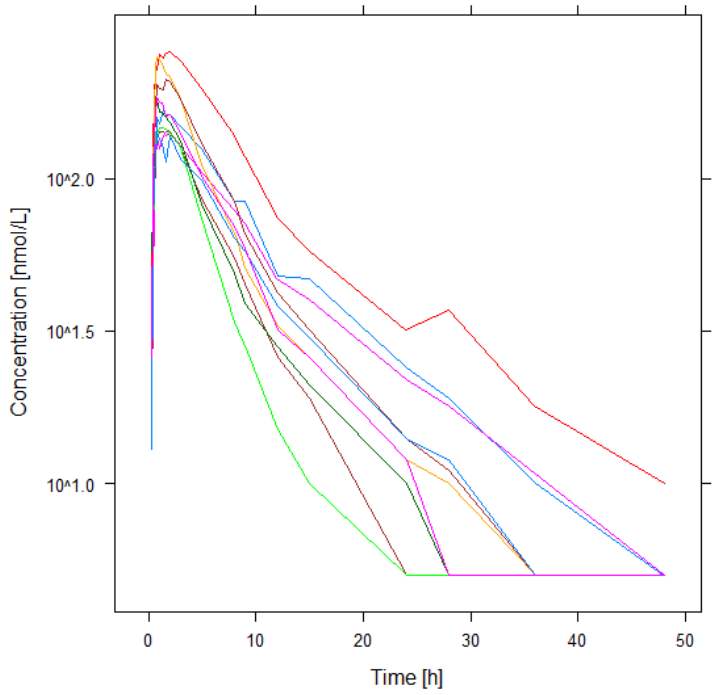

Fig. 6: Blood concentration versus time profiles for the investigational active compound, after 30 mg i.v. dose of the investigational prodrug. A different color is used for each idividual.

4.4. Software and computing tools.

Data were analysed using the nonlinear mixed effects modelling (NLMEM) software [5], NONMEM (version VII). NLMEMs approach has become increasingly common in population PK/PD analysis.

During this work a toolbox for population PK/PD model building, Perl-speaks-NONMEM (PsN) [17, 18] was used with NONMEM. It has a broad functionality ranging from parameter estimate extraction from output files, data file sub setting and resampling, to advanced computer-intensive statistical methods and NONMEM job handling in large distributed computing systems [19]. The PsN functions used were mainly execute, sumo, update intits, runrecord and vpc.

Numerical and graphical diagnostics will be generated using Xpose4 [20]. Xpose 4 is an open-source population PK/PD model building aid for NONMEM. Xpose tries to make it easier for a modeler to use diagnostics in an intelligent way, providing a toolkit for dataset checkout, exploration and visualization, model diagnostics, candidate covariate identification and model comparison.

5. Results

The different models developed are firstly presented as schematic drawing of the different compartments and parameters estimated. Secondly for each model the results of the evaluations tools presented earlier in the materiel and methods section (goodness of fit plots and predictive check) will be presented as final result.

5.1. First model: active compound

The disposition of drug was described by a three compartment model (Fig. 7) with a central and two peripheral compartments. To match the constraint of this project a hepatic compartment where the clearance process occurs is added. The parameters estimated in this model are a central (CVC) and two peripheral (CVP) volumes, two inter-compartmental clearances (CQ), the hepatic extraction ratio (CEH) and a proportional residual error (Prop. ruv).

An inter-individual variability is estimated for all the parameters except CQ1, which is fixed to zero. The intra-individual or residual variability is fixed to 1. The event dose and observation are done in the central compartment and the extraction ratio underwent a logit transformation. The logit transformation is used to allow any value from negative infinity to positive infinity as input, whereas the output is confined to values between 0 and 1. This containment gives a meaning to the ratio (and the fraction, see above).

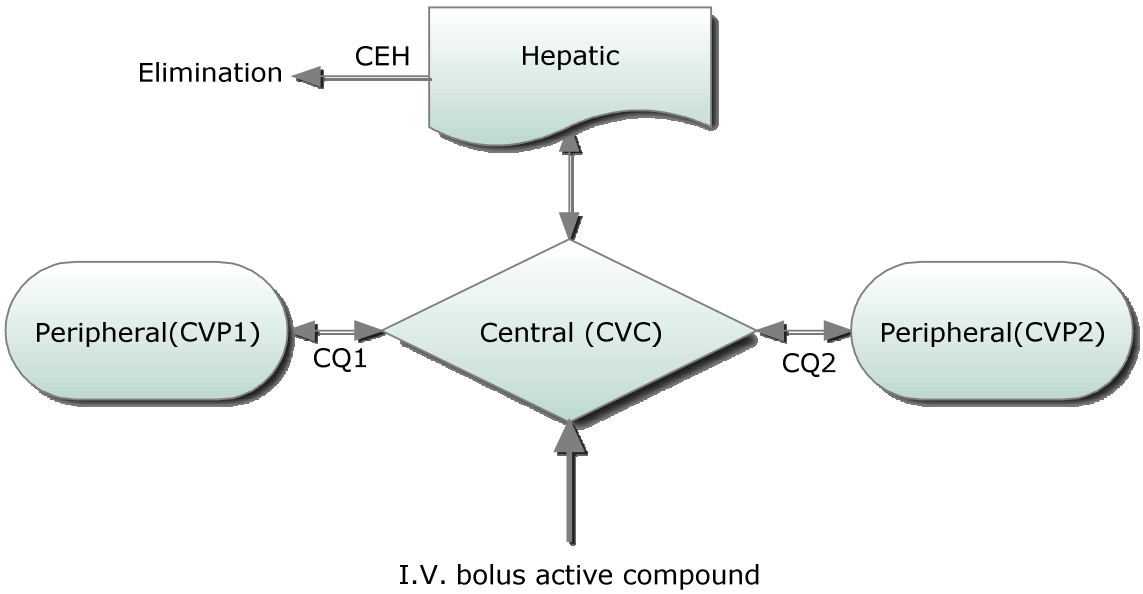

Fig. 7: Schematic illustration of the PK model of the active compound. The observation and the treatment events are done in the central compartment. The constraint of hepatic compartment and the relation between central and hepatic is described by physiological value to minimize the impact of this compartment on the model.

Table 2: Estimated parameter values for the model of the active compound model.

Parameter	Mean	Standard error (%)	Coefficient of variation (%)	Standard error (%)	Eta shrinkage (%)
CVC	11.22 L	1.44	0.46	168.25	8.54
CVP1	66.30 L	0.15	0.007	219.06	27.51
CVP2	40.29 L	0.61	0.012	228.68	3.42
CEH	0.188	50.07	2.96	120.18	3.95
CQ1	8.56 L/h	3.66			
CQ2	36.62 L/h	0.18	0.012	319.04	12.5
Prop. ruv	0.125	177.95			

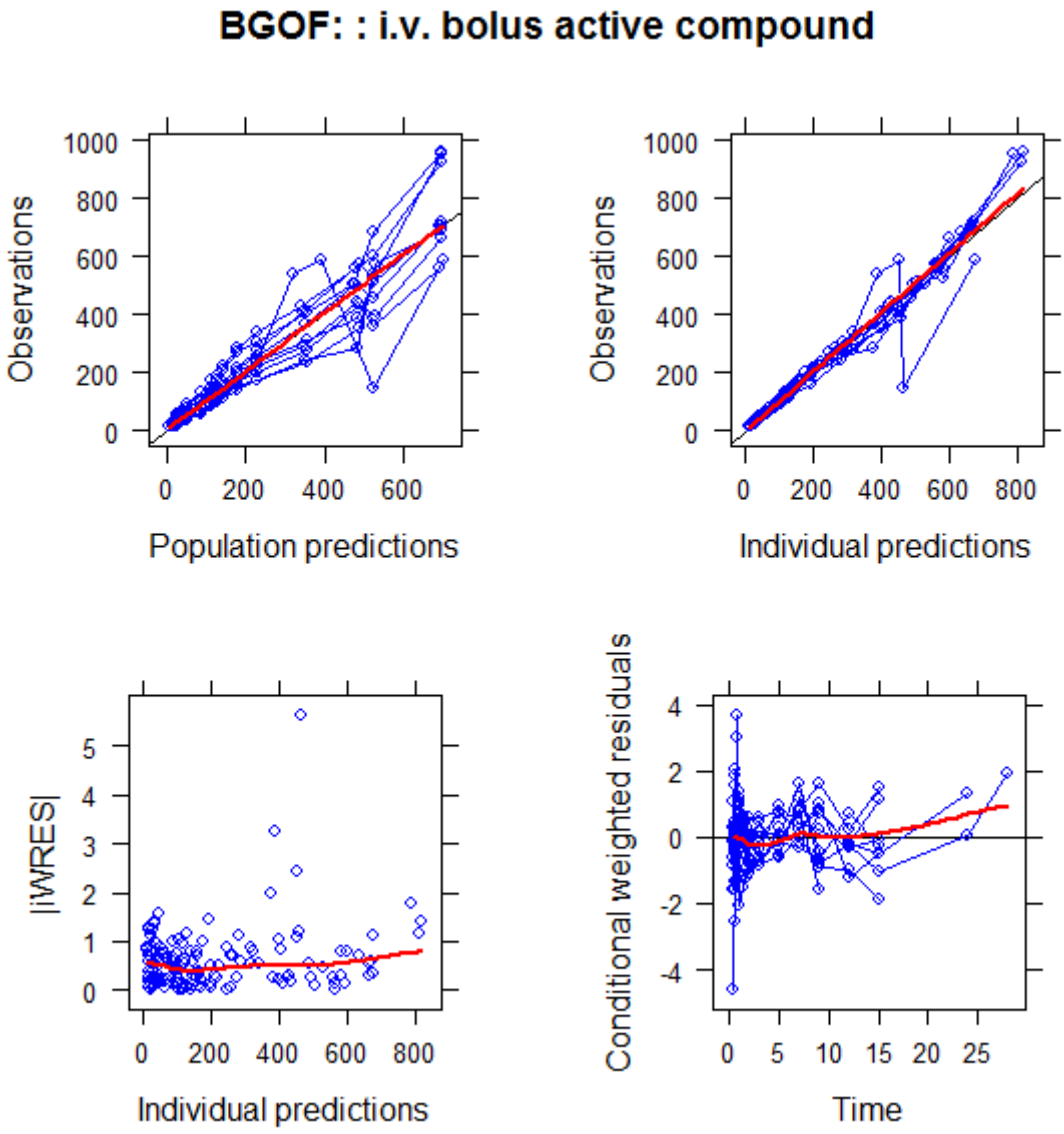

Fig. 8: Basic goodness of fit-plots: the red line is a non-parametric smoothing spline of the data points; the blue dots are the data points; the solid black line in each plot is the line of identity. Data points from the same individual are linked by lines.

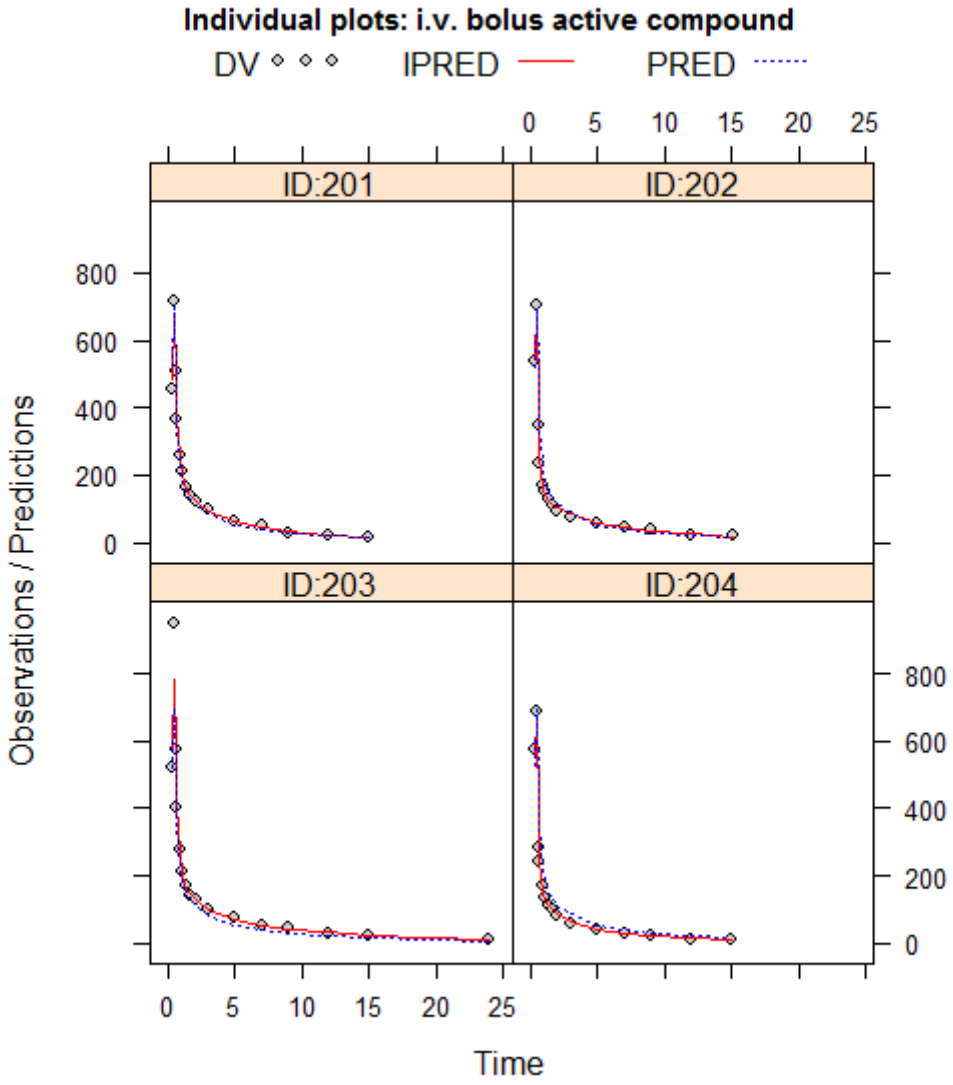

Fig. 9: Individual plots for the first four individuals. Each dot represents a data point; the red line is the individual prediction given by the model and the blue line is the population prediction of the model.

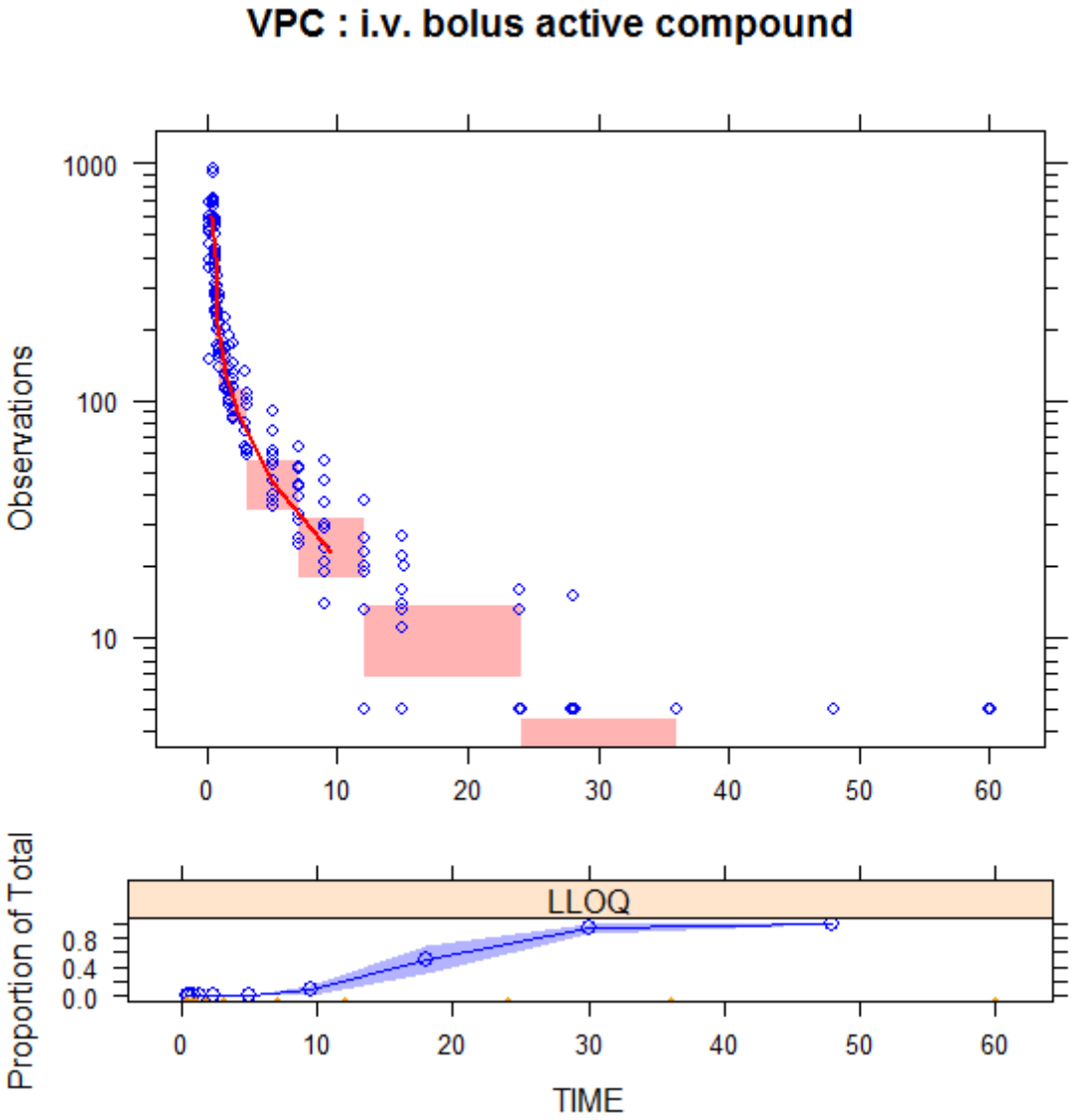

Fig. 10: Visual predictive check from 1000 simulated data sets. The 95% confidence interval of the median of the simulated data is represented by the pink square in the upper plot and the blue area in the plot down below. The real data are the blue dots. The median of the observed data is the red line in the upper plot and the blue line in the plot down below.

5.2. Second model: Pro-drug

As stated previously, the aim of this work is to develop a model which describes the pharmacokinetics of the prodrug. It means developing a linked model with the prodrug-metabolite-active compound data.

The disposition of the pro-drug appears to be adequately described by a six compartments model (Fig. 11) with the same active compound compartment as described before and the new metabolite and pro-drug compartments. This includes a central volume (BVC), a metabolite metabolised fraction (BFM), a proportional (Prop. ruv), and an additive residual error (Add. ruv) for the metabolite. For the prodrug, the new parameters are a central (AVC) and peripheral (AVP) volume, an inter-compartmental clearance (AQ), a hepatic extraction ratio (AEH), a prodrug metabolised fraction (AFM), a proportional (Prop. ruv) and an additive residual error (Add. ruv).

A slope-intercept (additive and proportional) residual error model is used to describe the metabolite and prodrug data. The active compound model part was adequately described by a proportional residual error. An inter-individual variability is estimated for all the parameters excepting AQ1, CVP2, CQ1 and CQ2 fixed to zero. The intra-individual or residual variability is fixed to 1. The event doses are done in the central compartment of the prodrug and active compound (Fig. 11). The observation or concentration-time measurement event happens in the central compartment of each compound. The metabolised fraction and the extraction ratio underwent a logit transformation.

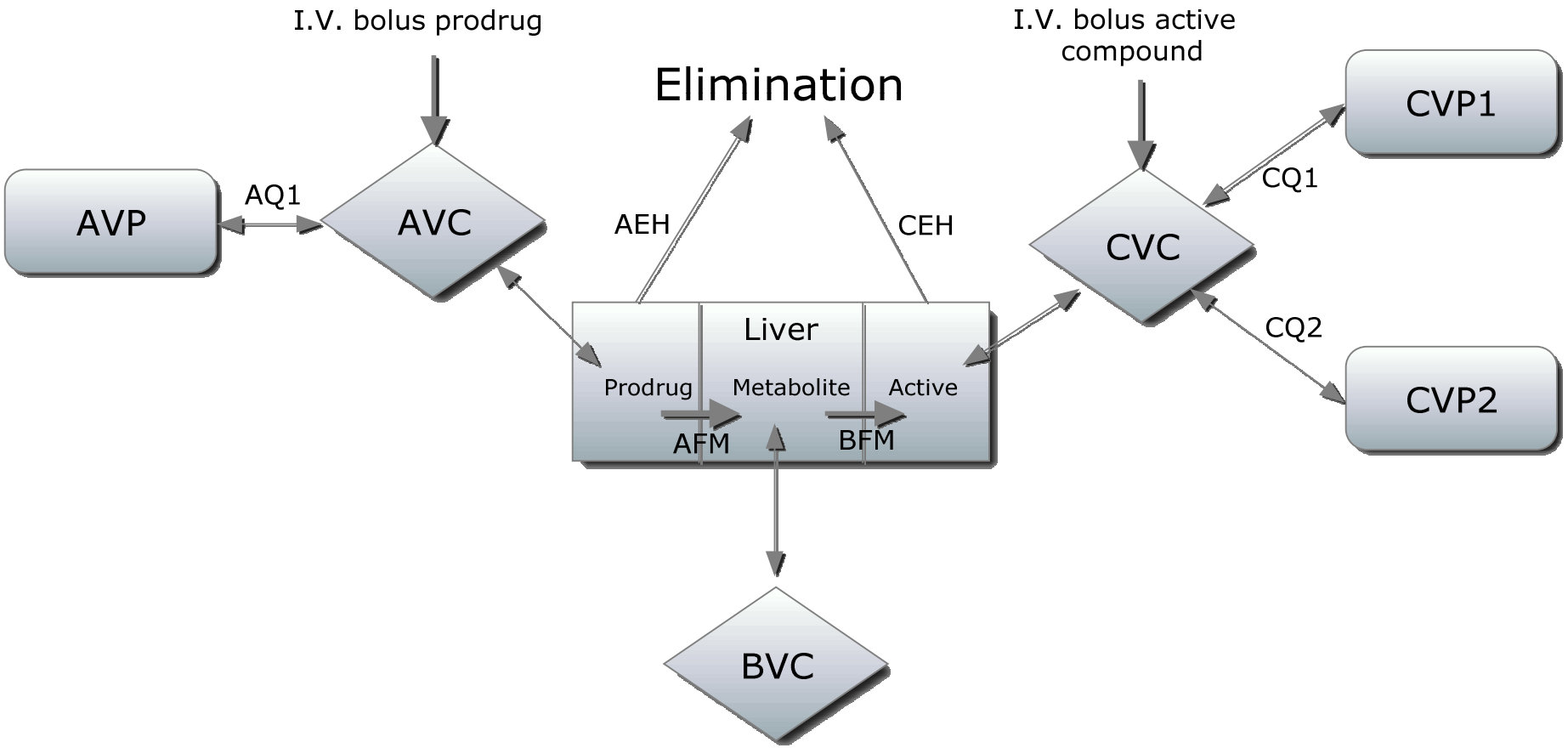

Fig. 11: Schematic illustration of the pro-drug PK model. The data from the two treatments were used to develop the model. The constraint of hepatic compartment and the relation between central and hepatic is described by physiological value to minimize the impact of this compartment on the model.

Table 3: Estimated parameter values of the prodrug model.

Parameter	Mean	Standard error (%)	Coefficient of variation (%)	Standard error (%)	Eta shrinkage (%)
AEH, pro-drug	0.172	25.79	2.35	166.95	4.48
AVC, pro-drug	5.94 L	1.11	0.07	315.45	3.66
AQ1, pro-drug	19 L/h	0.234			
AVP1, pro-drug	9.52 L	0.388	0.03	635.03	7.75
AFM, pro-drug	0.462	12.08	1.01	175.88	8.27
BVC, metabolite	7.88 L	0.88	0.05	330	6.53
BFM, metabolite	0.282	20.87	1.45	173.93	1.68
CVC, drug	16.7 L	0.58	0.03	228.41	18.61
CVP1, drug	51.3 L	0.19	0.01	149.31	4.02
CVP2, drug	81.7 L	0.16			
CQ1, drug	13.7 L/h	2.94			
CQ2, drug	2.33 L/h	2.95			
CEH, drug	0.162	48.33	3.39	137.51	3.14
Addi ruv, pro-drug	5.51	2.39			
Prop ruv , pro-drug	0.0557	172.35			
Addi ruv, metabolite	8.92	152.33			
Prop ruv, metabolite	0.0663	153.32			
Prop ruv, drug	0.206	38.83			

5.2.1. Prodrug data results

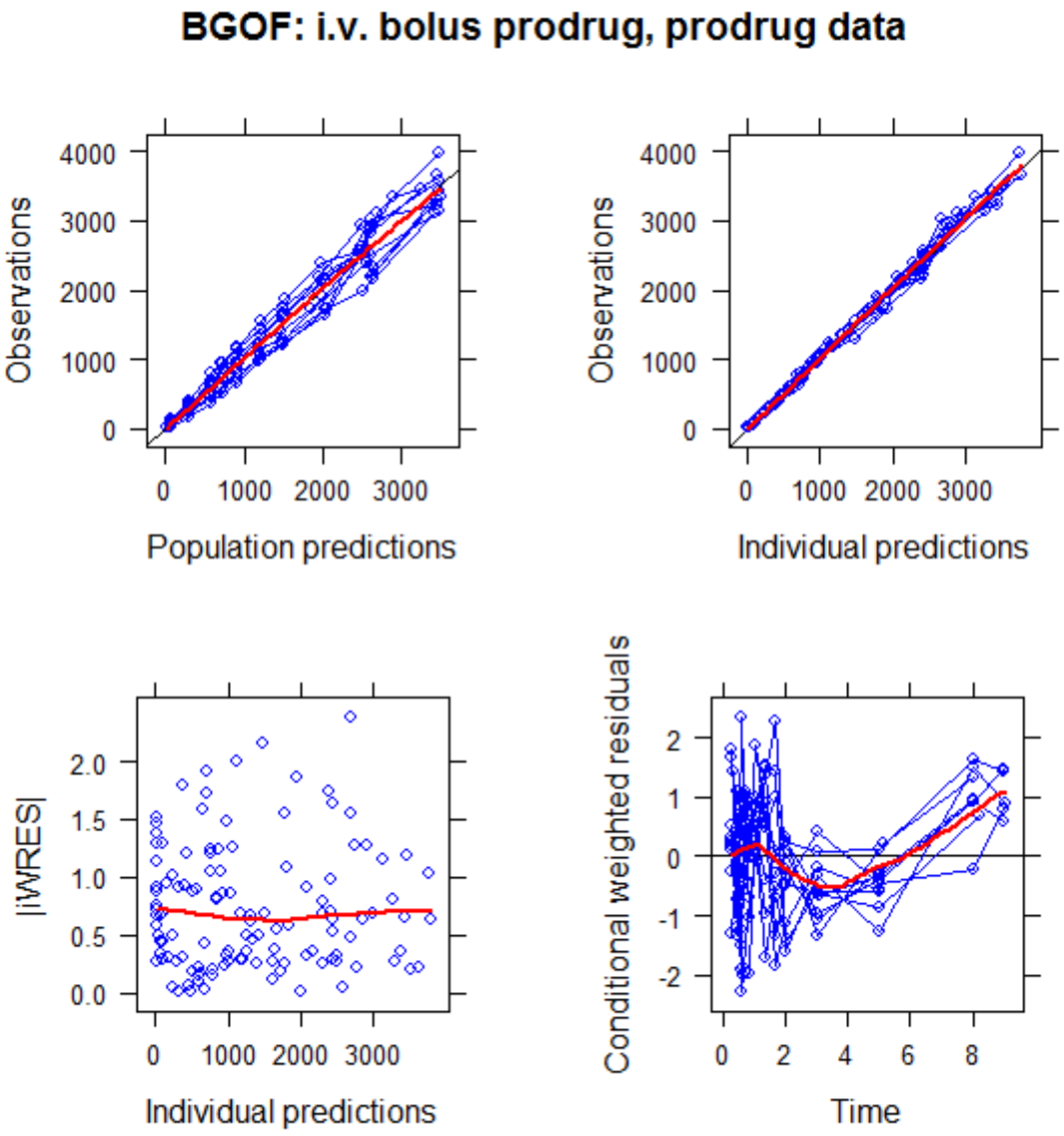

Fig. 12: Basic goodness of fit-plots for prodrug i.v. bolus treatment and prodrug data. For description see Fig. 8.

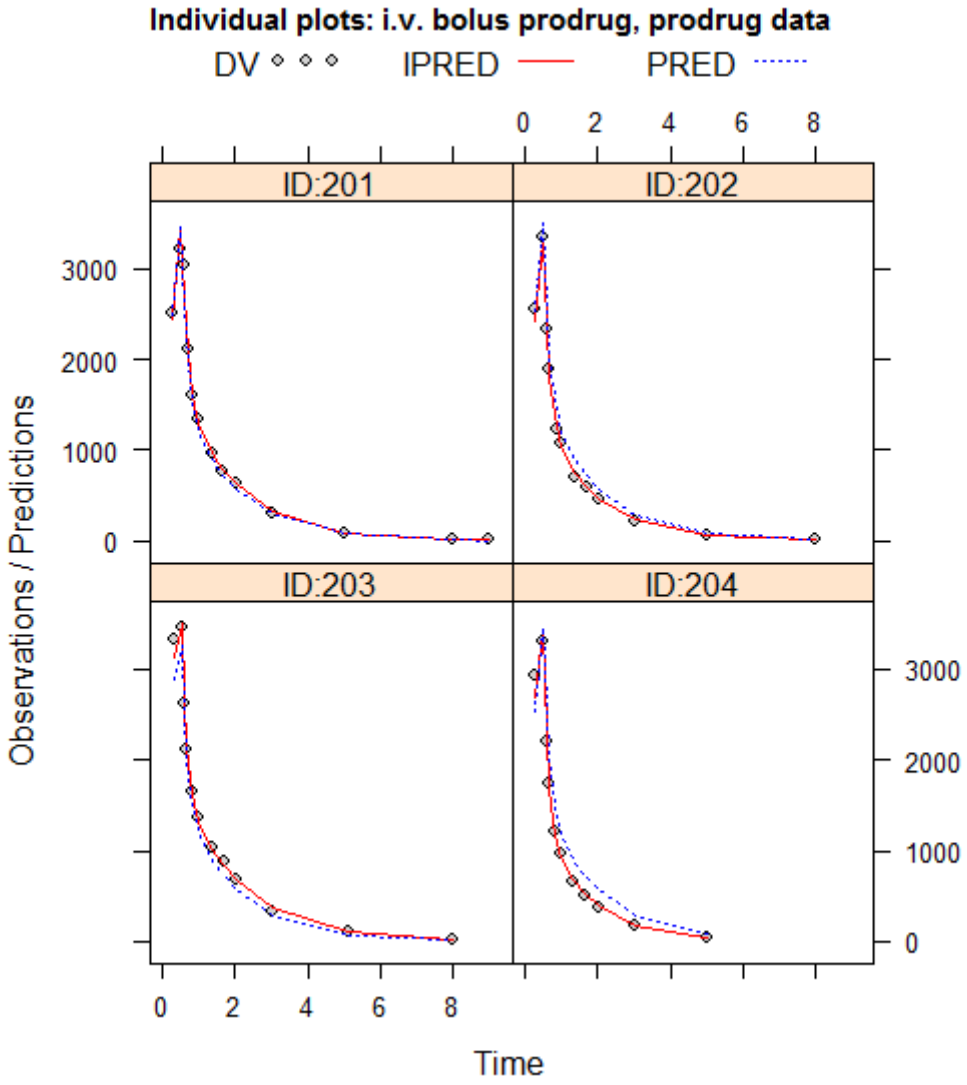

Fig. 13: Individual plots prodrug i.v. bolus treatment and prodrug data. For description see Fig. 9.

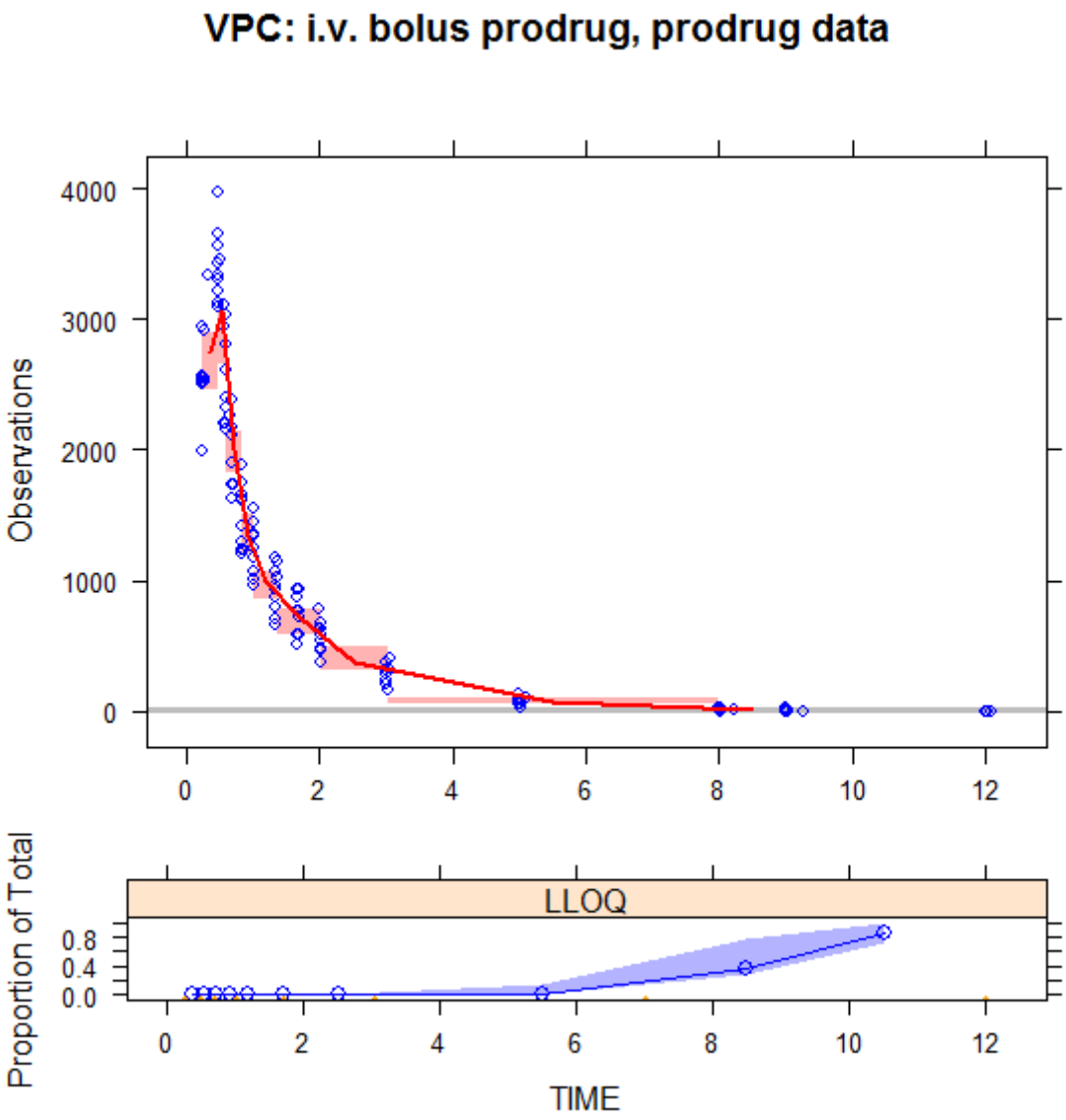

Fig. 14: Visual predictive check for prodrug i.v. bolus treatment and prodrug data. For description see Fig. 10.

5.2.2. Metabolite data results

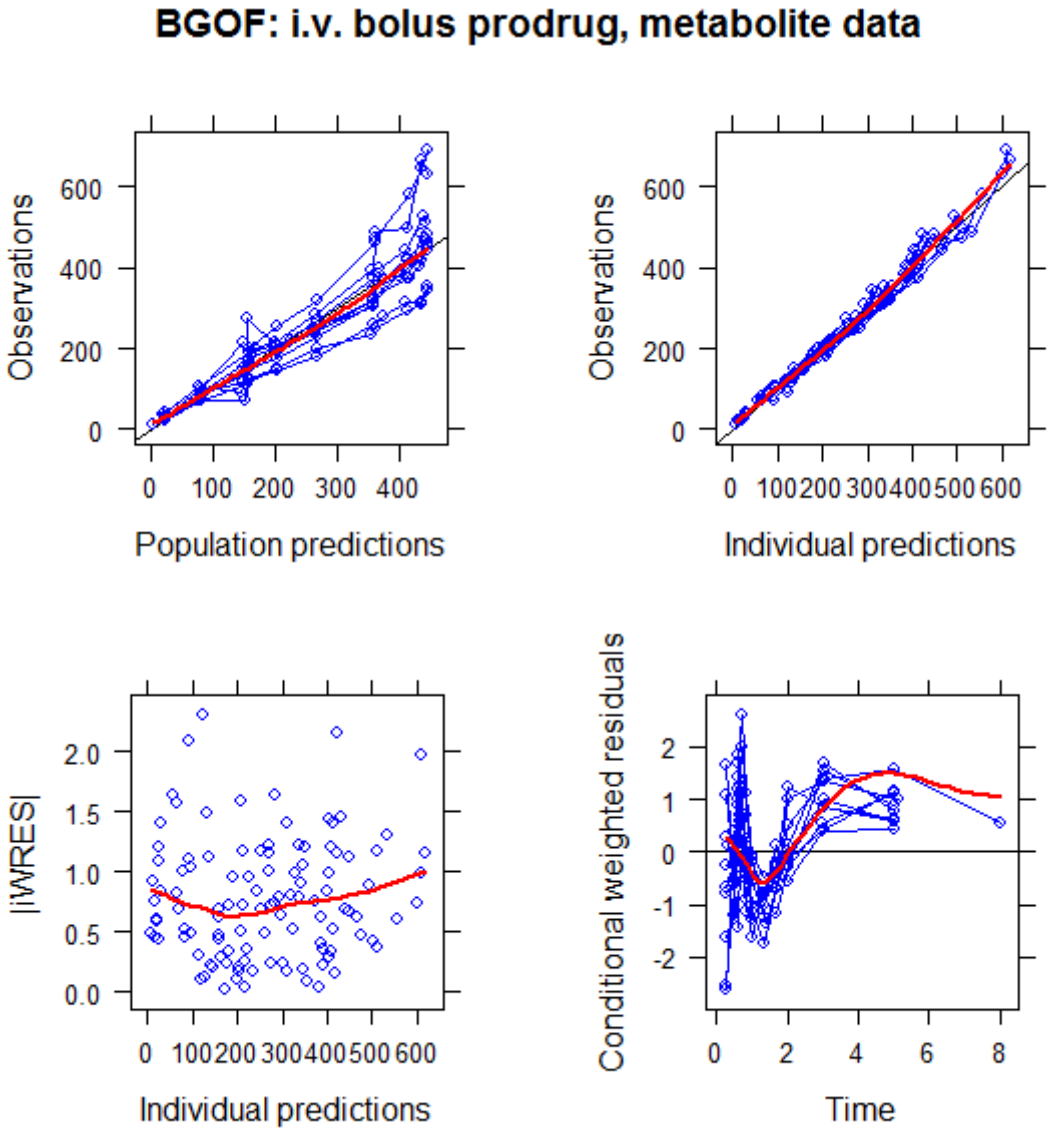

Fig. 15: Basic goodness of fit-plots for prodrug i.v. bolus treatment and metabolite data. For description see Fig. 8.

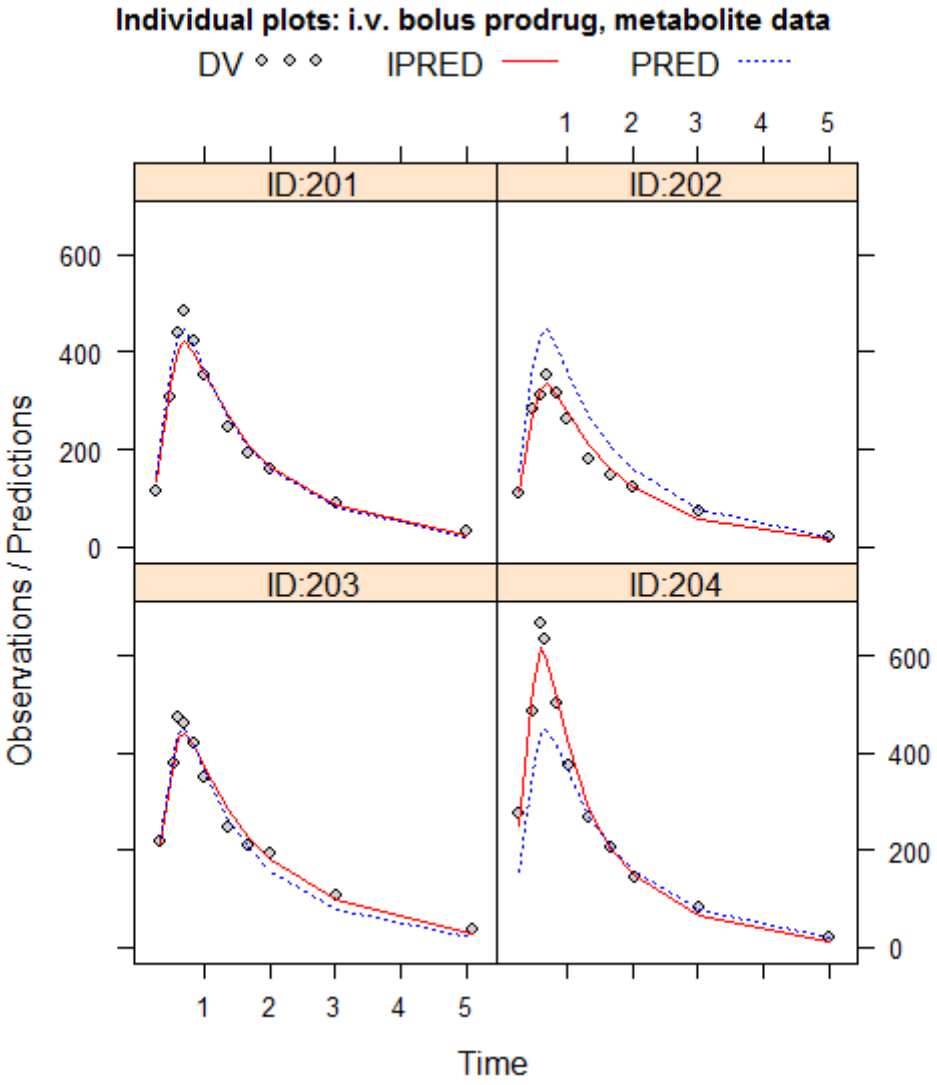

Fig. 16: Individual plots for prodrug i.v. bolus treatment and metabolite data. For description see Fig. 9.

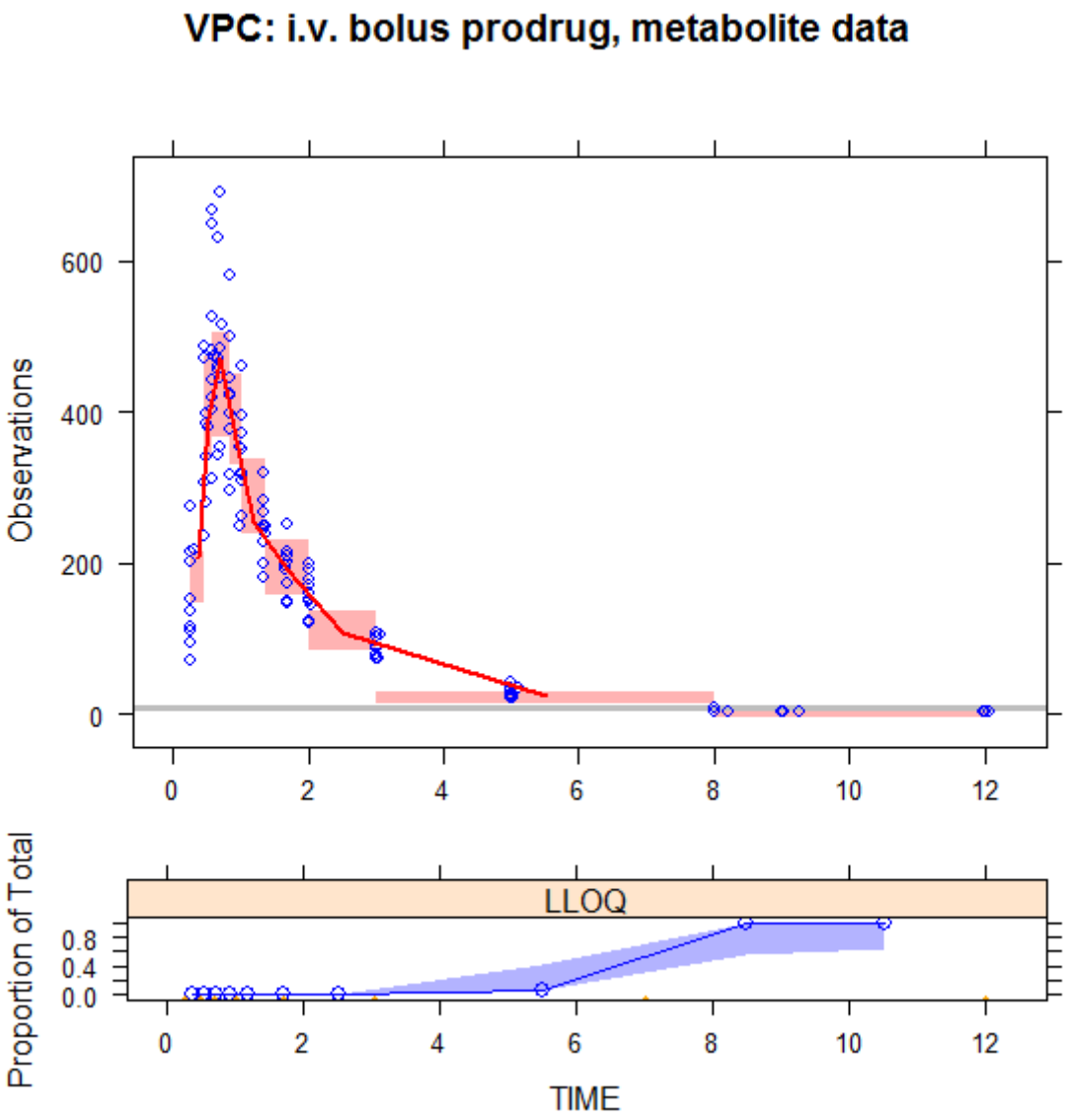

Fig. 17: Visual predictive check for prodrug i.v. bolus treatment and metabolite data. For description see Fig. 10.

5.2.3. Active compound data results, prodrug treatment

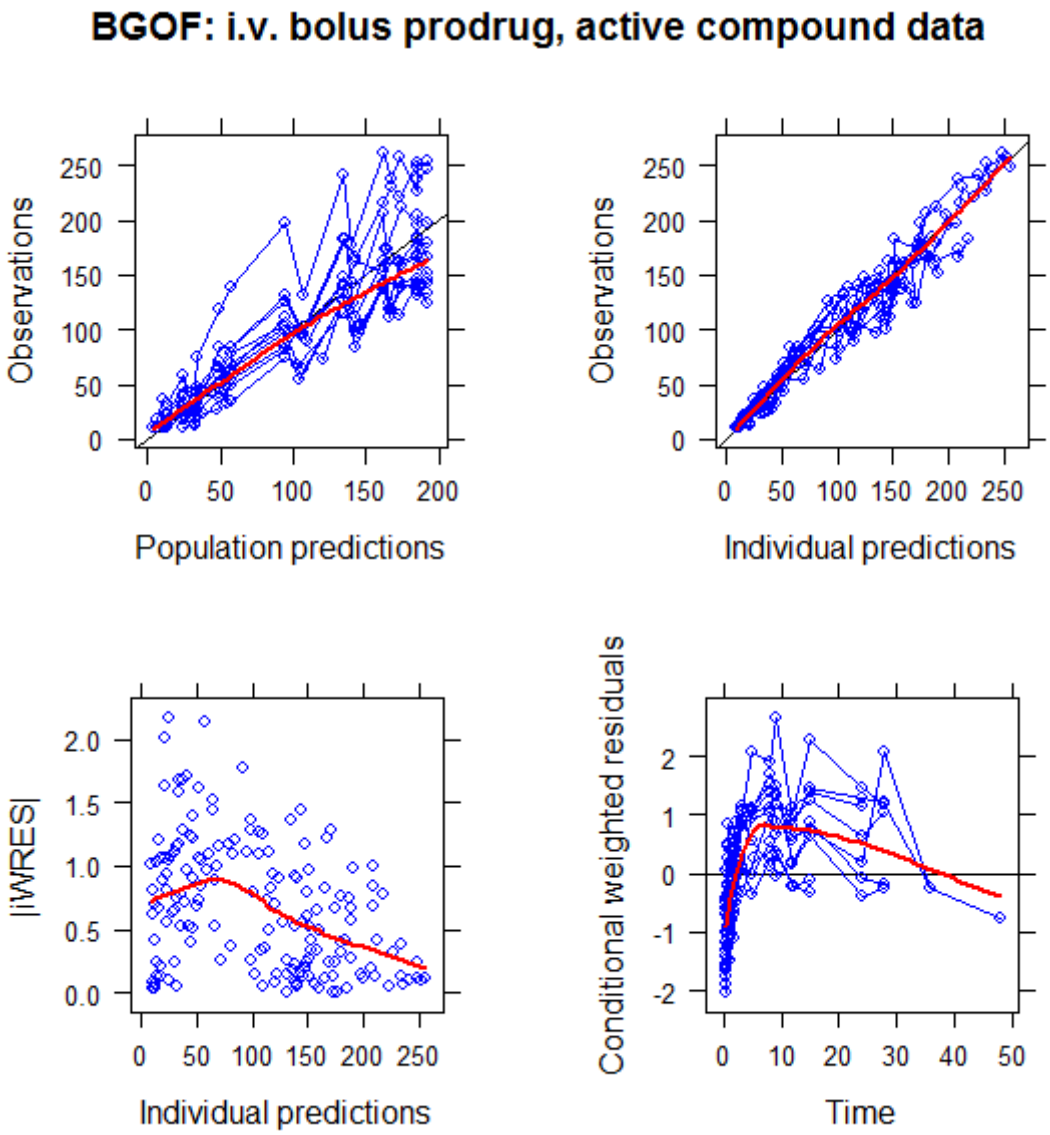

Fig. 18: Basic goodness of fit-plots for prodrug i.v. bolus treatment and active compound data. For description see Fig. 8.

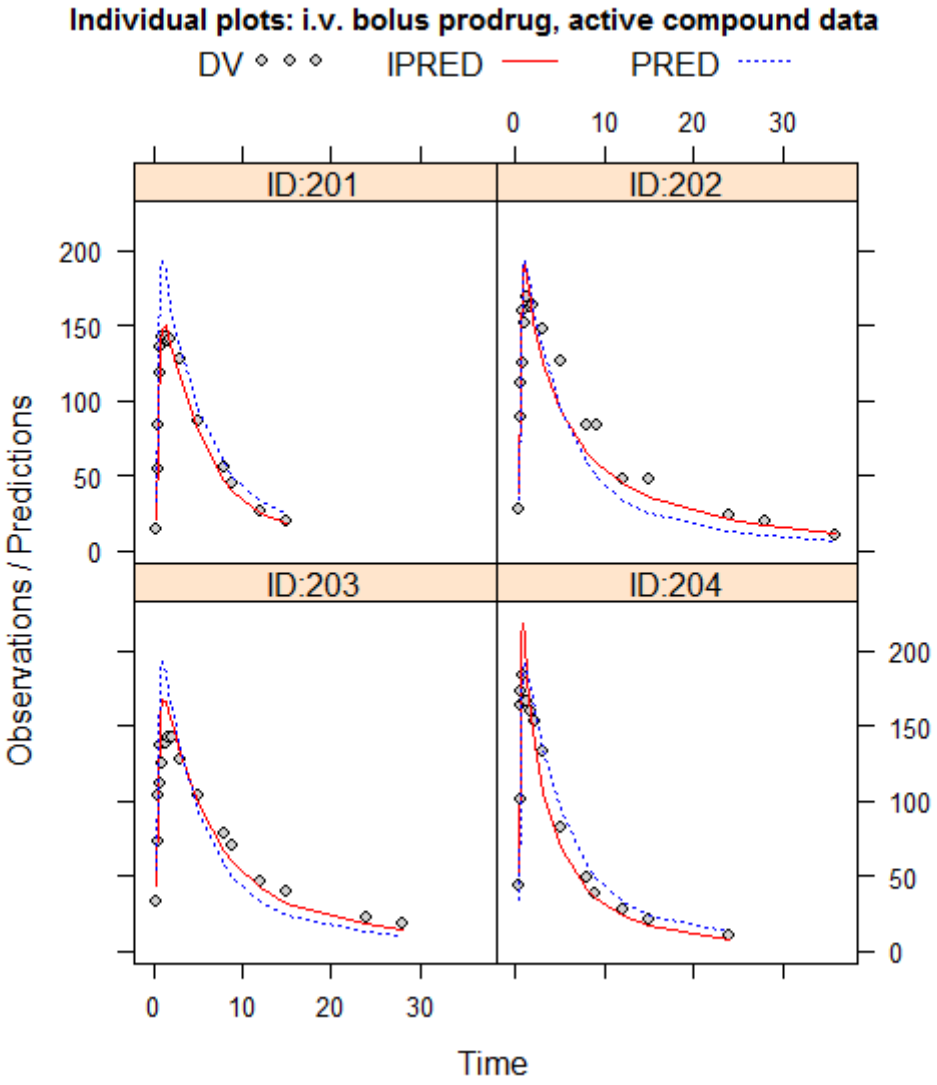

Fig. 19: Individual plots for prodrug i.v. bolus treatment and active compound data. For description see Fig. 9.

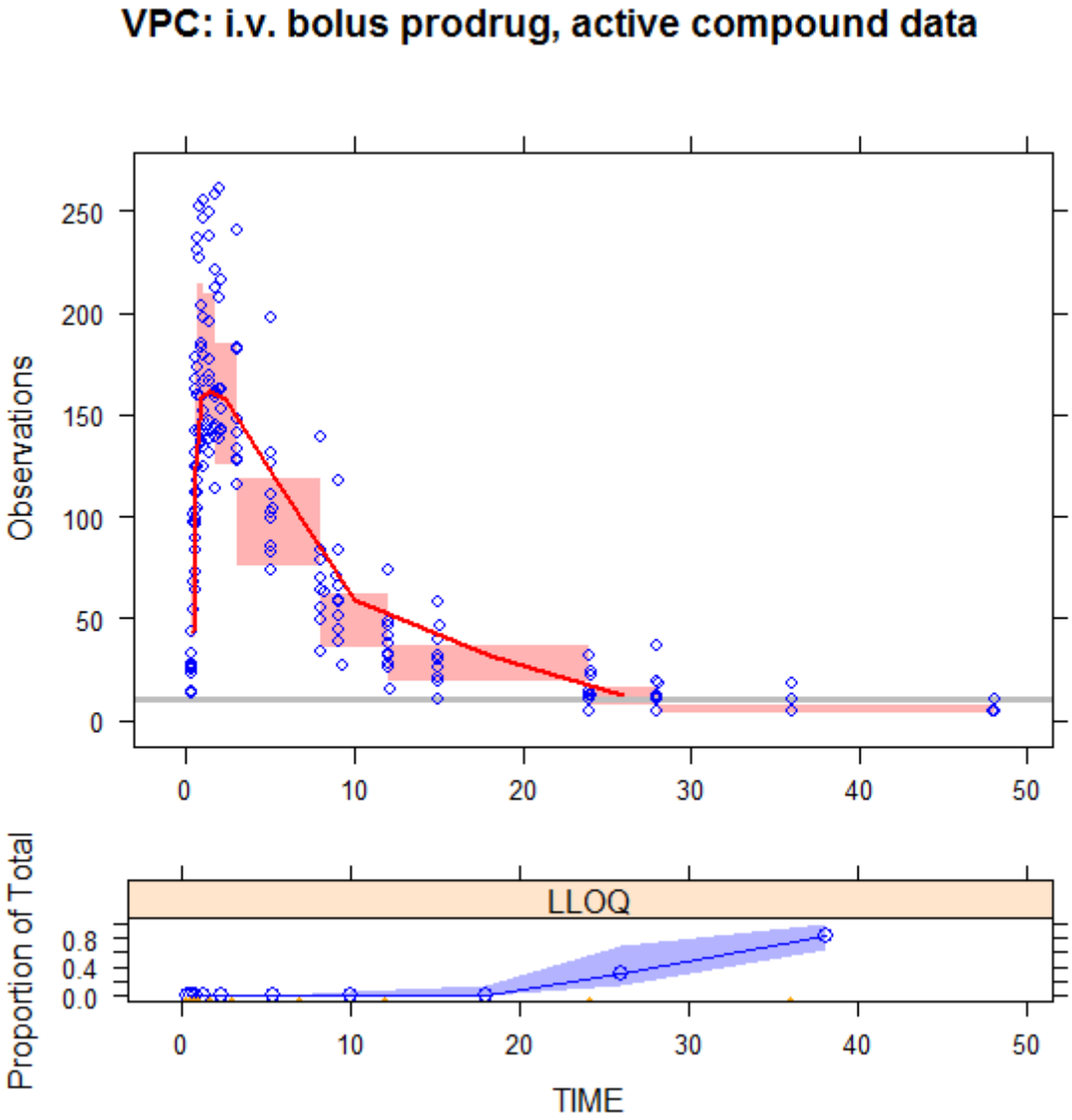

Fig. 20: Visual predictive check for prodrug i.v. bolus treatment and active compound data. For description see Fig. 10.

5.2.4. Active compound data results, active compound treatment

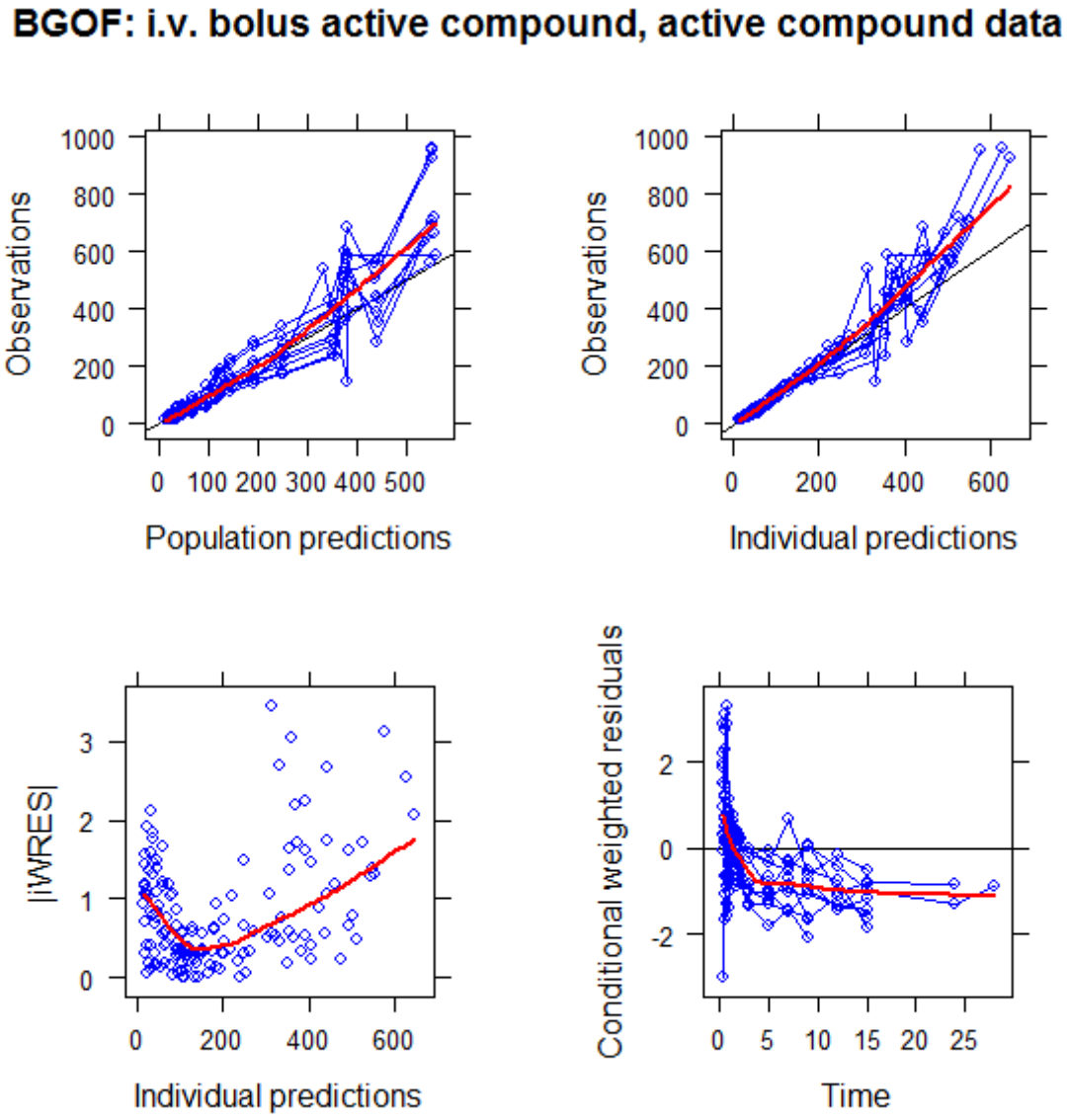

Fig. 21: Basic goodness of fit-plots for active compound i.v. bolus treatment and active compound data. For description see Fig. 8.

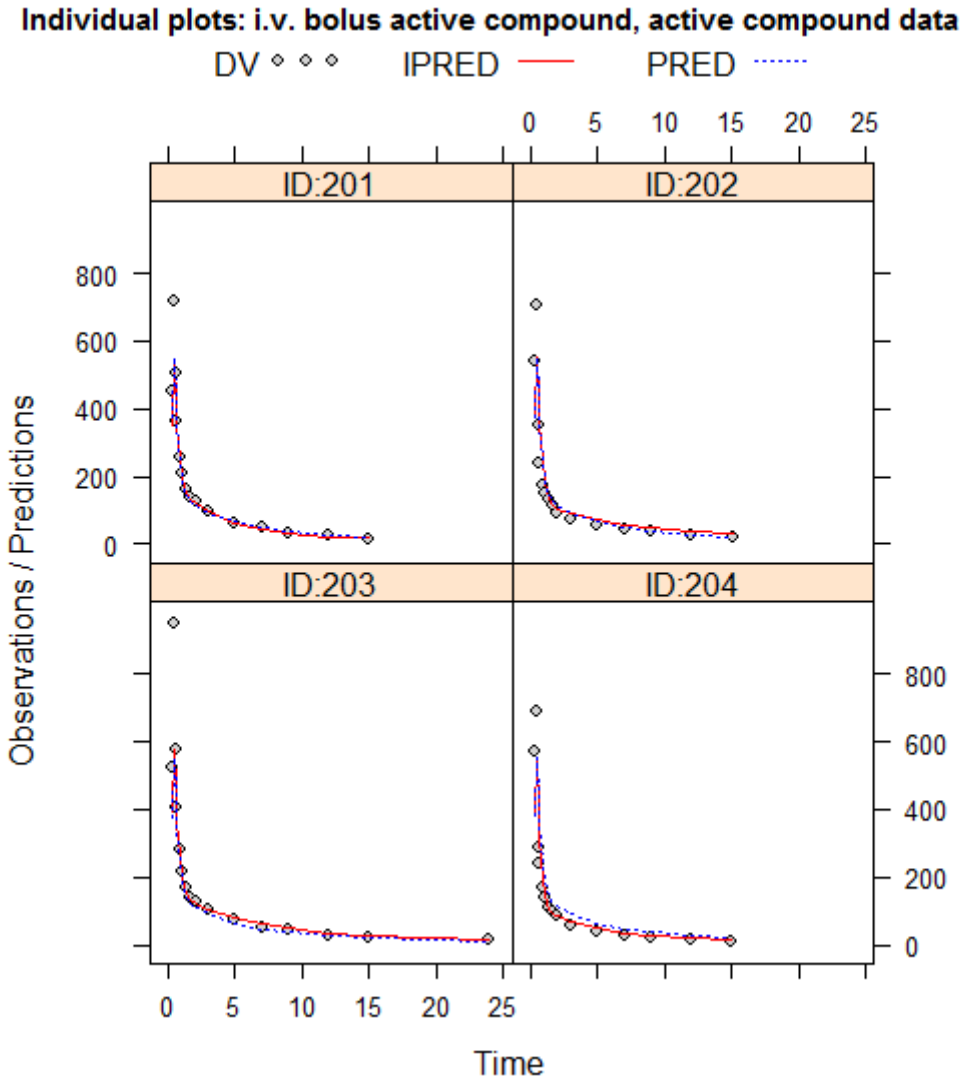

Fig. 22: Individual plots for active compound i.v. bolus treatment and active compound data. For description see Fig. 9.

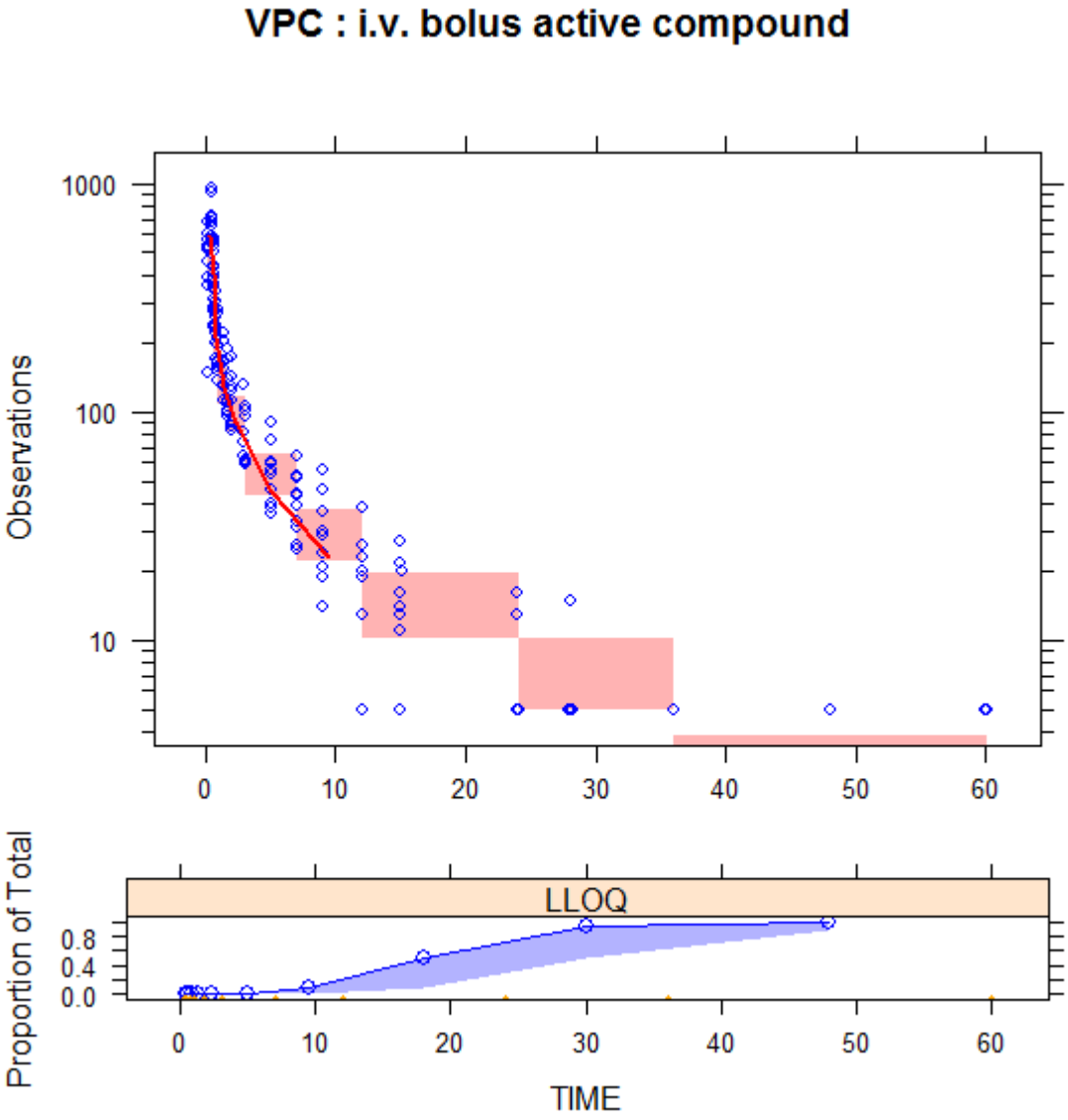

Fig.23: Visual predictive check for active compound i.v. bolus treatment and active compound data. For description see Fig. 10.

6. Discussion

The following section is structured according to the results part, with each section of the results being addressed in a separate section.

6.1. First model: active compound

All four basic goodness of fit plots (Fig. 8) illustrate that the model is describing the data adequately. There is no major trend in magnitude of |IWRES| and the CWRES vs time regression line is close to the zero line. The short rise of the curve after fifteen minutes is driven by tree data points and considered insignificant. For both the DV vs PRED and DV vs IPRED plots, the data points scatter evenly around the line of identity. For higher concentrations a slightly higher deviation is observed. One possible explanation is perhaps inaccuracies in the application of the infusion that can't be described by the model. Notably, one of the individuals displays a bigger discrepancy between the individual predicted and the observed value. However, since the deviations are on both sides of the line of identity (i.e. representing under and over prediction) this is less worrying.

The individual plots (Fig. 9) represent a very easy to interpret and natural representation of the model predictions and underline the very good performance of the model to describe the observations.

In contrast, VPCs might be harder to interpret, but constitutes a very powerful tool to evaluate a model. In Fig. 10 it can be seen that the median of the observed data is always contained in the 95% confidence interval for the simulated medians. This further illustrates that the model is describing the data adequately. In addition, this plot allows diagnosing how BLQ data is predicted by the model. Since the observed fraction is entirely contained in the predicted interval, this can also be considered as satisfactory.

The parameter estimates (Table 2) for the model have a low standard error for all fixed effects except for the hepatic extraction (CEH). The latter can be explained by its logit transformation. Due to the low number of individuals in the study, the random effect parameters (including the RUV) have a very high standard error. This might limit the usage of the model for simulations of large populations. However, the inclusion of each random effect was tested using the likelihood ratio test and only significant random effects were included in the final model.

All parameter estimates seem physiologically plausible with a low standard error for the fixed effects. This together with the excellent performance of this model in the graphical diagnostics, justifies the use of model 1 to describe the PK of the active compound in the full model.

6.2. Second model: Prodrug

6.2.1. Prodrug data results

The BGOF plots (Fig. 12) illustrate that the model is describing the data adequately except for the CWRES vs time plot. There are irregularities in the trend of the curve but it remains between -1 and 1 and it is not deemed significant. The individual plots (Fig. 13) show the good performance of the model to describe the observations. For the VPC (Fig. 14), it can be seen that the median of the observed data is always contained in the 95% interval for the simulated median. However, it also shows a few data points not covered by the prediction at the high concentration. The prediction (blue line) for the data below limit of quantification (LLOQ) is close to the center of the simulated data (blue area). The analysis of these plots supports this model to describe the first part of this complex model.

6.2.2. Metabolite data results

The population prediction (Fig. 15) is centerred on the mean but the data are scattered around this prediction. With the links between the dots that show the individual, we can clearly see some individuals are not well described. Overall, the individual prediction looks good. There is a trend in the |IWRES| vs IPRED but it is a small one and deemed acceptable. The CWRES vs time is problematic but we can go further and check the result of the other diagnostic tools. The individual plots (Fig. 16) show difficulty in matching the highest concentrations but the IPRED looks globally good. The VPC (Fig. 17) shows problems in describing the high concentrations and few data points at the beginning of the observation. After this analysis we can see few model misspecifications. We think these are mostly due to the active compound misspecification as described below.

6.2.3. Active compound data results, prodrug treatment

The BGOF (Fig. 18) shows a population prediction close to the mean but few individuals are mismatched and the data points are too sparse around the mean. The data points of the individual prediction are also scattered but the general shape of the curve is satisfactory. The |IWRES| vs IPRED show a clear trend, that could indicate a bad choice of residual error model but the slop-intercept or additive models were investigated without success. The CWRES vs time is not satisfactory and further work will be necessary. The individual plots (Fig. 19) present a good shape but some data points are not matched. In the VPC (Fig. 20) the prediction is not in the center of the simulated data and data points are not matched especially at the high concentrations. To do the modelling of this part, data from two different treatments were available. The model selected to describe this part of the prodrug model is the one developed for the I.V. bolus active compound treatment data. However, with a simple parameter comparison we can already expect problems in the model specification. One possible explanation of these problems is a saturation process in the degradation of the active compound in the prodrug treatment. The molecular weight of the different compounds is very close and in the prodrug treatment the degradation of the three compounds could use the same enzyme. This enzyme saturation could diminish the degradation rate of the active compound and produce the model misspecification.

6.2.4. Active compound data results, active compound treatment

The predictions vs observation (Fig. 21) plots show problems in matching the high concentrations. The |IWRES| vs IPRED and CWRES vs time show like the previous BGOF plot(Fig. 18) a real model misspecification. The individual plots (Fig. 22) present a bad prediction at the beginning of the observation. The VPC for this observation is produced on the log scale to allow the comparison with the first model (Fig. 10). We can see that the prediction is no longer in the center of the simulated data. The first model developed is a relevant model to describe this data. However, due to the bad capability of the model to describe the active compound of the prodrug, the estimation methods used try to find new parameter estimates. These parameters are a compromise between the two models and thus give a worse result for each of them.

7. Conclusion

For the first time a full population pharmacometric model, describing the complete PK of this investigational prodrug, including the active compound and an intermediate has been developed. In general, the model developed in this work describes the observed data to a satisfactory degree. Data from the intra-venous application of the pro-drug and the active compound are especially well described. The description of the intermediate form and the active compound after an i.v. dose of the prodrug may need further improvement. As discussed, one possibility would be the inclusion of a saturation phenomenon to the conversion process between the different compounds.

8. References

1. Ette Ene I. , W.P.J., *Pharmacometrics : the science of quantitative pharmacology*. Hoboken ed. 2007, Hoboken, N.J.: John Wiley & Sons. xix, 1205 p.

2. Gobburu, J.V., *Pharmacometrics 2020.* J Clin Pharmacol, 2010. 50(9 Suppl): p. 151S-157S.

3. Machado, S.G., R. Miller, and C. Hu, *A regulatory perspective on pharmacokinetic/pharmacodynamic modelling.* Stat Methods Med Res, 1999. 8(3): p. 217-45.

4. Bergstrand M, S.E., Eriksson U, Weitschies W, Karlsson MO. *Semi-mechanistic modeling of absorption from extended release formulations - linking in vitro to in vivo*. in *PAGE*. 2010. Berlin.

5. Beal, S., Sheiner, L.B., Boeckmann, A., & Bauer, R.J., *NONMEM User's Guides. (1989-2009)*. 2009, Icon Development Solutions: Ellicott City, MD, USA.

6. Grasela, T. and L. Sheiner, *Pharmacostatistical modeling for observational data.* Journal of Pharmacokinetics and Pharmacodynamics, 1991. 19(0): p. 25S-36S.

7. Ueckert, S., *A Population Disease Progression Model for Osteoporosis*, in *Department of Mathematics and Computer Science*. 2009, Freie Universität Berlin: Berlin. p. 91.

8. Foracchia, M., et al., *POPED, a software for optimal experiment design in population kinetics.* Comput Methods Programs Biomed, 2004. 74(1): p. 29-46.

9. Wang, Y., *Derivation of various NONMEM estimation methods.* J Pharmacokinet Pharmacodyn, 2007. 34(5): p. 575-93.

10. Pharmacometrics, U.u.g., *Introduction to the PHARMACOMETRICS GROUP at Uppsala University*. Vol. 1. 2010, Uppsala. 143.

11. Ahn, J.E., et al., *Likelihood based approaches to handling data below the quantification limit using NONMEM VI.* J Pharmacokinet Pharmacodyn, 2008. 35(4): p. 401-21.

12. Bonate, P.L., *Pharmacokinetic-Pharmacodynamic Modeling and Simulation*. 2006: Springer US.

13. Hooker, A.C., C.E. Staatz, and M.O. Karlsson, *Conditional weighted residuals (CWRES): a model diagnostic for the FOCE method.* Pharm Res, 2007. 24(12): p. 2187-97.

14. Neyman, J. and E.S. Pearson, *Joint statistical papers*. 1967, Berkeley,: University of California Press. 299 p.

15. Holford, N., *The Visual Predictive Check – Superiority to Standard Diagnostic (Rorschach) Plots*, in *PAGE*. 2005: Pamplona, Spain.

16. Karlsson MO , H.N., *Tutorial on Visual Predictive Checks.* , in *PAGE*. 2008: Marseille, France.

17. Lindbom L, R.J., Jonsson EN. , *Perl-speaks-NONMEM (PsN)--a Perl module for NONMEM related programming.* Comput Methods Programs Biomed, 2004. 75(2): p. 85-94.

18. Lindbom L, P.P., Jonsson EN. , PsN-Toolkit--a collection of computer intensive statistical methods for non-linear mixed effect modeling using NON MEM. Comput Methods Programs Biomed., 2005. 79(3): p. 241-57.

19. K. Harling, S.U., A. C. Hooker, E. N. Jonsson and M. O. Karlsson, *Xpose and Perl speaks NONMEM (PsN)*, in *PAGE*. 2010: Berlin, Germany.

20. Jonsson, E.N.K., M.O., *Xpose--an S-PLUS based population pharmacokinetic/pharmacodynamic model building aid for NONMEM.* Computer Methods and Programs in Biomedicine, 1999. 58(1): p. 51-64.

9. Appendix

Model file developed for NONMEM.