A PHARMACOKINETIC POPULATION MODEL FOR CYCLOSPORIN A IN RENAL TRANSPLANT RECIPIENTS

Development of a model for whole blood- and intracellular concentrations

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TABLE OF CONTENT

AKNOWLEDGEMENT **2**

TABLE OF CONTENT **3**

ABBREVIATIONS **6**

ABSTRACT **8**

1. INTRODUCTION **10**

1.1 PHARMACOKINETICS 10

1.1.1 Introduction *10*

1.1.2 Variability *11*

1.1.3 Compartmental theory *13*

1.1.4 Population pharmacokinetics *13*

2. PHARMACOKINETIC POPULATION MODELING **15**

2.1 INTRODUCTION 15

2.1.1 Standard 2-stage approach *16*

2.1.2 Naïve pooled data approach *16*

2.1.3 Nonlinear mixed-effect model approach *16*

2.2 NONMEM 17

2.2.1 Background *17*

2.2.2 Modelling population PK using NONMEM *18*

2.2.3 How to find the best model using maximum likelihood approach *20*

3. CYCLOSPORIN A **22**

3.1 HISTORY 22

3.2 APPLICATION AND MECHANISM OF ACTION 22

3.3 KNOWN PROBLEMS WITH CYCLOSPORIN A 23

3.4 ADME OF CSA 23

3.4.1 Administration ... *23*

3.4.2 Distribution ... *24*

3.4.3 Metabolism ... *24*

3.4.4 Elimination ... *25*

3.5 THERAPEUTIC DRUG MONITORING ... 25

3.6 POPULATION KINETIC MODELS OF CSA IN LITERATURE ... 26

3.7 GOALS OF THE THESIS ... 26

4. METHODS AND MATERIALS ... 27

4.1 MATERIALS FOR THE WHOLE BLOOD MODEL ... 27

4.2 MATERIALS FOR THE WHOLE BLOOD MODEL AND INTRACELLULAR CONCENTRATIONS ... 29

4.3 DEVELOPING AND BUILDING THE MODELS ... 30

4.3.1 The whole blood model ... *31*

4.3.2 The whole blood and intracellular model ... *31*

5. RESULTS ... 35

5.1 RE-ANALYZING FOR COVARIATES FOR THE WHOLE BLOOD MODEL ... 35

5.2 TESTING FOR INTEROCCACIONAL VARIABILITY ... 37

5.3 COVARIATE ANALYSIS BASED ON VISUAL PREDICTION ... 38

5.5 COMPARING THE OLD VERSUS THE NEW MODEL ... 43

5.6 THE WHOLE BLOOD AND INTRACELLULAR CONCENTRATIONS ... 44

5.6.1 Model building results ... *44*

6. DISCUSSIONS ... 52

6.1 RE-ANALYZING FOR COVARIATES FOR CSA PLASMACONCENTRATIONS ... 52

6.2 TESTING FOR INTEROCCASIONAL VARIABILITY FOR THE WHOLE BLOOD MODEL ... 54

6.3 WHOLE BLOOD AND INTRACELLULAR MODEL ... 55

7. CONCLUSIONS ... 58

8. REFERENCES 59

9. APPENDIX 63

9.1 FORMULAS USED IN DEMOGRAPHICS MODEL 63

9.2 PARTIAL INPUT FILE FOR WHOLE BLOOD MODEL 64

9.3 CONTROL FILE FOR FINAL MODEL WHOLE BLOOD 66

9.4 INDIVIDUAL FITTING MADE BY R FOR FINAL WHOLE BLOOD MODEL 68

9.5 PARTIAL INPUT FILE FOR WHOLE BLOOD AND INTRACELLULAR MODEL 77

9.6 CONTROL FILE FOR FINAL MODEL WHOLE BLOOD AND INTRACELLULAR CONCENTRATIONS ..80

9.7 DIAGNOSTIC PLOT MADE BY R FOR WHOLE BLOOD AND INTRACELLULAR MODEL 82

9.8 DIVERSE FIGURES FOR WHOLE BLOOD AND INTRACELLULAR MODEL WITH A LOWER KA AND OFV COMPARING WITH THE FINAL MODEL 85

ABBREVIATIONS

- 2LL	minus two log likelihood, the objective function of NONMEM
ADME	absorption, distribution, metabolism and elimination
ALAG	absorption lagtime
AUC	area under curve
BMI	body mass index
BOV	between occasion variability
C_0	trough concentration
C_n	concentration “n” hours post-dose
CL	clearance
CsA	Cyclosporine A
CYP450	cytochrom P450
DV	dependent variable, observed concentration
DVID	dependent variable identification
F	bioavailability
FDA	food and drug administration (USA)
FO	first-order
FOCE	first-order conditional estimation
GOF	goodness of fit
ID	identification
IOV	interoccasional variability
IPRED	individual predicted concentration
Ka	absorption rate constant
LBM	lean body mass
LN	natural logarhytm
NA	not applicable
NM-TRAN	NONMEM-translator
NONMEM	nonlinear mixed-effect modeling
NPD	naïve pooled data
OFV	objective function value
P-gp	P-glycoprotein
PD	pharmacodynamic

PK	pharmacokinetic
PPK	population pharmacokinetic
PRED	predicted concentration
Q	inter-compartmental clearance
QOF	quality of fit
RH	Rikshospital University Hospital
SD	standard deviation
STS	standard two-stage
TDM	therapeutic drug monitoring
TV	typical value
Tx	transplantation
V_c	central volume
V_d	distribution volume
V_p	peripheral volume
WRES	weighted residuals

ABSTRACT

Background: Cyclosporine A (CsA) has since its introduction in the 1980's played a substantial part of the success in solid organ transplantation. Like many other immunosuppressive drugs, CsA has a narrow therapeutic window and a large inter-individual variability. Drug exposure above the therapeutic window is associated with adverse events like nephrotoxicity, infection and cancer while drug exposure below will yield a lack of effect and increased risk for acute rejection episodes. Obtaining an optimal drug concentration will prevent acute organ rejections and optimize survival of the grafts and ultimately the patients.

The primary aim of this study is to implement a T-cell compartment to an already existing whole blood model. Another goal was to further develop the basic whole blood model after the inclusion of 20 new patients followed for at least 8 weeks, by re-evaluating for relevant covariates and include estimation of interoccasional variation in the model.

Methods: Data was gathered from four separate clinical trials, performed by the department of Pharmaceutical Biosciences at the University of Oslo in co-operation with the Medical Department at Rikshospitalet, Oslo University Hospital, for the whole blood model. In all 70 patients and a total of 1276 whole blood samples were included in the whole blood model.

Of the 70 patients, 20 patients also had intracellular concentrations measured. These 430 intracellular samples were included in the development of the extended model.

By using the nonlinear mixed-effect modelling program NONMEM two population pharmacokinetic models were developed.

Results: When re-analyzing for significant covariates, many similar results as earlier tested for the whole blood model was found. Age was a significant co-factor on the parameters: clearance (CL), absorption (Ka) and compartmental volumes (V_1), while cytochrom P450 3A5 (CYP3A5) genotype had a significant impact on clearance. The

steroid dose and weight influenced the inter-compartmental clearance (Q), while BMI had an effect on volume (V_1) and absorption (Ka). Interoccasional variability was found significant on the parameter V_2, and included in the final model.

An intracellular population pharmacokinetic kinetic (PPK) model was developed for CsA. The concentrations were homogenized to the same unit (ng/ml), by estimating the T-lymphocyte volume, and LN-transformed because of the large concentration range difference. The developed model predicts whole blood and intracellular concentrations, but does not predict accurately or stable enough in its current state.

Conclusion: Two models were developed, one for whole blood concentrations and one extended model also including intracellular concentrations of CsA. There is no unambiguous answer if the whole blood model gave a significant improvement on the already existing model, but the model showed somewhat improvement in the visual plots and also included prednisolone and CYP3A5 as a covariate. Interoccasional variability was found significant and further included for the whole blood model. The whole blood and intracellular model is still in an early stage and needs to be further developed, tested for covariates and interoccasional variability, and finally validated.

1. INTRODUCTION

1.1 Pharmacokinetics

1.1.1 Introduction

Pharmacokinetics (PK) describes the relationship between drug concentrations attained in different body compartments with time, during and after drug input. The drug level-time relationship is related to adjustable elements such as route of administration, dose, dosage form, frequency etc. Pharmacokinetics is simply put how the body affects the drug. It differs from pharmacodynamic (PD) which says something about the relationship between the drug concentrations effect on the body with time. Simply put, PD is how the drug affects the body. [1]

In order to develop a PK/PD evaluation it would be ideally to take samples from the site of action, but because of practical difficulties samples are normally acquired from more accessible sites like blood and urine who are two of the most commonly sampled fluids. [1]

All drugs have a therapeutic window where the drug has optimal effect and acceptable side effects. (Figure 1) Below this range the drug exposure is too low to give the desired effect, and above the concentration range the drug will result in undesired adverse effects. This therapeutic window differs from drug to drug and is individual from patient to patient. For drugs with a large therapeutic window it is easy to stay in this window, but for drugs with a narrow therapeutic window it can be difficult to obtain the ideal concentration where the wanted effect is achieved. [1]

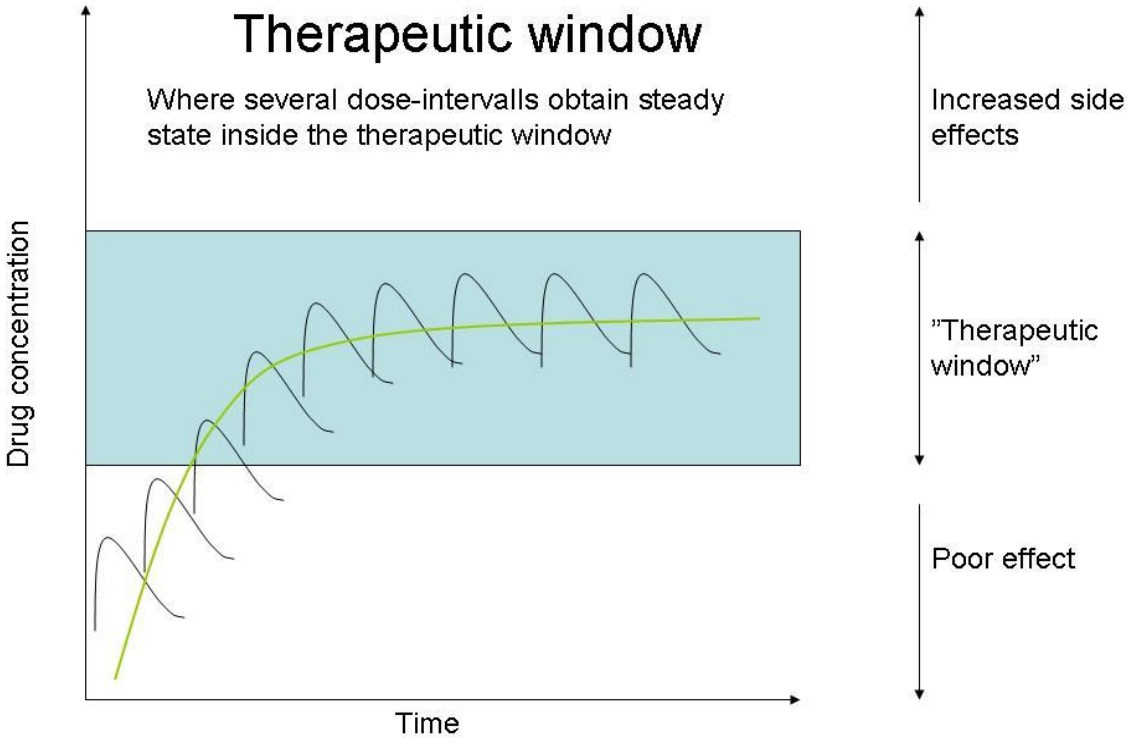

Figure 1. A representation of an optimal dose input so that steady state lies between the minimum and maximum levels of the therapeutic window

1.1.2 Variability

It is often seen a difference between the expected outcome and true values in PK/PD evaluations. This can be attributed to inter-individual variability and residual variability. [1]

Inter-individual variability is the true biological variability that exists between subjects. When calculating parameter values based on past experience and research, the parameter value for a specific individual will differ from the expected value because of true biological differences between individuals. Covariates can account for some of this variability, and searching for these factors is an important feature of population pharmacokinetics.

Residual variability is a common name for several variations including intra-individual variability (variability in between the same patient), interoccasion

variability (IOV) (day-to-day or week-to-week) and errors in measurement, dosage and modeling. Because the mathematical calculations made are an oversimplification of the reality, residual variability can arise. An increase of unexplained random variability can cause insecurity when predicting and controlling the drug concentrations and this can further lead to a decrease of drug safety and efficacy. It is however important to remember that the drug response variability also applies to side effects. [2-6]

Other variabilities in pharmacokinetics that may influence the dose concentration relationship are:

- environmental factors: smoking, diet, exposure to pollutants etc.
- interactions with other drugs, co-medication
- physiological factors: pregnancy
- demographics: gender, age, weight etc.
- genetic phenotype of polymorphism in cytochrom P450 isoforms that can effect both metabolism and clearance of drugs
- pathophysiological factors: renal- and hepatic impairment, CHD
- other factors: circadian rhytm, adherence, food effect, timing of meals, physical activity, posture and stress

We differ between two different variabilities, fixed- and random effects. Fixed effects are properties of each individual that causes them to be different from the average, while random effects can not be predicted. Random effect consists of inter-individuality and residual variability. [3, 7]

It is well known that individual pharmacokinetic may vary over time. Some of the variations can be attributed to physiological processes by means of surrogate variables, e.g. serum creatinine, co-medications with known enzyme inhibitors etc. Most variability in pharmacokinetic parameters within individuals are however not predictable. It could be that the governing processes are not understood or appropriate surrogate variables are lacking. Such apparently random intra-individual variability

can be arbitrary, but practically, divided into variations interoccasion variability (IOV) or sometimes called between-occasions variability (BOV). [8]

Neglecting IOV may result in a high incidence of statistically significant spurious period effects and most importantly, ignoring IOV can lead to a falsely optimistic impression of the potential value of therapeutic drug monitoring. [8]

1.1.3 Compartmental theory

Human anatomy and physiology is very complex, making it very challenging to model how the body uses the drug. It is however possible to simplify the body into few compartments in regards of PK modeling. [1]

A compartment is not a real physiologic or anatomic region. It represents unspecific tissue or group of tissues that have similar blood flow and drug affinity. Within each compartment the drug is presumed to be uniformly distributed and to reach distribution equilibrium simultaneously. [1]

The simplest model consist of one compartment, which assumes that changes in plasma levels of a drug reflect proportional fast changes in tissue drug level. In more advanced multi compartment models the drug distributes into the central compartment and one or several more tissue/peripheral compartments. The central compartment often represents the blood, extracellular fluid and highly perfused tissues that rapidly equilibrate with the drug. The tissue/peripheral compartment represents tissues where the drug equilibrates to. [1]

1.1.4 Population pharmacokinetics

The main goals of population pharmacokinetic (PPK) are to quantitatively assess the pharmacokinetic parameters, the inter-individual- and residual variability in drug absorption, distribution, metabolism and elimination (ADME). It can be defined as the study of variability in plasma drug concentrations between individual representatives for the target population group receiving the drug. PPK highly contrasts with traditional pharmacokinetics. With PPK the goal is not to homogenize

and standardize the patients, whom the data was gathered from, but rather to seek as much relevant information as possible and tailor individual dosages based on this. [3-5]

While traditional pharmacokinetic studies rarely account for the random effects, but rather PK-averages, population pharmacokinetics has that important feature of quantitatively estimating the residual variability in the patient population. This may give important information regarding drug efficacy and safety. PPK is therefore often used in both drug development and individual dosing regimens. In drug development, population pharmacokinetics can help designing dose guidelines. The approach is recommended in the US Food and Drug Administration (FDA) guidance for Industry as part of the development process. [3, 4, 9]

PPK makes it possible to collect integrated information on relatively sparse data, dense data or from a combination of both. Data can be divided into two groups:

- Experimental data: Data collected from traditional studies, with a controlled design and blood samples i.e. dens data.
- Observational data: Data gathered through routine clinical care or as a supplement for traditional studies. These data are often limited, collected at various times and unbalanced.

PPK is most valuable in situations where the drugs have a narrow therapeutic window and shows a complex pharmacokinetic relationship. [4, 5]

2. PHARMACOKINETIC POPULATION MODELING

2.1 Introduction

The requirement for population modeling has roots in the need for a system to predict pharmacokinetic and/or pharmacodynamic parameters in new patients based on patient-characteristics and often limited sampling. Individual predictions of drug exposure will increase the chances of successful therapy and reduce the chances for dose dependent side effects. [2, 10-12]

In PPK there are several parametric and nonparametric methods for estimating the different parameters. Parametric models have a continuous parameter distribution, and the distribution is assumed to be either normal or lognormal. It obtains means and standard deviations (SD) of the parameters, and correlations between them. Parametric models are able to distinguish inter- and intra-individual and assay error. One weakness of this method is that it lacks mathematical consistency, and it makes assumptions about the shape of the parameter distribution. [11]

Nonparametric methods makes no assumptions about the shape of the parameter distribution, meaning that no specific parameters such as means and standard deviations are used to describe the distribution of the parameters within a population *a priori*. The shape of the distribution is instead exclusively determined from the population raw data and can therefore detect any possible subpopulation with other distributions. It is mathematical consistent, but it lacks a feature to distinguish the various sources of variability. [11]

Of the most common methods for doing population pharmacokinetic analyses are the standard 2-stage (STS) method and the nonlinear mixed-effect model approach, which both are parametric, and the naïve pool data approach. [11, 13]

2.1.1 Standard 2-stage approach

The standard 2-stage approach is traditionally used in data rich situations. It consists of two stages where the first phase is to estimate each individual's PK and/or PD parameters from that individual's dense concentration time data, using a method of weighted nonlinear least squares.

During the second stage the populations mean and variance are derived from individual measurements and the relationship between covariates, and the parameters explored.

STS is easy to implement and quick to run, but gives poor prediction of parameters in data poor situations. [4, 5, 10, 11, 14]

2.1.2 Naïve pooled data approach

In the naïve pooled data (NPD) approach, all data gathered from every individual are considered coming from one unique individual. NPD is a general approach and can easily deal with experimental data, non-standard data and routine pharmacokinetic data. Parameter estimates are obtainable after a unique fitting of all data at concurrently. [4, 10]

NPD may be a good method when inter-individual variability is small. However, since the data is recognized as coming from one individual, imbalance and confounding correlations may occur. Only mean parameters are given in this approach so the inter-individual variability is lost and an imbalance in data per individual could lead to biased estimates. [4, 10]

2.1.3 Nonlinear mixed-effect model approach

The nonlinear mixed-effect modeling considers the population sample instead of the individual. They make foundation to estimate the distribution of parameters, the covariates and correlation between them. Similar to the NPD approach, nonlinear mixed-effect modeling analyzes data from all individuals simultaneously. The

difference is that the variability within and between patients is kept. An advantage compared to STS is that the nonlinear mixed-effect modeling finds the best set of parameters and one can perform formal testing of covariates. However, the method is slower to run and more advanced to implement compared to STS. [2, 4, 10, 11, 15, 16]

NOMEM was the first true nonlinear mixed-effect modeling program and is currently the most used program in the pharmaceutical industry for this purpose.

2.2 NONMEM

2.2.1 Background

Shreiner et al. suggested as early as the 1970's to use nonlinear mixed-effect regression models to quantify inter- and intra-individual variability. The concept further developed into a computer program, NONMEM, which was released in the early 1980's by Lewis Shreiner and Stuart Beal. [2]

NONMEM is a computer program written in FORTRAN77, used together with two programs, PRED for population pharmacokinetics (PREDPP) and NONMEM translator (NM-TRAN). Besides being the oldest, NONMEM is probably the most widely used population analyze program today. NONMEM is validated and a well accepted program for PK/PD analysis and allows large flexibility in the building of models as well as in the data input.

NONMEM was the first modeling program designed to analyze large amounts of PK data using nonlinear mixed-effect modeling.

In the NONMEM program, linearization of the model in the random effects is effected by using the first-order (FO) Taylor series expansion with respect to the random effect variables η_i and ε_{ij}. NONMEM implements two alternative estimation methods; the Laplacian method which uses second-order expansions about conditional estimates of random effects, and the first-order conditional estimation

(FOCE) which uses Taylor series expansion method. FOCE uses an expansion about conditional estimates (empirical Bayesian estimates) of the inter-individual random effects rather than about zero. [17, 18]
FOCE is the most widely used approach in PPK, and is also applied in this thesis.

2.2.2 Modelling population PK using NONMEM

NONMEM requires two specific files for modelling. Both are created by the user and they are called the input- and control file. The input file is where the data are stored. They are often arranged as follows: the first column is the patient ID e.g. 1, 2, 3 etc, and in the next correlating columns are other PK data such as when the drug was delivered (time), drug amount given, concentrations measured etc. This is also where information about other variables (covariates) that might be relevant are included, for example creatinine clearance, weight, height, sex etc. [7]

The other file used by NONMEM is the control file. This file describes the structural model and states what NONMEM shall do with the input data. If it is to believe that the model has one or several compartments, zero, first or multiple order absorption or elimination etc. It contains the model and parameter specifications. [7]

Population modeling with NONMEM means that besides describing the PK parameters for the population, inter-individual and residual variability also needs to be described. The inter-individual variability (ε) in the PK-parameters *i* is described exponentially shown in equation 1 where θ is the individual j pharmacokinetic parameter.

Equation 1: $P_{ij} = \theta_j * \exp(\varepsilon_{ij})$

Residual variability can be described by a number of different models: additive models, proportional (CCV; Constant Coefficient of Variation) models, exponential models, power function model, and combined additive and proportional model (slope-intercept model).

The additive error model is described with the following equation:

Equation 2: $Y = \hat{Y} + \varepsilon 1$

The proportional error model is described with the following equation:

Equation 3: $Y = \hat{Y} \times (1 + \varepsilon 1)$

The combined model describes the residual variability with the following equation:

Equation 4: $Y = \hat{Y} \times (1 + \varepsilon 1) + \varepsilon 2$

Where $\hat{Y}$ is the predicted concentration, and the randomly distributed terms $\varepsilon 1$ and $\varepsilon 2$ have zero mean and variances $\sigma 1$ and $\sigma 2$.

Testing for covariates can be carried out using several methods. [7] Covariates are often divided into two groups; continuous- (weight, creatinine clearance, height, age etc.) and categorical variables (gender, diabetes, CYP-genotype etc.).

In this thesis the following methods were used: proportional, linear, power function, mean-centered model and if/else model:

Equation 5: Linear model: $TV_{pop} = \theta_p \pm \theta_1 \times covariate\ value$

Equation 6: Proportional model: $TV_{pop} = \theta_p \times / \div covariate\ \ value$

Equation 7: Power function: $TV_{pop} = \theta_p \times \theta_1{}^2 / \div covariate\ \ value$

Equation 8: Mean centered model:

$$TV_{pop} = \theta_p + \theta_1 \times (covariate\ \ value \pm mean\ covariate\ value)$$

Equation 9: IF/ELSE model:

IF (OBSERVATION.EQ.X) THEN

$$TV_{pop} = \theta_p + \theta_1$$

ELSE

$$TV_{pop} = \theta_p + \theta_2$$

ENDIF

2.2.3 How to find the best model using maximum likelihood approach

Parameter estimation in a model is often done with the maximum likelihood approach by minimizing the -2log likelihood (-2LL)-function:

Equation 10: $$-2\log(L) = n\log(2\pi) + \sum_{i=1}^{n}\left(\log\left(\sigma_i^2\right) + \frac{\left(Y_i - \hat{Y}_i\right)^2}{\sigma_i^2}\right)$$

Where Y is the measured observation, $\hat{Y}$ is the prediction of that observation by the model, and σ^2 is the variance of the model. The second part of the equation: $\sum_{i=1}^{n}\left(\log\left(\sigma_i^2\right) + \frac{\left(Y_i - \hat{Y}_i\right)^2}{\sigma_i^2}\right)$ is sometimes called the "extended least squares" objective function, and from this equation the objective function value (OFV) can be obtained. To maximize the likelihood -2LL has to be minimized. Since the first part $n\log(2\pi)$ is a constant focus has to be set on the last part of the equation.

The likelihood ratio test is a common test for statistical significance. It allows a possibility to compare two models that are nested with each other and one can test the significance of the parameter which differs between the two models. The difference between -2LL values follows a chi-square distribution, with the degrees of freedom being the difference in the number of parameters. With a probability of 0.05 and 1

degree of freedom the value of the chi-distribution is 3.84. Accordingly, if the difference in -2LL values (OFV) for two models that differ with 1 parameter exceeds 3.84, then the parameter is significant at $p<0.05$. [7]

It is important to remember that the model with the lowest OFV is not necessarily the best model. OFV differs from model to model and a comparison can not be justified when more than one/two parameters are changed at a time. Depending on the purpose of the model, several factors should be involved in deciding which model is better; run-time vs. visual plots vs. OFV etc.

3. CYCLOSPORIN A

3.1 History

Cyclosporin A is a small hydrophobic cyclic polypeptide of 11 amino acids, among them a characteristic unsaturated C-9 amino acid, with a molecular weight of 1202.6 dalton. CsA was first discovered through screening of lower fungus extracts. Active metabolites from the fungus *Cylindrocarpon lucidum* showed both mild antifungal activity and antibody depression in mice. [19, 20]

Oral administration in mice and rats showed a strong depression of the appearance of both direct and indirect plaque-forming cells and produced an obvious dose-dependent, yet reversible inhibition of haemagglutinin. Skin graft rejection in mice and graf-versus-host disease in mice and rats was considerably delayed by CsA. Soil samples collected in Norway in March of 1970 showed that the fungus *Tolypocladium inflatum* also contained CsA. This fungus was originally classified as *Trichoderma polysporurn*. In 1972 CsA proved to have powerful immunosuppressive properties. Since then much research has been performed on this drug. [19, 21]

3.2 Application and mechanism of action

Cyclosporin A was introduced to the market in the early 1980's and has since then been a cornerstone of solid organ transplant procedures. CsA led to for example an improvement in transplant kidney graft outcome, and made it possible to transplant hearts. [22-24] It has played a major part in the success of immunosuppression in the clinical setting since its introduction. [24]

CsA acts by forming a complex with the intracellular protein cyclophilin A, a protein localized in the cytoplasm of lymphocytes. This complex binds to and inhibits calcineurin that will ultimately lead to interference with activation of T-cells and production of interleukin-2. [20, 25-28]

CsA gives a better response to infection compared to other immunosuppressive agents because it suppresses T-cells partially, while it to some extent spares B-lymphocyte activity. [25, 26, 28]

3.3 Known problems with Cyclosporin A

When administrating immunosuppressive agents to patients it is important to obtain an optimal exposure of the drug. The most important reason for this is to prevent acute rejection, which secondarily will prolong the survival of the grafts and ultimately, the patients. Since CsA has a narrow therapeutic window it is challenging to keep the concentration levels within the therapeutic window.

Besides having a narrow therapeutic window CsA has a large inter-individual variability. This is especially visible after oral administration where observations show great variability. Below the therapeutic window there is a high risk of acute rejection, while concentrations above the therapeutic window are associated with minor and severe side effects such as anorexia, gastrointestinal disturbances, nephrotoxicity, infection, hepatotoxicity, dyslipidemia, hypertension and development of diabetes and cancer. [29-31]

There are also a wide range of drugs and other agents that interact with CsA pharmacokinetic which can cause a decrease or increase in concentration levels. All of these factors make it important to make a representative PPK-model to obtain an optimal treatment. [25, 26]

3.4 ADME OF CsA

3.4.1 Administration

CsA exists in two administration forms, infusion or orally. Oral administration can further be divided into capsules and mixture. [32]

The plasma peak concentration is obtained after 1-2 hours. The absorption profile is often characterized by a lag phase followed by rapid absorption. The site of absorption is predominately in the small intestine and due to its lipophility the absorption is dependent of bile flow, gut motility, food and time after transplantation. [33, 34]

Bioavailability of CsA normally ranges from 30-60 %. [32]

3.4.2 Distribution

Because CsA is highly lipophilic the distribution will to a large degree bind outside the blood circulation. Within whole blood CsA will distribute highly to erythrocytes 41-58%, plasma proteins 33-47%, granulocytes 5-12% lymphocytes 4-9%. In plasma approximately 90% is bound to plasma proteins, mainly lipo proteins. [32]

3.4.3 Metabolism

CsA has an extensive metabolism. It is metabolized in liver, small intestine and kidney to approximately 30 metabolites. The reactions involved in phase 1 metabolism are oxidation, hydroxylation and demethylation. [35, 36]

Cytochrome P450 system, in particular CYP3A4 and CYP3A5 are responsible for the Phase 1 biotransformation. CsA is also a substrate and inhibitor for the ATP-binding cassette transporter protein, P-glycoprotein (P-gp, mdr-1/ABCB1). [37, 38] CYP-enzymes and P-gp work together in hindering CsA to access the systemic blood circulation. Since both systems are present in a large degree in both intestines and liver CsA is subject to a large first pass metabolism and accordingly shows a low oral bioavailability.

Patients with geno typical differences in CYP3A protein expression will therefore have large variations in CsA PK. [35, 38-40]

3.4.4 Elimination

CsA is mainly eliminated through the biliary system. 6% of the oral dose is eliminated renally while less than 1% excretes unchanged through the urine. [32]

Depending on the population and method used, half life varies to a great extent. Half life varies from 6 hours for healthy volunteers to 20 hours for patients with severe liver complications. [32]

3.5 Therapeutic drug monitoring

Due to the complex reasons for variability of CsA it is subject for therapeutic drug monitoring (TDM) to maximize the effect of the immunosuppressive therapy.

The parameter most closely linked to the therapeutic effect and the toxic effect is thought to be the area under the whole blood versus time curve from 0 to 12 hours ($AUC_{0\text{-}12}$). This way of measuring drug exposure is both time consuming and expensive and is rarely done. An often used method is measuring concentrations at trough level (C_0), and/or 2 hours after dosing (C_2) which is considered a better marker for toxic effects. [41, 42]

Studies have shown that there are valid arguments for monitoring intra-lymphocytic CsA trough levels ($C_{0\text{-intracellular}}$). Since CsA's effect is initiated by its binding to its lymphocyte receptor, a measurement here would be "at the site of action" and a more advantageous way to monitor. [29, 30, 43, 44]

The super CsA-study showed that by measuring the intracellular concentration, one may potential to detect acute rejection several days earlier than possible with traditional methods, [45] making it an attractive option to monitor CsA-concentrations inside the T-lymphocytes as well as in whole blood concentrations. The intracellular concentration appears to provide information about processes important to rejection which whole blood concentrations do not provide. CsA whole blood concentrations actually tend to be slightly higher for the rejection patients

during that study and did not correlate with the intracellular concentrations that were declining days before rejection. [45]

Developing a successful PPK-model for CsA can prove useful. General dosing regiments today is based on the physicians experience and knowledge. With a PPK-model it will hopefully be possible to give more correct doses to each individual at an earlier time.

3.6 Population kinetic models of CsA in literature

Through the history there have been many attempts to model the PK of CsA. Different attempts have resulted in different conclusions. Both 1- and 2- and 3-compartments have been used and different Erlang distribution and absorption lag-time have given a good fit. This also applies to covariates where a wide range has been found significant. [38, 46-51]

3.7 Goals of the thesis

The objective of this thesis is to include a T-lymphocyte compartment to the whole blood model and continue develop the previous CsA model made by Truc van Le [48] and Live Storhagen [49] by including 20 more patients, followed for at least 8 weeks, re-evaluate for covariates and test the model for interoccasional variation which has not yet been tested.

4. METHODS AND MATERIALS

4.1 Materials for the whole blood model

The whole blood model consists of totally 70 patients from 4 different studies. [45, 52-54]

There are differences in the amount of information gathered from each study. The medical records included information about date, time, CsA dosage, CsA concentration, gender, weight, serum creatinine, urea, current co-medication and transplantation date. A full PK population design was used to allow blood samples to be drawn at different times. [5] A total number of 1276 measured drug concentrations were used in the model development.

All patients received renal transplantation at Rikshospitalet University hospital HF, Oslo, Norway. CsA (Sandimmun Neoral®, Novartis Pharmaceuticals Corporation, Switzerland) was administered orally twice daily in soft gelatin capsule formulation, along side other routine protocol medication.

Patients 1-5, 8-11, 16-18, 20-22, 24-25, 31, 34 and 38 were from the POPDOC study. [54]

Patient 101-120 were from the super-CsA study. [45] This was a single prospective pilot study following patients from 0-17 weeks after transplantation, with measurements made sporadically at trough level C_0 and C_2 (2 hours after CsA administration). Nine of these 20 patients had a 12-hours pharmacokinetic profile done once in this period. [45]

Patient 130-137 originated from the MIMPARA-study [53] which was an interaction study between Cinacalcet and immunosuppressive drugs. Only CsA data from before Cinacalcet was administrated was used in this model. [53]

The remaining 21 patients (151-165, 167-172) were from a CsA study performed to screen for possible age effect on PK of CsA. [52]

Whole blood samples drawn specifically for the clinical trials were analyzed for CsA concentrations using a validated LC-MS/MS method [55], while routine clinical follow up samples were analyzed using Cedia Cyclosporine PLUS Assay (CEDIA+) (Cloned Enzyme Donor ImmunoAssay; Microgenetic Corporation, Fremont, CA) method at the clinical chemistry department at Rikshospitalet. All blood concentrations used in the development of the model were transformed to CEDIA+ equivalent concentrations. [55]

Whole blood samples for the 12-hours PK-profiling were analyzed at both the study center, Rikshospitalet University Hospital HF and by the Department of Pharmaceutical Biosciences, University in Oslo, while whole blood samples taken sporadically were analyzed by Rikshospitalet University Hospital HF. Analysis results showed that there was significant inter-laboratory variability. This may be the result of the different analysis methods. All CsA concentrations analyzed by the Department of Pharmaceutical Biosciences were therefore adjusted to the correct concentration, as defined by Rikshospitalet University Hospital HF, with the following equation:

Equation 11: $RH=DPB \times 0.88$

Where *RH* is the adjusted concentration according to Rikshospitalet University Hospital HF, and *DPB* is the concentration obtained from analysis performed by the Department of Pharmaceutical Biosciences. This equation was obtained from correlation of concentrations measured at both laboratories in the three studies. [48]

As data was gathered at various times, it was no missing data points so to speak. In the NONMEM input file C_0 levels was computed for morning doses at 06.00 hours and at 20.00 hours for evening doses while C_2 levels was coded at 08.00 hours and at 22.00 hours for evening doses.

Table 1. Patient demographics whole blood		
		Range
Number of patients	70	
Number of male/female patients	47/23	
Age (years)	56.4	21-78.6
Weight (kg)	79.7	49-124
Height (m)	1.77	1.53-1.92
Body mass index (kg/m^2) *[1]	25.3	16.7-34.3
Lean body mass (kg) **[2]	53.8	75.6
Gender male	47	
Gender female	23	
CYP 3A5 genotype;		
*1/*3	9	
*3/*3	61	
Time after transplantation (weeks)	5.6	1.0-17.0
Estimated creatinine clearance (ml/min) ***[3]	70.8	18.3-162.5
Cyclosporine A		
Observed whole blood concentrations (ng/mL)	937.1	30-3240
Total number of samples	1276	
Average number of samples per patients	18	

*[1] Estimated using BMI-formula, **[2] estimated using LBM-formula, ***[3]estimated using Cockgroft-Gault equation (Formulas found in Appendix 9.1)

4.2 Materials for the whole blood model and intracellular concentrations

Data for the combined whole blood and intracellular concentration was based on the patients from the super-CsA study. [45] From the same patients 20 patients there was also obtained intracellular concentrations. From these 20 patients, nine patients had a 12-hour PK-profile done once in the study period.

The intracellular samples were measured in T-lymphocytes. T-lymphocytes were isolated from 7 ml whole blood using Prepacyte (BioE, St. Paul, MN). CsA concentrations were measured in freshly isolated T-lymphocytes using a validated liquid chromatography (LC) double mass spectrometry (MS/MS) method. The intracellular levels of CsA were then related to the number of T-lymphocytes (ng/10^6 cells). [45]

The data was computed similar to the whole blood model where C_0-levels was coded at 06.00 hours for morning doses, and at 20.00 hours for evening doses, while C_2-doses was coded at 08.00 hours for morning doses and at 22.00 hours for evening doses.

Table 2. Patient demographics whole blood and intracellular model		
		Range
Number of patients	20	
Number of male/female patients	13/7	
Age (years)	53.6	21-74
Weight (kg)	77.9	58.5-100.5
Height (m)	1.78	1.65-1.88
Body mass index (kg/m^2) *[1]	24.7	19.3-32.9
Lean body mass (kg) **[2]	55.5	46.3-66.6
Gender male	13	
Gender female	7	
CYP 3A5 genotype;		
*1/*3	2	
*3/*3	18	
Time after transplantation (weeks)	6	1.0-17.0
Estimated creatinine clearance (ml/min) ***[3]	76	18.3-162.5
Cyclosporine A		
Observed whole blood concentrations LN (ng/mL)	6.76	3.4-8.1
Observed intracellular concentrations LN (ng/mL)	10	6.5-13.6
Total number of whole blood samples	510	
Total number of intracellular samples	420	
Average number of samples per patients	52	

*[1] Estimated using BMI-formula, **[2]estimated using LBM-formula, ***[3]estimated using Cockgroft-Gault equation. (Formulas found in Appendix 9.1)

4.3 Developing and building the models

All computations were done using NONMEM (version VI; GloboMax LLC, Hanover, MD, USA). Graphical diagnostics plots were obtained from the program R (http://www.r-project.org) and in some situations drawn using Microsoft® Office Excel 2003 (USA) and Minitab® Statistical Software version 15.1.20.0 (State College, Pennsylvania, USA).

4.3.1 The whole blood model

For the whole blood model, there was no model development process. The model had already been developed and undergoing clinical testing in the POPDOC-study when this thesis was begun. The model was tested and validated to being a 2-compartment model with lagtime. Significant covariates had already been identified, but these were now re-validated with more patients using forward inclusion and backwards deletion process.

Testing for interoccasional variability required a new column to be added to the data set. This column identifies the different visits each patient had when samples were taken. IOV was later coded in the control file by using the separate visits to equal different etas. The BLOCK(1) option was also included. (Appendix 9.3)

It was made several attempts of modeling IOV into the model. The first attempt was made by marking each date with a measured sample as different visits and tested on one parameter at the time. The number of visits ranged from 22-46 for the different patients.

NONMEM had problems with too many etas and NM-TRAN gave an error statement when too many visits were tried estimated, accordingly it was only possible to code the first 11 visits.

To avoid this problem every second dates (with measurements) was marked as a different visit. For example, the first two dates with samples were marked “visit 1” and the third and fourth samples marked “visit 2” and so on. This was done to keep the time perspective of the samples. IOV was then tested at one parameter at the time.

4.3.2 The whole blood and intracellular model

Developing a model with both whole blood and intracellular concentrations was a time consuming and demanding task.

The first process step was to include the intracellular concentrations to the data set including the whole blood. Then the different concentrations had to be separated using dependent variable identification (DVID). The data are divided by EVID/DVID, where DVID = 1 is whole blood concentrations, DVID = 2 is intracellular concentrations. EVID = 0 is no observation, EVID = 1 is whole blood observation and EVID = 2 is intracellular observations. Corresponding IPRED = 1 is whole blood individual prediction and IPRED = 2 is individual intracellular concentration predictions.

Building on the previous model the idea was to add another compartment which was the intracellular compartment (Figure 2). Clearance (CL) and volume (V) was parameterized and inter-compartmental rate constants were estimated by CL and V. (Appendix 9.6)

A $DES code was added to the control file to describe the absorption and elimination profiles for the different compartments. Previous studies on the same subjects indicated that the absorption process in the intracellular compartment was following a 1.order reaction.

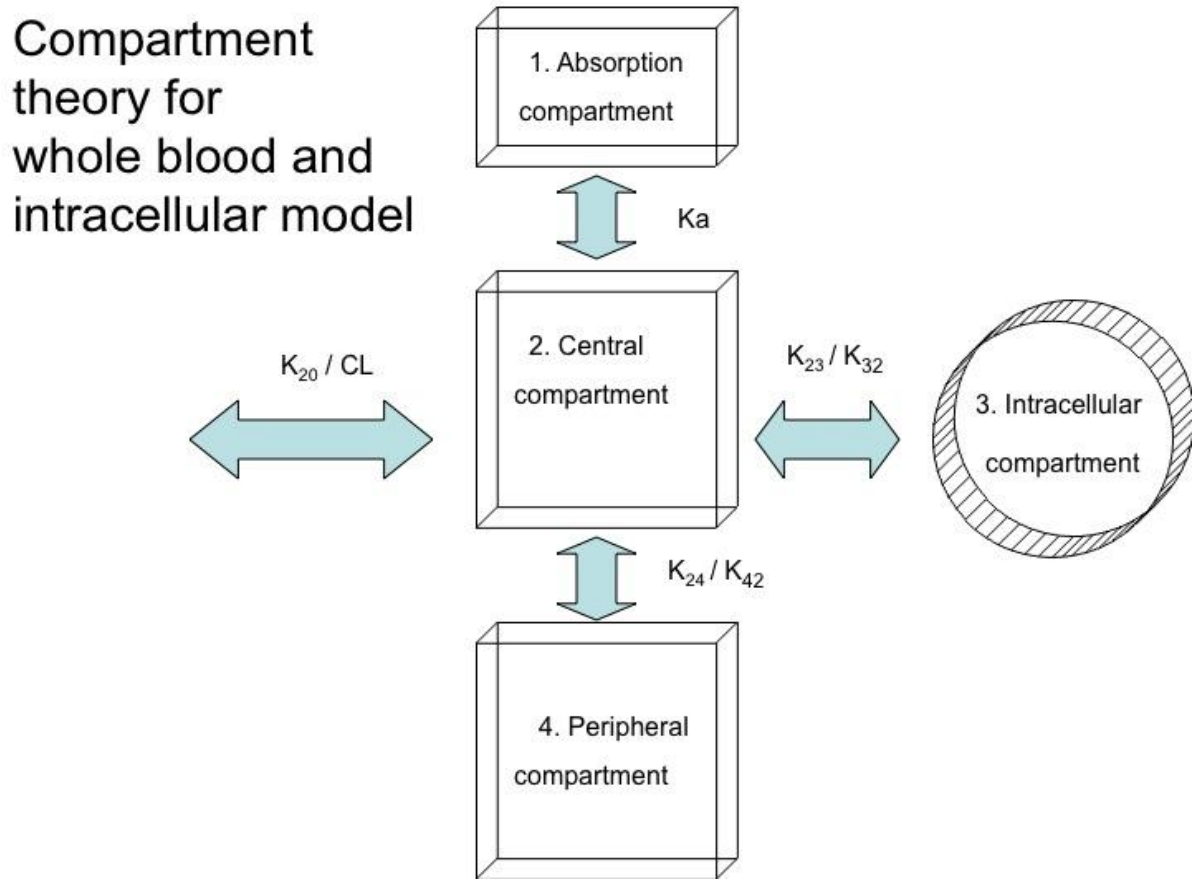

Figure 2: Compartment theory for the model including whole blood and intracellular concentrations. A 4-compartment model where the drug absorbs from the absorption compartment (1) into the central compartment (2) and from there, it is distributed into the peripheral compartment (4) and eliminated. The intracellular concentrations are represented in the intracellular compartment (3) where there is equilibrium with the central compartment (2).

The whole blood concentrations were measured in ng/ml while the intracellular concentrations were measured in ng/10^6 cells.

In an attempt to convert the intracellular concentrations to the same unit, an estimate of a T-cell volume had to be made. The T-cell's diameter was estimated to $8*10^{-6}$ m in diameter and the volume was estimated to have a spherical shape.

Diameter: 8 μm

Equation 11: Volume = $(4/3) * \pi * r^3$

Similar approaches were tested to the converted concentrations without significant luck. There were signs that NONMEM was able to predict that the observations was independent, but because of the very large concentration differences (intracellular ≈ 80000ng/ml vs. whole blood ≈ 3000ng/ml) NONMEM was not capable of reaching concentrations that were high enough.

As a result of this the data were LN-transformed and a necessary new residual error-code was included in the model. It was checked against both only proportional error and additive error, and the combined proportional and additive error.

5. RESULTS

5.1 Re-analyzing for covariates for the whole blood model

The fixed effects parameters estimated for the final 2-compartment model were CL/F (Θ1), V1/F (Θ2), Q/F (Θ3), V2/F (Θ4), Ka (Θ5), and ALAG (Θ6).

In the screening process all covariates (table 3) were tested individually on each parameter. All the positive covariates were then double checked for significance in the second screening (table 4). All the covariates found significant were further included in the model (table 4) before the backwards deletion process (table 5).

Table 3. Covariates tested

Lean body mass (LBM)	CYP P450 3A5 (3A5)	Steroid dose (STER)
Body mass index (BMI)	Height (HGT)	Gender
Post transplantation TXT)	Age (AGE)	Diabetes
Creatinine Clearance (CRCL)	Weight (WT)	

Table 4. Covariates found significant during forward inclusion for the whole blood model

Parameter	Covariate	Model	OFV	ΔOFV	P
CL/F	CRCL	Θ1-Θ7*CRCL	14723.41	-10.7	>0.01
	LBM	Θ1-Θ7*(LBM-53.8)	14702.31	-31.8	>0.01
	C3A5	IF (C3A5.EQ.1) THEN Θ1*Θ7 ELSE Θ1*Θ8 ENDIF	14722.24	-11.9	>0.01
	BMI	Θ1-Θ7*BMI	14724.21	-9.88	>0.01
	AGE	Θ1-Θ7*AGE/56	14722.79	-11.3	>0.01
V1/F	AGE	Θ2/Θ7*AGE	14712.37	-21.7	>0.01
	TXT	Θ2*Θ7*(TXT/5)	14702.99	-31.1	>0.01
	BMI	Θ2*Θ7*BMI	14699.29	-34.8	>0.01
Q	AGE	Θ3-Θ7*AGE	14721.85	-12.2	>0.01
	WT	Θ3*Θ7*WT	14710.95	-23.1	>0.01
	CRCL	Θ3+Θ7*CRCL	14709.53	-24.6	>0.01
	STER	Θ3-Θ7*(1+STER/100)	14718.7	-15.4	>0.01
V2	AGE	Θ4+Θ7*(AGE-56)	14719.72	-14.4	>0.01
	CRCL	Θ4-Θ7*CRCL	14721.24	-12.9	>0.01
	LBM	Θ4+Θ7*(LBM-53.8)	14723.34	-10.8	>0.01
	STER	Θ4-Θ7*(1+STER/100)	14729.07	-5.02	>0.01

Ka	AGE	Θ5-Θ7*AGE	14710.01	-24.1	>0.01
	CRCL	Θ5*Θ7*CRCL	14711.12	-23	>0.01
	STER	Θ5-Θ7*(1+STER/100)	14724.99	-9.1	>0.01
	BMI	Θ5-Θ7*BMI	14700.16	-33.9	>0.01
	WT	Θ5-Θ7*(WT-78.5)	14717.26	-16.8	>0.01
	TXT	Θ5*Θ7**TXT/5	14686.4	-42.8	>0.01
ALAG	AGE	Θ6+Θ7*AGE	14719.06	-15	>0.01

From the early screening process many of the same covariates were found as expected.

When all covariates from the forward-inclusion were added the OFV was 14597.34. After the backwards deletion step, the following covariates were left in the model (table 5).

Table 5. Covariates found significant after backwards deletion for whole blood model

Parameter	Covariate	Model	OFV	ΔOFV	P
CL	CRCL	Θ1-Θ7*CRCL	14642.19	44.85	>0.01
	C3A5	IF (C3A5.EQ.1) THEN Θ1*Θ7 ELSE Θ1*Θ8 ENDIF	14852.22	254.88	>0.01
	AGE	Θ1-Θ7*AGE/56	14614.15	16.81	>0.01
V1/F	AGE	Θ2/Θ7*AGE	14712.37	14.97	>0.01
	BMI	Θ2*Θ7*BMI	NA	NA	NA
Q	WT	Θ3*Θ7*WT	14710.95	18.24	>0.01
	STER	Θ3-Θ7*(1+STER/100)	NA	NA	NA
Ka	TXT/5	Θ5*Θ7*TXT**5	14624.7	27.36	>0.01
	BMI	Θ5-Θ7*BMI	NA	NA	NA
	AGE	Θ5+Θ7*AGE	NA	NA	NA

OFV in the start model was 14734.09 and dropped to 14597.34 when all the significant covariates were added. After the backwards deletion the final model had an OFV of 14643.28 which is a significant improvement.

When comparing the final model to the first model several similarities of significant covariates were found. Both Truc's model and the model used in the POPDOC-study found these covariates to be significant in their models.

5.2 Testing for interoccacional variability

By looking at the data there is reason to suspect interoccasional variability (IOV). After the alteration of visits (every other visits where coded as different visits) there was a change in OFV for V_2.

Table 6. OFV change after inclusion of interoccacional variability on the different parameters

	OFV	ΔOFV
CL	14696.27	52.99
V1	14665.4	22.12
Q	14687.8	44.52
V2	14612.72	-30.56
Ka	14723.22	79.94
ALAG	NA	NA

Inclusion of OFV on the parameter V_2 gave a significant reduction of OFV. Remembering the OFV with covariates to be 14643.28, there was an OFV-change of 30.56 which makes the model with IOV significant better than the model with covariates and the model without covariates.

5.3 Covariate analysis based on visual prediction

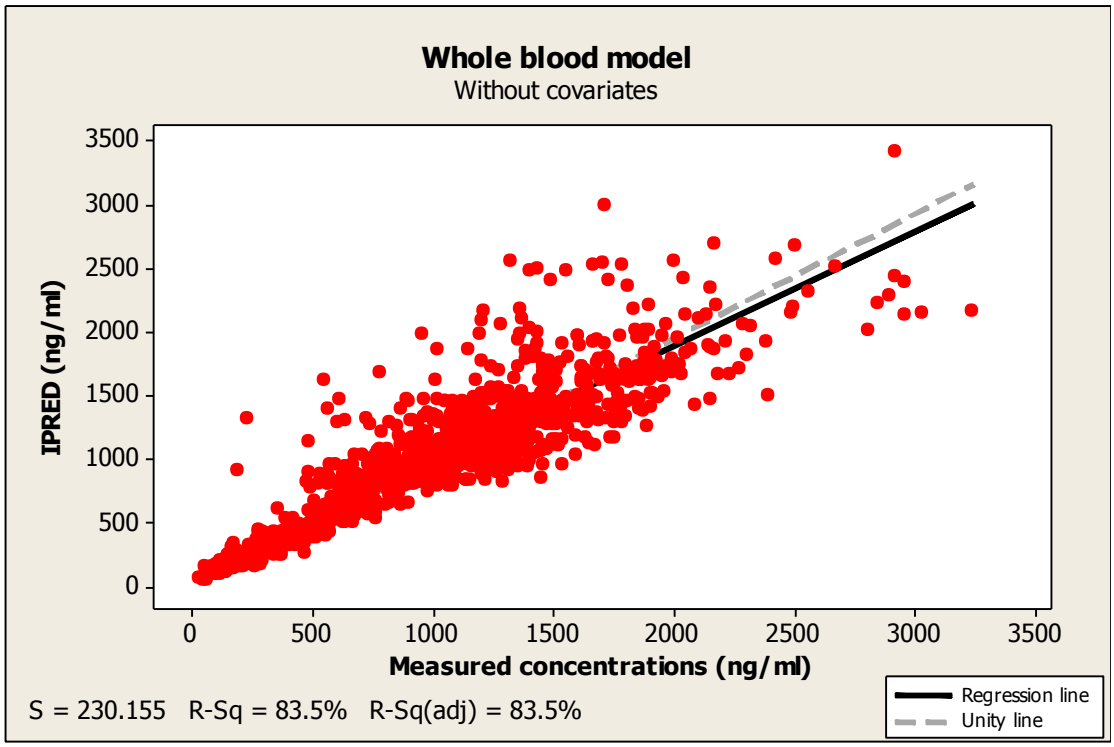

Figure 3. Scatterplot for whole blood model without covariates - IPRED versus Concentration

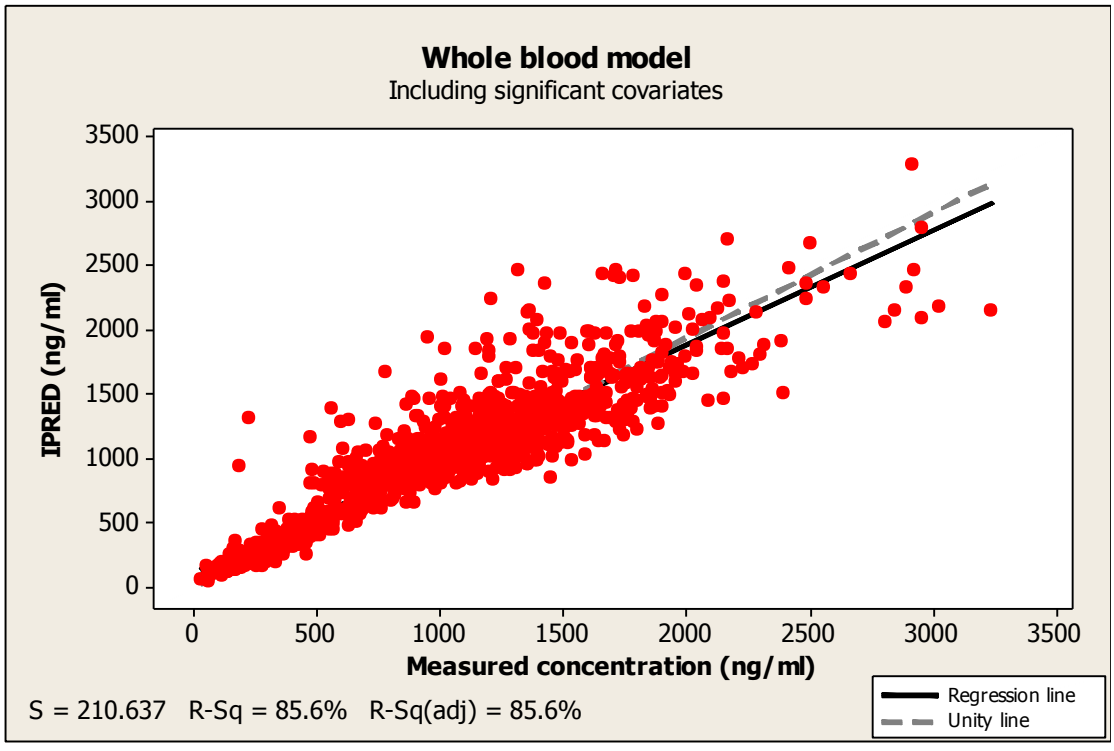

Figure 4. Scatterplot for whole blood model with significant covariates - IPRED versus Concentration

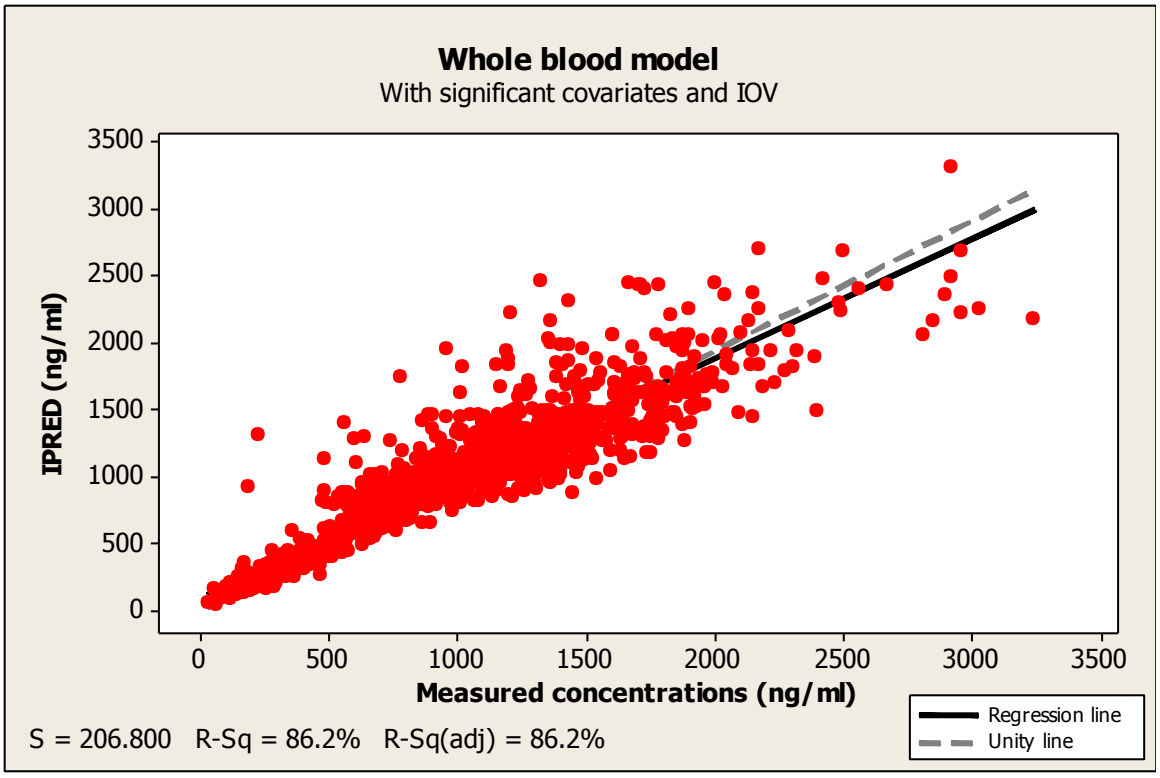

Figure 5. Scatterplot for whole blood model with significant covariates and interoccasional variability - IPRED versus Concentration

The first three scatterplots (figure 3-5) show the development from the model without covariates to the inclusion of significant covariates and finally with significant covariates and interoccasional variability. There is a gradually improvement as seen earlier by the decrease of OFV. The increasing R^2 together with the decrease of S show that there is a better fit and the regression line shows an improving description of the data.

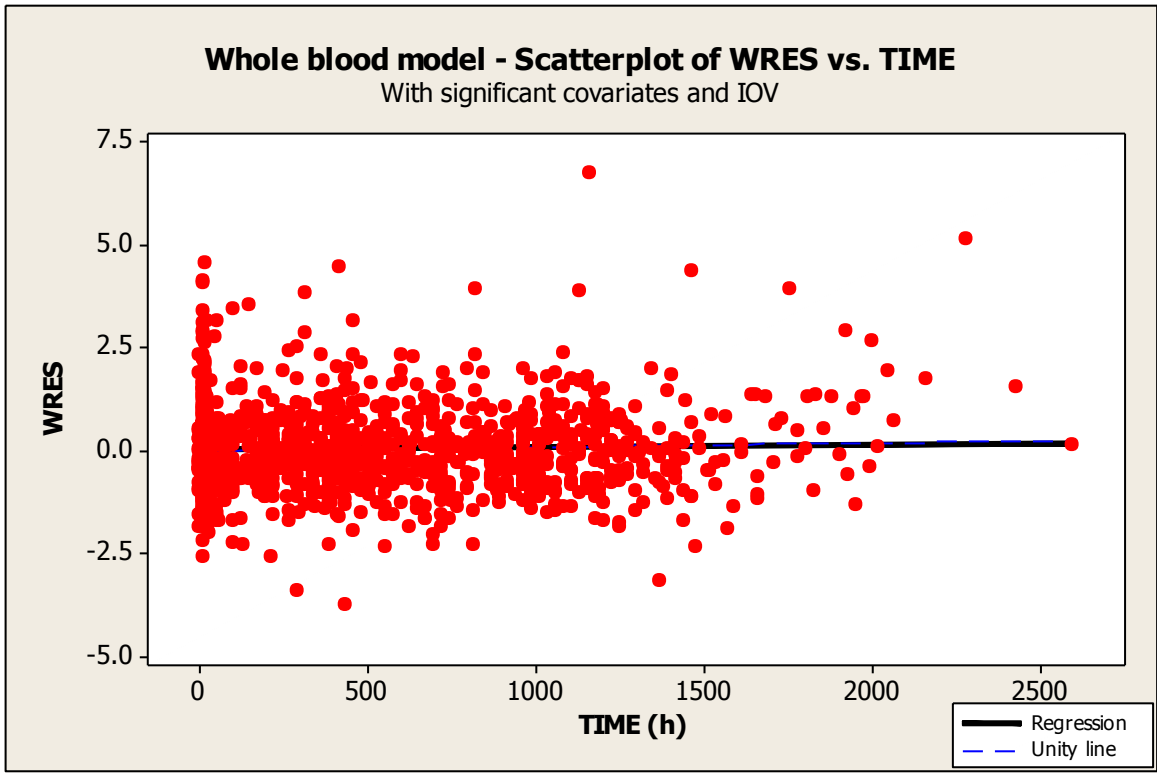

Figure 6. Whole blood model with significant covariates and IOV. Scatterplot of Wres vs. time.

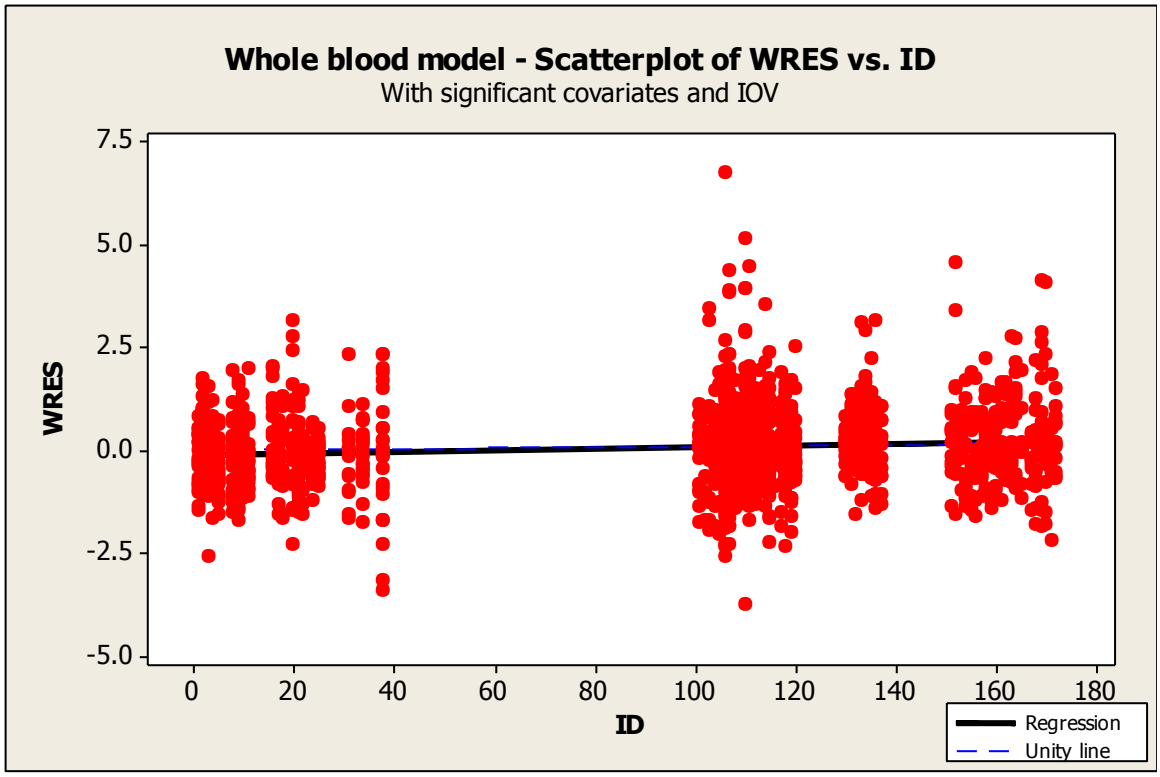

Figure 7. Whole blood model with significant covariates and IOV. Scatterplot of Wres vs. ID.

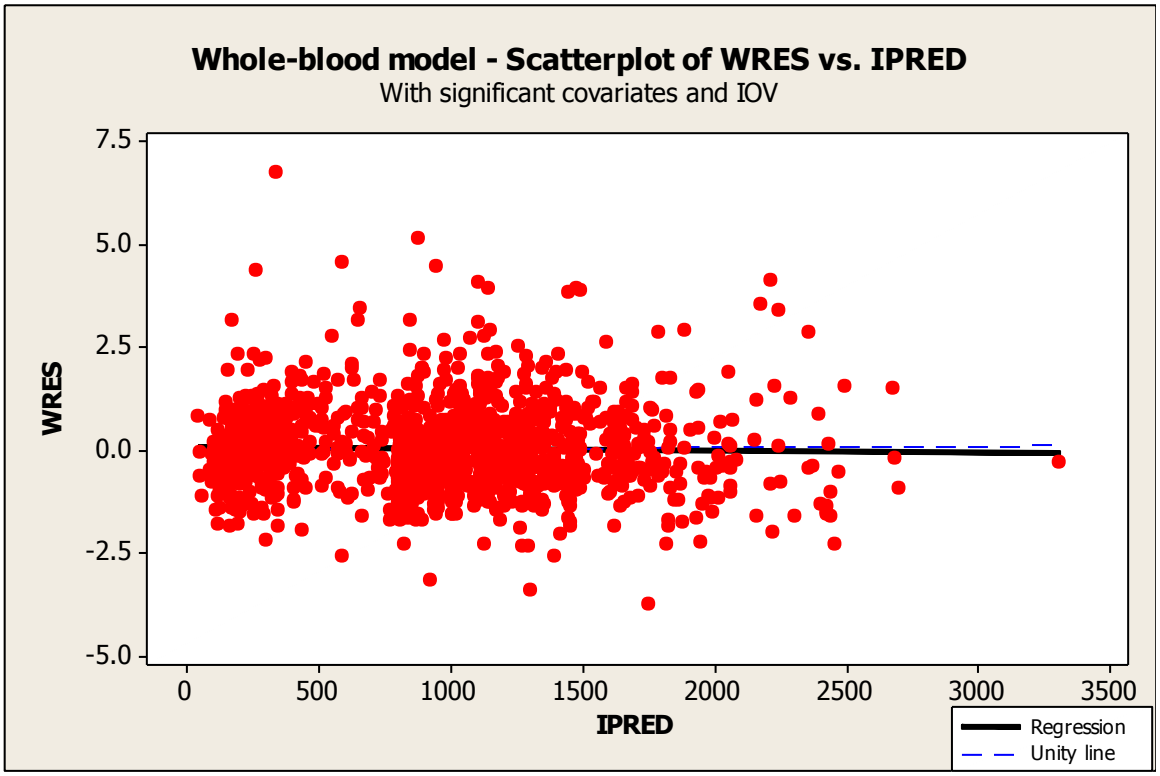

Figure 8. Whole blood model with covariates and IOV. Scatterplot Wres vs. ipred.

Figure 6-8 shows the final model with significant covariates and IOV, WRES versus time, ID and population prediction (PRED). The weighted residuals (WRES) are evenly distributed with time and identification (ID). WRES versus population prediction (PRED) shows that the weighted residuals are tending towards the negative side for the large concentration predictions implying that there is a small over-prediction for the large concentrations.

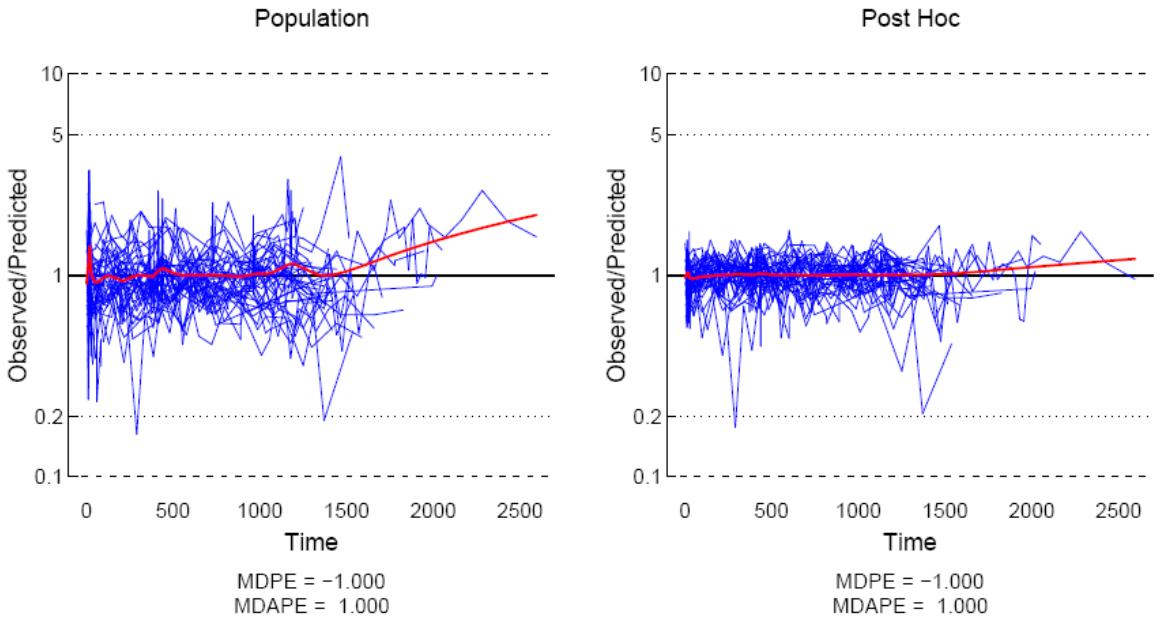

Figure 9. Quality of fit plot, whole blood model with all significant covariates and IOV

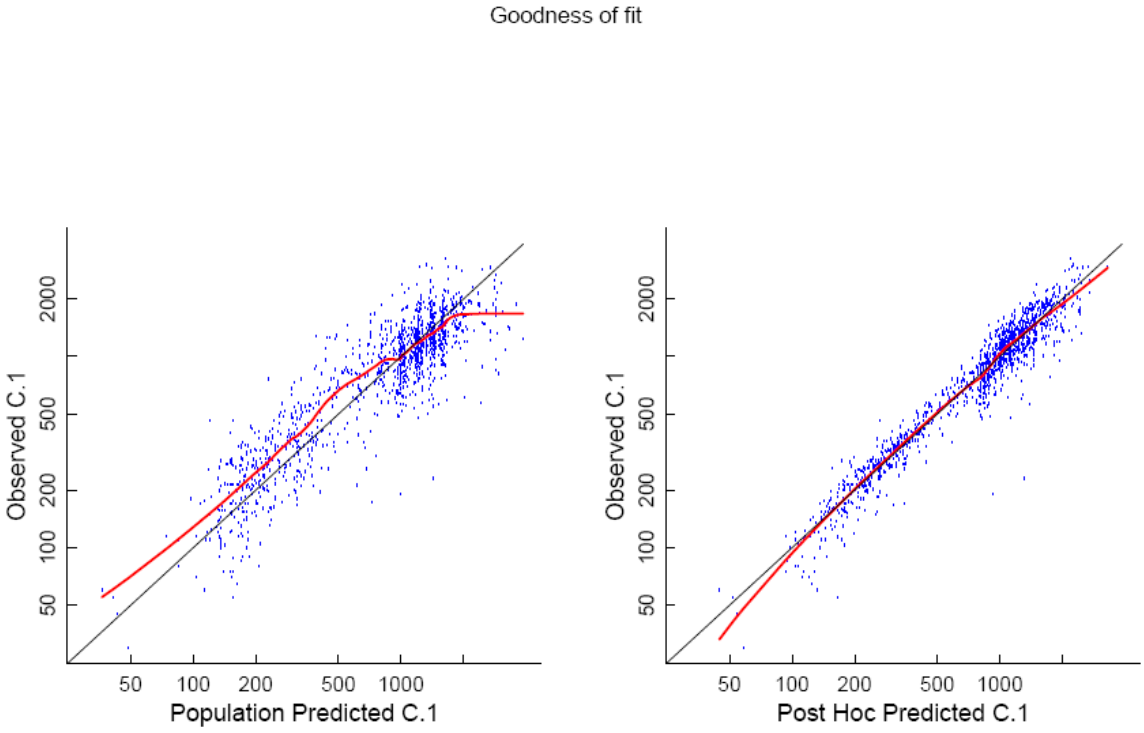

Figure 10. Goodness of fit, whole blood model with all covariates and IOV

The remaining figures (9-10) are parts of the diagnostics plots drawn by R-script of the final model. The figures show that POSTHOC gives an overall good prediction

for the final model. It also shows that NONMEM has small problems fitting the concentrations on the highest and lowest end of the scale. Overall there is a good prediction with an even spread around the regression line.

5.5 Comparing the old versus the new model

Table 7. Comparison of significant covariates from old model versus new model

Previous covariates*		Current covariates	
Parameter	Covariates	Parameter	Covariates
CL	Age	CL	Age CRCL Cyp3A5
V1	Age Weight	V1	Age BMI
KA	TXT Age Weight	KA	TXT Age BMI
		Q	Weight Steroid dose

*The last version of the model, the model used in the POPDOC-study.

Comparing the new model with the old model shows several similarities. Both models are pretty accurate for the low to normal levels of CsA, but have some difficulties predicting concentrations on the higher level of the scale. The covariates found significant are majorly the same (table 7). Furthermore the scatter-plot of weighted residuals are evenly distributed which is acceptable. The new model has IOV included, which is expected, and this factor may be decisive of small, but significant improvement of the model.

5.6 The whole blood and intracellular concentrations

5.6.1 Model building results

The data were LN-transformed and the necessary new error code was included in the model. Using a proportional and an additive error code for the inter-individual variability gave the best fit and lowest OFV.

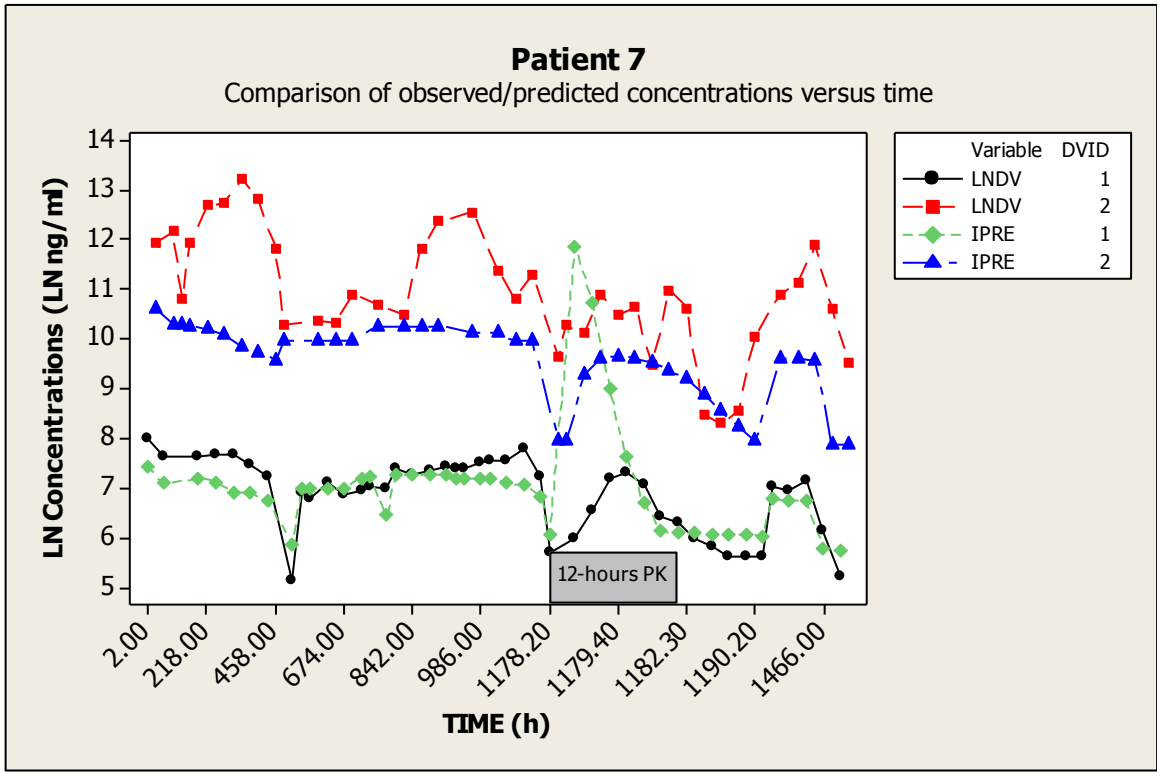

Figure 11. Patient 7, concentration vs. time. LNDV = 1 for whole blood concentrations, LNDV = 2 for intracellular concentrations and the corresponding IPRED = 1 for individual whole blood predictions and IPRED = 2 for individual intracellular predictions. The y-scale (concentrations) is presented on LN-scale while the x-scale is time (h). The time units are not homogenous, but rather time measured when the different samples were taken. The graph is showing C_0, C_2 and 12-hours profile data. The marked area represents the 12-hour profiles and enlarged in the figure below is the 12-hours profile for the same patient

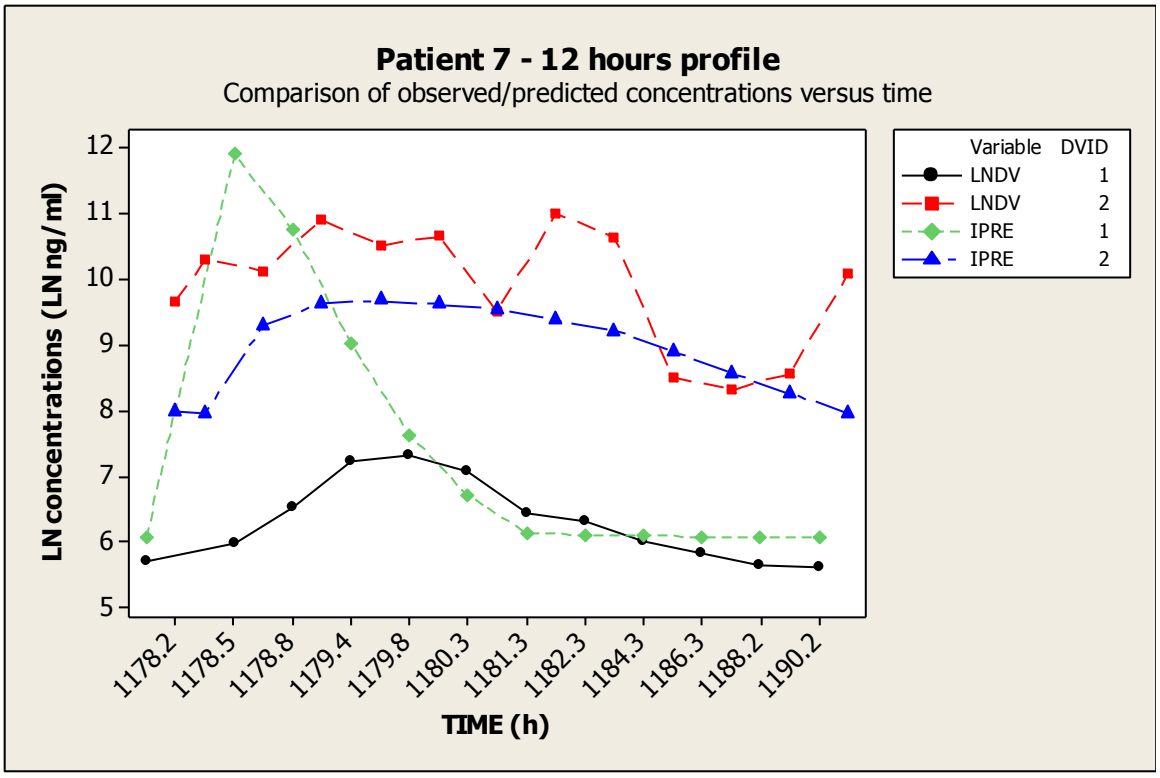

Figure 12. Patient 7 – *12-hours PK-profile*. For detailed description see Figure 11

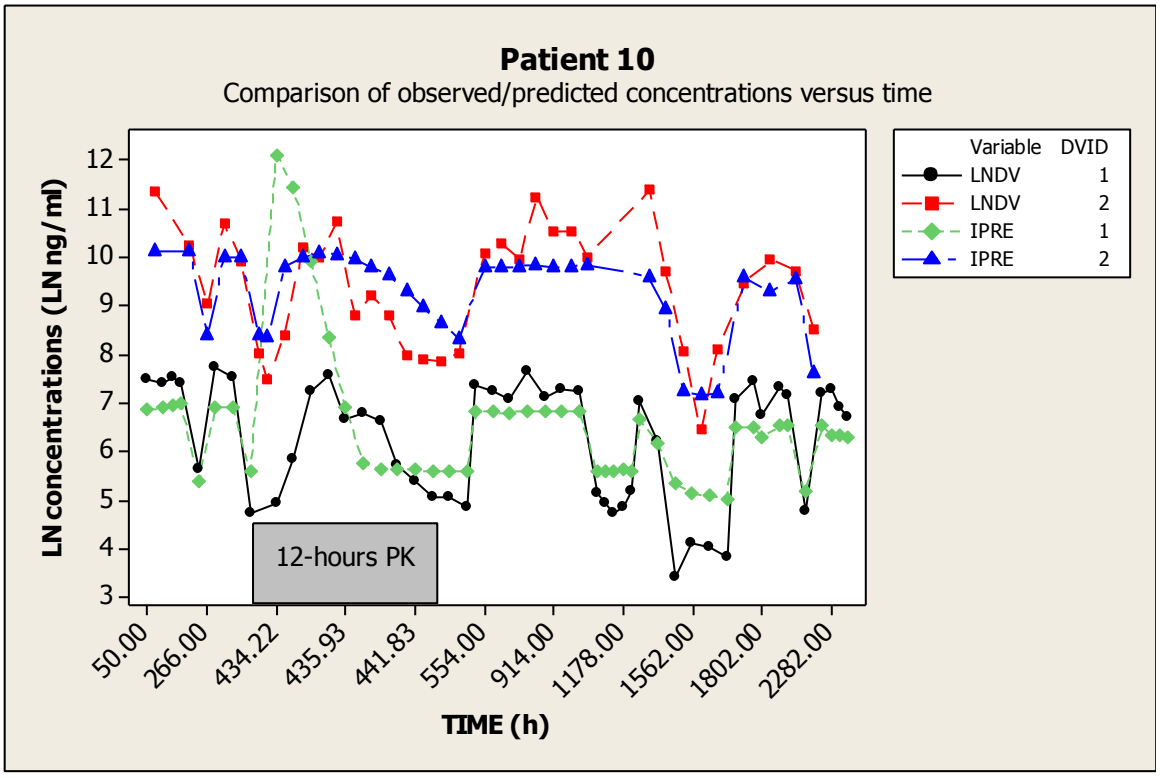

Figure 13. Patient 10 – concentrations vs. time. For detailed description see Figure 11

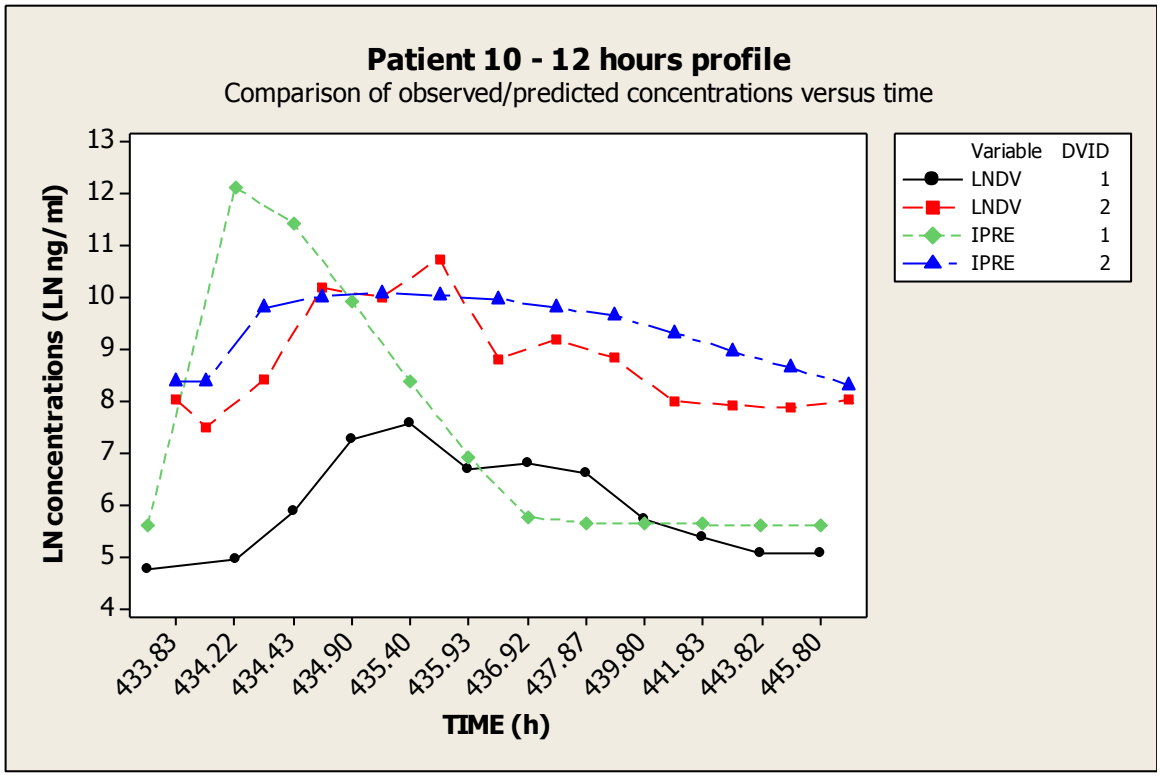

Figure 14. Patient 10 – *12-hours PK profile*. For detailed description see Figure 11

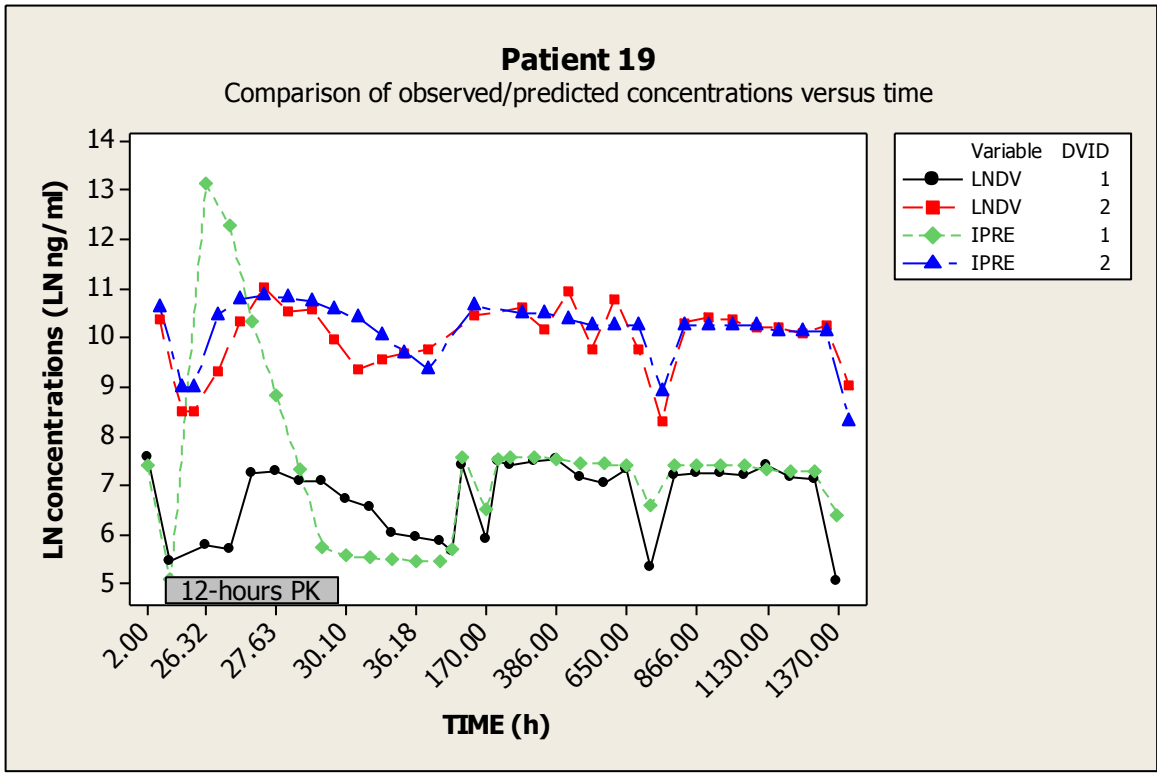

Figure 15. Patient 19 – concentrations vs. time. For detailed description see Figure 11.

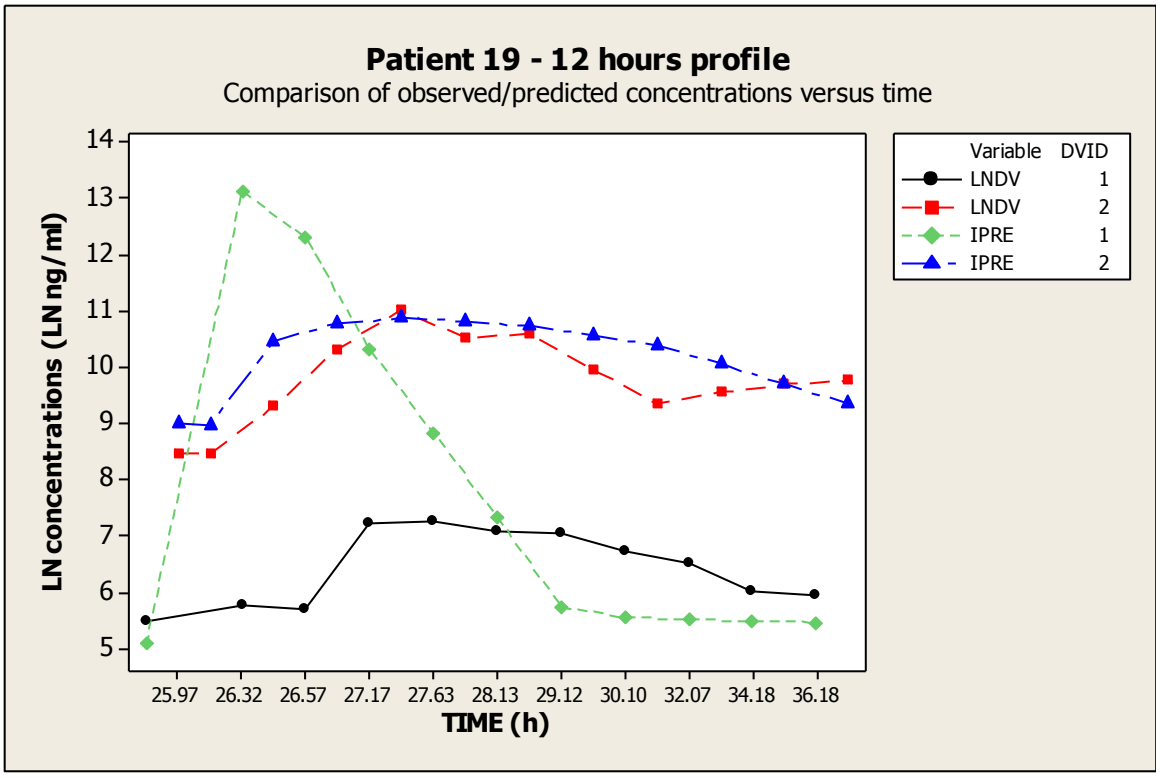

Figure 16. Patient 19 – 12-hours PK profile. For detailed description see Figure 11.

According to the graphical comparisons the whole blood and intracellular model is able to tell the difference between the different observations. It seems as if it predicts C_2-levels better the trough concentrations. The model is to some degree able to predict the large fluctuations of the observed concentrations. (Figure 11, 13 and 15) The 12-hours profile is however rather inaccurate. (Figure 12, 14 and 16)

The absorption phase is wrongly estimated both for the whole blood and the intracellular concentrations (Figure 12, 14, 16-19). The elimination phase is a little over-predicted for the whole blood concentrations, and it does not match the intracellular concentrations too well either.

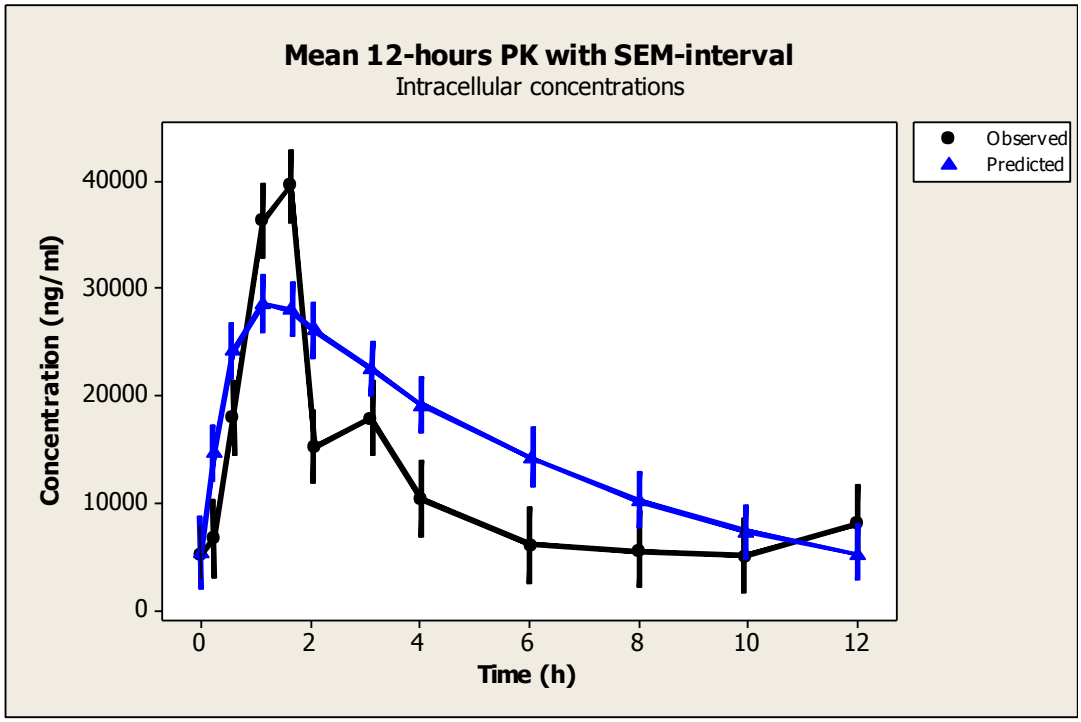

Figure 17. Mean intracellular 12-hours profile, 12-hour profile for the mean observed and predicted intracellular concentrations shown on a normal-scale versus a normal time-scale (h). The SEM-interval is represented at each measurement.

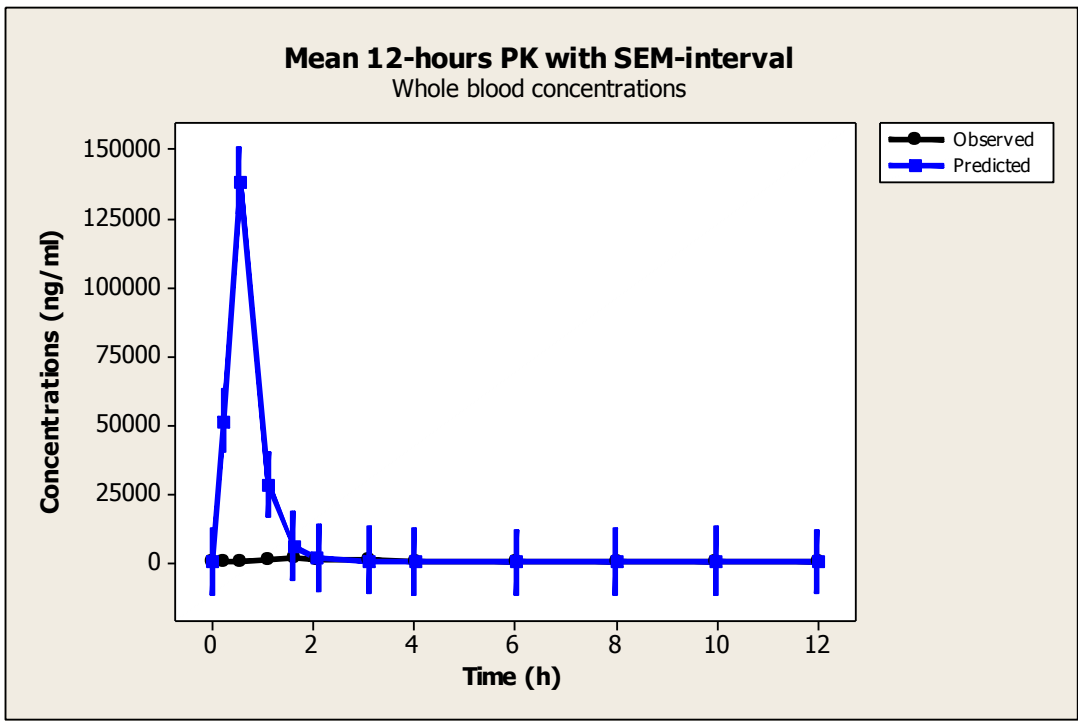

Figure 18. Mean whole blood concentrations 12-hours profile. 12-hour profile for the mean observed and predicted whole blood concentrations shown on a normal-scale versus a normal time-scale (h). The variations on the predicted concentrations are represented by the SEM-interval.

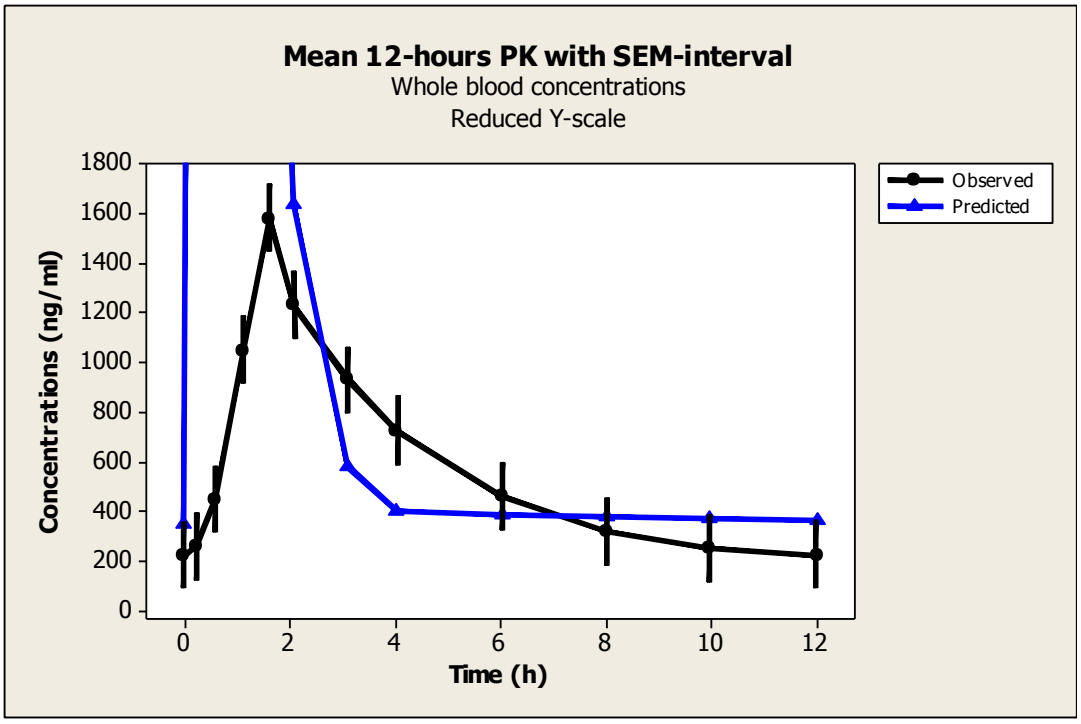

Figure 19. Mean whole blood concentrations 12-hours, DVID = 1 = Whole blood. 12-hour profile for the mean observed and predicted whole blood concentrations shown on a reduced normal-scale versus a normal time-scale (h). The variations on the observed concentrations are represented by the SEM-interval.

Figure 17-19 are mean whole blood- and intracellular 12-hours PK profiles for the 9 patients. The figures confirm the earlier findings that the absorption phase is wrongly estimated for both whole blood and intracellular measurements. The absorption phase for whole blood predictions is too fast with a lower T_{max} and a very increased C_{max}, but the elimination phase for the predicted is in the same ballpark-area as the observed. For the intracellular 12-hours PK the absorption phase is very similar for both the predicted and observed concentrations, with the predicted C_{max} a little lower than the observed and the T_{max} a little earlier. The elimination phase is unfortunately not similar for the predicted and observed concentrations.

The standard error of the mean (SEM) is represented as the interval for both the observed (SEM = 3448.31) and the predicted concentrations (SEM = 2543.99) for intracellular concentrations (figure 17). Regarding the whole blood measurements $SEM_{predicted}$ is very large (SEM = 11749.34) because of C_{max}. Therefore SEM for predicted whole blood concentrations are presented in figure 18 with a full Y-scale,

while SEM for the observed concentrations (SEM = 132.14) are presented in figure 19 with a reduced Y-scale.

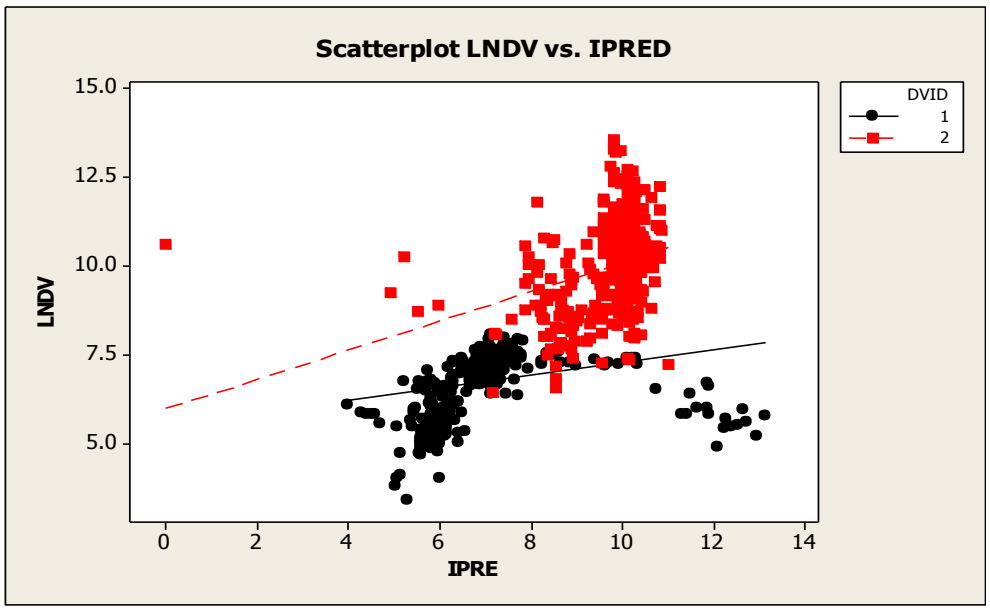

Figure 20. Scatterplot of LNDV vs. IPRED. DVID = 1 is whole blood predictions, DVID = 2 is intracellular predictions.

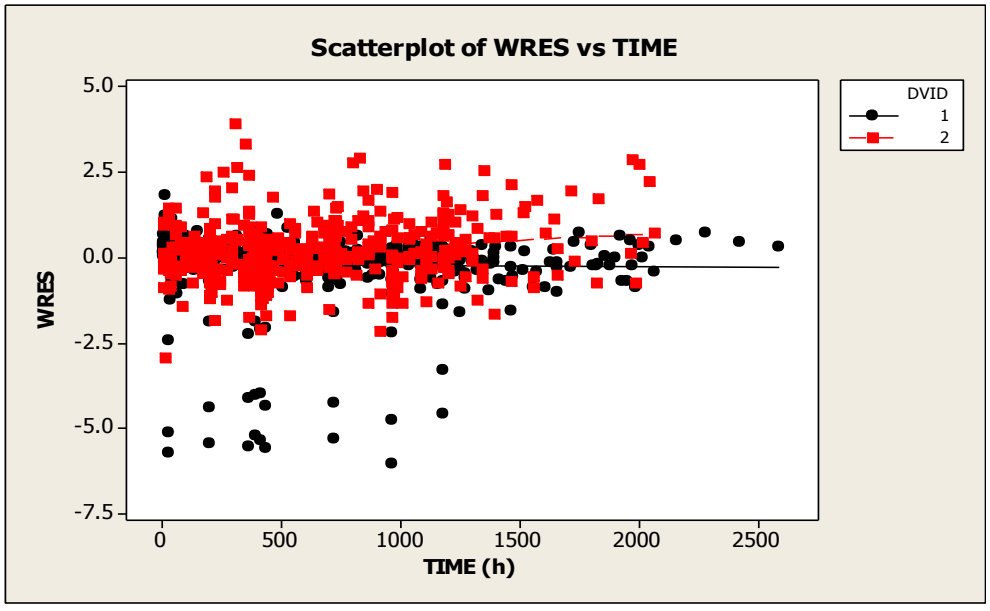

Figure 21. Scatterplot of WRES vs. time. DVID = 1 is whole blood predictions, DVID = 2 is intracellular predictions.

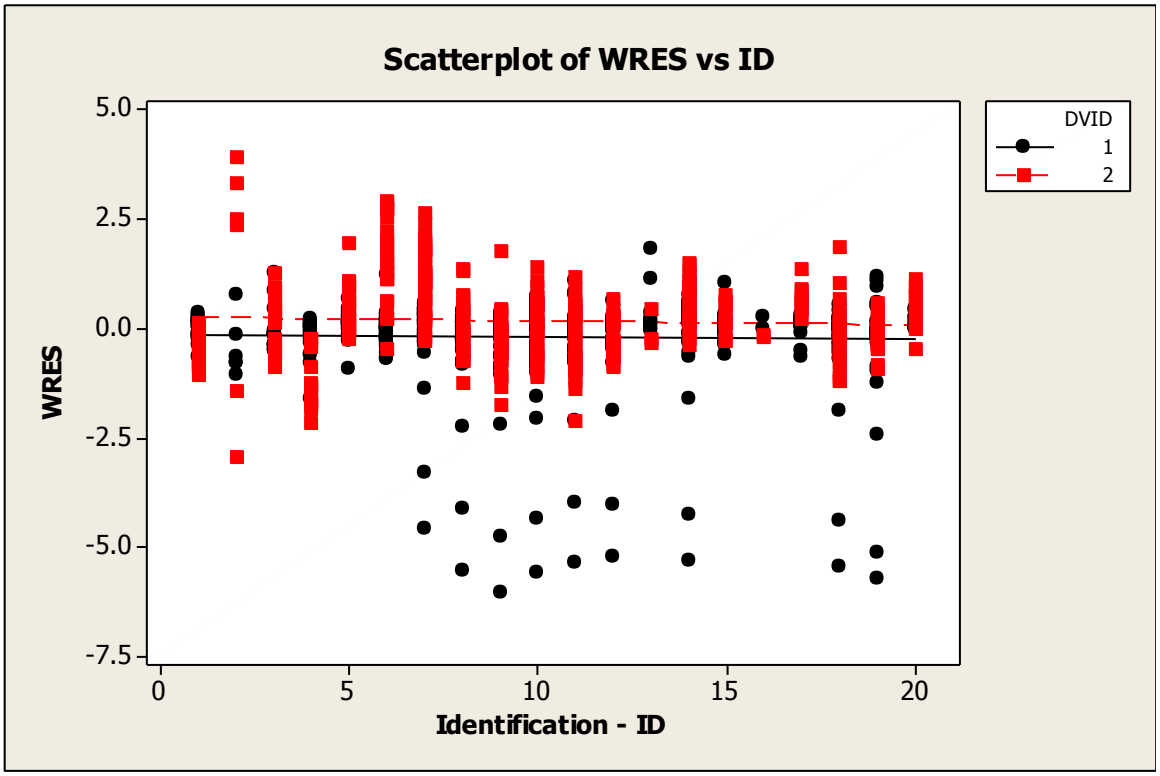

Figure 22. Scatterplot of WRES vs. ID. DVID = 1 is whole blood predictions, DVID = 2 is intracellular predictions.

The scatterplot of LNDV versus IPRED (Figure 20) shows that the predictions are still rather inaccurate. Figure 21 shows that the weighted residuals are stable over a period of time, but the scatterplot of WRES versus ID (figure 22) shows for all patients that the weighted residuals are negative for whole blood concentrations which give an indication that the predictions are over-predicted.

6. DISCUSSIONS

6.1 Re-analyzing for covariates for CsA plasmaconcentrations

As expected there were many covariates influencing different PK-parameters. During forward inclusion several covariates showed statistical significant, but most proved not to be significant after the backwards deletion process.

During backwards deletion (table 5) the model would not run without some covariates. Due to the recommendation that only one or two parameters should be changed in order to compare with the previous OFV, those covariates were kept and marked “not applicable” (NA). [7]

Storehagen [49] and Le [48] used some of the same data for their thesis and it was expected to find similar significant covariates. The covariate screening for their theses included: age (years), gender (FLAG = 1 male, FLAG 2 = female), diabetes (FLAG = 1 diabetic, FLAG 2 = non-diabetic), weight (kilos), height (centimeters), post-transplantation (weeks), steroid dose at the pharmacokinetic day (mg), CYP3A5-enzyme genotype (*1/x vs. *3/*3) and estimated creatinine clearance (ml/min).

The surgery performed in renal transplantation patients will most likely affect the intestinal motility and hence absorption and bioavailability of CsA in the early post-transplant phase. Therefore the post-transplantation time will presumably influence the absorption constant Ka which also is found.

Patients gain up to 10% more bodyweight after transplantation and it is not surprising that parameters like weight, lean-body-mass and body-mass-index can influence PK-parameters like Ka, Q and distribution volume. After transplantation, patients are less catabolic and they are able to eat more which might increase the body-fat. This is supported by the findings of weight and weight-related (BMI) covariates influence on Ka, Q and distribution volume.

Storehagen [49], Le [48], Falck [56] and Wu [57] have shown earlier that age has a significant covariate for CL/F. With increasing age pharmacological changes such as loss of liver-mass and the blood flow to liver is reduced, which influences the metabolism and clearance of CsA. Physiological changes can also explain the significance of age on parameters like volume and absorption. These factors strengthen the findings in this thesis that age has a real effect on CL, V_1 and Ka.

Cyclosporin A is metabolized by the cytochrom P450-system as mentioned earlier. CYP 3A5 genotypes will most likely affect the clearance of CsA. In earlier studies Storehagen [49] did not test this covariate because of various reasons, Le found this covariate to be significant in a later study, but it was not included in the final model because of clinical relevance.[48] It is therefore no surprise that this was shown to be a significant covariate on clearance in this present version of the model.

Diabetes was tested and found non-significant in this thesis. It has been shown in literature that diabetes may affect the absorption rate of CsA. [58] One reason why it was not found significant in the present model may be due to the lack of detailed description of diabetes in patients. It was only marked as FLAG = 1 diabetes and FLAG = 2 non-diabetes. Some patients had diabetes before undergoing transplantation and some patients developed diabetes after transplantation thus a more detailed coding could have given a different result. This should be tested in the future development of the model.

The covariates found to be statistical significant were mostly the same as previously discovered by Storehagen, Le and Falck (table 7). This further supports the theory that those covariates are statistical significant for this drug and drug model.

Overall the OFV decreased significantly during the development of the model, which gives a strong indication that the inclusion of covariates improves the model. However, the graphs showing concentration vs. individual predictions do not give an unambiguous answer. There might be a slightly better population prediction with the inclusion of covariates but this is very difficult to see from the figures. There are minor signs indicating that the model with covariates has less spread and that the

divergence starts a little later. A more secure way of determining this is by looking at the decrease of OFV. Along with the R^2 value and S-value for the regression line for the observation versus predictions (Figure 3-4) shows that there is an improvement of the model.

6.2 Testing for interoccasional variability for the whole blood model

Based on the results from the POPDOC-study there is reason to suspect significant inter-occasional variability. [54] Due to many visits per patients the chances increased to detect if there is something "unexplainable" that happens between different visits. There are patients that have a variation that cannot be explained with covariates and intra- and inter-individual variability. This gives a strong indication that there is something more that influences patients PK-parameters.

NONMEM has a limit of etas before there is an error statement from FORTRAN. There are ways to avoid this problem, but it requires a degree of competence in NONMEM coding that is beyond the scope of this research group. Because of this limitation it was only possible to code 11 visits per patients which could be a reason why the visits were not shown more significant than when coding each measurement-date as one separate visit. The maximum number of visits one patient had was 34 visits, thus that way of coding accounted for roughly 40% of the visits.

When coding every other date as separate visits there was an improvement (decrease) in OFV. There was a decrease of 30.56 when including IOV to the parameter V_2 (table 6), making the model significant better with IOV than without. Another advantage of coding every other visit as one visit comparing to coding every visit as its own, is that about 65% of the visits are included, and the time perspective is kept.

Still there are some visits that are not accounted for. A hypothesis is that coding every visit as "its own visit" will enhance the model further. Another way to do this is by

looking at the data and picking the data where there is suspicion of IOV and code only these visits.

Moreover there are also some covariates that are believed to influence the different parameters e.g. diabetes. [58] Including all of these factors may improve the model and make it even better than it is today.

From a graphical inspection it is hard to determine which model gives the best fit. Due to a large data set there are too many observations to give an unambiguous answer. Still, by looking again at the R^2- and S-value for the regression line an improvement is visible from the starting model without covariates to the model including covariates and finally the model including covariates and IOV. Similarly, by looking at the OFV the value is decreased by first including covariates and then including IOV.

Another way to determine the best model would be a visual prediction check. [59] One way to do this is to program R-script. There also exist different add-on programs to NONMEM for example xPose, Wings, PsN, which gives a possibility to draw more complex graphs. To do this it is necessary to have to have a stand-alone NONMEM installation, which was not available. More detailed graphs would provide an even better foundation to determine which model that had the best data fit.

6.3 Whole blood and intracellular model

The first step to overcome was to make NONMEM to differ between the whole blood observations from the intracellular observations. This was an extremely challenging task and was more difficult than first imagined. An unexpected hinder was the run time the model had. In the beginning most runs used approximately 1 day. After the transformation of intracellular concentrations from ng/10^6 cells to the same unit (ng/ml), as the whole blood concentrations were measured in, there was an enormous range from whole blood concentrations to intracellular concentrations. This large range made data fitting nearly impossible. Finally after LN transforming the

concentrations there were difficulties finding the correct error code to include in the model. In the end when the data fittings at last seemed promising, time was running out.

The final model seems capable of predicting the concentrations at two different sites. The WRES vs. time/ID shows an overall over-prediction for the intracellular concentrations and an under-prediction for the whole blood values. The over-prediction of whole blood concentration is largely due to the C_{max} in the 12-hours PK. The difference between predicted value and observed concentration is so high that it will affect the mean prediction power. It seems as though the model fits the C_2-levels slightly better than the C_0-concentrations, but it still has trouble fitting the large variety of concentrations.

For the 12-hours whole blood profile it is visible that the model has large problems in the absorption phase, which indicates that the Ka is over estimated. The elimination however seems to be rather accurate compared to the absorption phase. It is obvious that Ka is estimated wrongly when NONMEM predicts it to be 55 on a LN-scale. The high Ka is the reason for the high predicted C_{max} for whole blood (140000 ng/ml – 1600 ng/ml – figure 18-19). The high C_{max} increases the SEM which makes the predictive power worse as mentioned earlier.

An attempt to force/lock Ka to a lower value resulted in a more correct C_{max} for whole blood. C_{max} went from 140000 ng/ml to 22000 ng/ml compared to 1600 ng/ml, but resulted in a more identical whole blood and intracellular prediction with a lack of concentration-variety. (Appendix 9.8) The absorption-phase is also delayed with T_{max} estimated to be approximately 1 hour later than the true value. (Figure 17-19)

The intracellular 12-hours profile has predictions in the same range as the measured observations. The absorption phase is however slightly delayed. The absorption into the intracellular compartment is estimated to be a 1.order reaction. This is a reasonable assumption by looking at the measured concentrations and considering there is a passive diffusion of CsA into T-lymphocytes and not an active transport. It could be an idea to test other absorption-profiles to find a more fitting description.

The concentrations/LNDV vs. Ipred did not attain the desired degree of consistent prediction. (Figure 20) The “inclusion” of the whole blood model has not been optimal. One way to possibly improve this model is the inclusion of covariates. However, before the covariates are tested the basic model needs to be improved, especially the absorption rate constant, and the absorption and elimination processes. The model is still in an early stage and needs to be further developed.

Since the model is still in a development phase it is premature to validate the model. After further development with the inclusion of covariates and IOV the model should be validated following FDA’s guidelines. [5] The validation process consists of external validation (including new patients) or internal (using the existing data set). A useful and common validation method for this data set could be cross-validation, bootstrapping and Jackknife.

In a retrospective view it should be possible to develop a model based on the original intracellular data. NONMEM does not understand units, meaning it only reads numbers. Accordingly it should be possible to develop a model based on the original data and unit (ng/10^6 cells). Doing this has many benefits i.e. there is no need to transform data, future data can be measured the same way and included directly into the model. Because the range is similar, plotting graphs will also be easier. When both whole blood and intracellular concentrations are in the same graphs it will be easier to interpret the plots as well.

7. CONCLUSIONS

Re-analyzing for covariates in the whole blood model was performed using the traditional forward-inclusion criteria and the backwards-deletion process. The result cohered with similar finding in earlier results. This substantiates earlier findings that these covariates are relevant for this drug/model.

Interoccasional variability was included and found significant for V_2. With both covariates and IOV included, the model for whole blood has improved. The model is accurate for low and normal concentrations, but has a tendency to over-predict concentrations at the higher end of the scale. The predictions are however stable and show little spread.

A model for whole blood- and intracellular concentration was initially developed from the previous model. The model seems capable of predicting different concentrations at different sites of measurements. There is generally a better prediction of C_2-levels compared to C_0-levels. The model has however its weaknesses however that is quite visible in the 12-hours PK. The absorption phase for both whole blood and intracellular concentrations are not optimal and the elimination phase is wrongly predicted. There is a need to continue working on the basic model before covariates are to be included, IOV have to be checked and finally the model has to be validated.

There are several reasons to continue working on this model. In theory a PPK-model will predict concentrations more accurately and stable than in the current clinical setting and a prediction of intracellular concentrations may prevent more rejections from kidney transplants.

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9. APPENDIX

9.1 Formulas used in demographics model

Equation 12: BMI-formula:

$$BMI = weight\ (kg) / (height\ (m))^2$$

Equation 13: Hume LBM-formula [60]:

(Male): L.B.M = 0.32810 W (Weight/kg) + 0.33929 H (Height/cm) – 29.5336

(Female): L.B.M = 0.29569 W (Weight/kg) + 0.41813 H (Height/cm) – 43.2933

Equation 14: Cockgroft-Gault Equation:

$$GFR = \frac{(140\text{-}age(years) \times weight\ (kg)) \times (0.85\ for\ women)}{72 \times Serum\ creatinine\ (mg/dl)}$$

9.2 Partial input file for whole blood model

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X
1	#ID	AMT	RATE	DAT1=DROP	TIME	C=DV	VIST	WT	UREA	SCR	CRCL	MDV	SS	II	CMT	FLAG	AGE	GENDER	HEIGHT	TXT	STER	BMI	C3A5	LBM
2	1	300000	0	09.08.2008	6	0	.	104.6	10	148	76.3	1	2	12	1	1	52	1	1.85	1	40	30.6	3	67.6
3	1	0	0	09.08.2008	6	235	1	104.6	10	148	76.3	0	0	0	2	1	52	1	1.85	1	40	30.6	3	67.6
4	1	300000	0	09.08.2008	20	0	.	104.6	10	148	76.3	1	0	0	1	1	52	1	1.85	1	40	30.6	3	67.6
5	1	0	0	09.08.2008	22	0	.	104.6	10	148	76.3	1	0	0	2	1	52	1	1.85	1	40	30.6	3	67.6
6	1	300000	0	10.08.2008	6	0	.	103.4	10	131	85.2	1	0	0	1	1	52	1	1.85	1	40	30.2	3	67.2
7	1	0	0	10.08.2008	8	0	.	103.4	10	131	85.2	1	0	0	2	1	52	1	1.85	1	40	30.2	3	67.2
8	1	300000	0	10.08.2008	20	0	.	103.4	10	131	85.2	1	0	0	1	1	52	1	1.85	1	40	30.2	3	67.2
9	1	0	0	10.08.2008	22	0	.	103.4	10	131	85.2	1	0	0	2	1	52	1	1.85	1	40	30.2	3	67.2
10	1	300000	0	11.08.2008	6	0	.	101	10	125	87.2	1	0	0	1	1	52	1	1.85	1	40	29.5	3	66.4
11	1	0	0	11.08.2008	6	230	1	101	10	125	87.2	0	0	0	2	1	52	1	1.85	1	40	29.5	3	66.4
12	1	300000	0	11.08.2008	20	0	.	101	10	125	87.2	1	0	0	1	1	52	1	1.85	1	40	29.5	3	66.4
13	1	0	0	11.08.2008	22	0	.	101	10	125	87.2	1	0	0	2	1	52	1	1.85	1	40	29.5	3	66.4
14	1	300000	0	12.08.2008	6	0	.	101	10	146	74.7	1	0	0	1	1	52	1	1.85	1	40	29.5	3	66.4
15	1	0	0	12.08.2008	8	0	.	101	10	146	74.7	1	0	0	2	1	52	1	1.85	1	40	29.5	3	66.4
16	1	300000	0	12.08.2008	20	0	.	101	10	146	74.7	1	0	0	1	1	52	1	1.85	1	40	29.5	3	66.4
17	1	0	0	12.08.2008	22	0	.	101	10	146	74.7	1	0	0	2	1	52	1	1.85	1	40	29.5	3	66.4
18	1	275000	0	13.08.2008	6	0	.	99.8	10	134	80.4	1	0	0	1	1	52	1	1.85	1	40	29.2	3	66
19	1	0	0	13.08.2008	8	0	.	99.8	10	134	80.4	1	0	0	2	1	52	1	1.85	1	40	29.2	3	66
20	1	275000	0	13.08.2008	20	0	.	99.8	10	134	80.4	1	0	0	1	1	52	1	1.85	1	40	29.2	3	66
21	1	0	0	13.08.2008	22	0	.	99.8	10	134	80.4	1	0	0	2	1	52	1	1.85	1	40	29.2	3	66
22	1	275000	0	14.08.2008	6	0	.	99.5	10	132	81.4	1	0	0	1	1	52	1	1.85	1	40	29.1	3	65.9
23	1	0	0	14.08.2008	8	0	.	99.5	10	132	81.4	1	0	0	2	1	52	1	1.85	1	40	29.1	3	65.9
24	1	275000	0	14.08.2008	20	0	.	99.5	10	132	81.4	1	0	0	1	1	52	1	1.85	1	40	29.1	3	65.9
25	1	0	0	14.08.2008	22	0	.	99.5	10	132	81.4	1	0	0	2	1	52	1	1.85	1	40	29.1	3	65.9
26	1	275000	0	15.08.2008	6	0	.	99.5	10	132	81.4	1	0	0	1	1	52	1	1.85	1	40	29.1	3	65.9
27	1	0	0	15.08.2008	8	0	.	99.5	10	132	81.4	1	0	0	2	1	52	1	1.85	1	40	29.1	3	65.9
28	1	275000	0	15.08.2008	20	0	.	99.5	10	132	81.4	1	0	0	1	1	52	1	1.85	1	40	29.1	3	65.9
29	1	0	0	15.08.2008	22	0	.	99.5	10	132	81.4	1	0	0	2	1	52	1	1.85	1	40	29.1	3	65.9
30	1	275000	0	16.08.2008	6	0	.	99.5	10	132	81.4	1	0	0	1	1	52	1	1.85	1	40	29.1	3	65.9
31	1	0	0	16.08.2008	8	0	.	99.5	10	132	81.4	1	0	0	2	1	52	1	1.85	1	40	29.1	3	65.9
32	1	275000	0	16.08.2008	20	0	.	99.5	10	132	81.4	1	0	0	1	1	52	1	1.85	1	40	29.1	3	65.9

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X
10018	170	0	0		18.01	286	1	49			109.3	0	0	0	2	1	73.8	2	1.59	7.3	15	19.4	1	37.68
10019	170	0	0		19.83	217	1	49			109.3	0	0	0	2	1	73.8	2	1.59	7.3	15	19.4	1	37.68
10020	170	0	0		21.81	206	1	49			109.3	0	0	0	2	1	73.8	2	1.59	7.3	15	19.4	1	37.68
10021	170	0	0		23.4	139	1	49			109.3	0	0	0	2	1	73.8	2	1.59	7.3	15	19.4	1	37.68
10022	171	300000	0		0	0	.	94			36.6	1	2	12	1	2	48.8	1	1.92	2.3	20	25.5	3	66.45
10023	171	0	0		11.4	168	1	94			36.6	0	0	0	2	2	48.8	1	1.92	2.3	20	25.5	3	66.45
10024	171	300000	0		11.45	0	.	94			36.6	1	0	0	1	2	48.8	1	1.92	2.3	20	25.5	3	66.45
10025	171	0	0		11.7	353	1	94			36.6	0	0	0	2	2	48.8	1	1.92	2.3	20	25.5	3	66.45
10026	171	0	0		11.97	642	1	94			36.6	0	0	0	2	2	48.8	1	1.92	2.3	20	25.5	3	66.45
10027	171	0	0		12.47	1872	1	94			36.6	0	0	0	2	2	48.8	1	1.92	2.3	20	25.5	3	66.45
10028	171	0	0		12.97	2289	1	94			36.6	0	0	0	2	2	48.8	1	1.92	2.3	20	25.5	3	66.45
10029	171	0	0		13.45	1877	1	94			36.6	0	0	0	2	2	48.8	1	1.92	2.3	20	25.5	3	66.45
10030	171	0	0		14.38	1347	1	94			36.6	0	0	0	2	2	48.8	1	1.92	2.3	20	25.5	3	66.45
10031	171	0	0		15.43	1009	1	94			36.6	0	0	0	2	2	48.8	1	1.92	2.3	20	25.5	3	66.45
10032	171	0	0		17.57	637	1	94			36.6	0	0	0	2	2	48.8	1	1.92	2.3	20	25.5	3	66.45
10033	171	0	0		19.42	393	1	94			36.6	0	0	0	2	2	48.8	1	1.92	2.3	20	25.5	3	66.45
10034	171	0	0		21.42	386	1	94			36.6	0	0	0	2	2	48.8	1	1.92	2.3	20	25.5	3	66.45
10035	171	0	0		22.98	309	1	94			36.6	0	0	0	2	2	48.8	1	1.92	2.3	20	25.5	3	66.45
10036	172	125000	0		0	0	.	73			56.7	1	2	12	1	2	75.1	1	1.76	6.6	15	23.6	3	54.13
10037	172	0	0		11.48	269	1	73			56.7	0	0	0	2	2	75.1	1	1.76	6.6	15	23.6	3	54.13
10038	172	125000	0		11.57	0	.	73			56.7	1	0	0	1	2	75.1	1	1.76	6.6	15	23.6	3	54.13
10039	172	0	0		11.8	228	1	73			56.7	0	0	0	2	2	75.1	1	1.76	6.6	15	23.6	3	54.13
10040	172	0	0		12.05	200	1	73			56.7	0	0	0	2	2	75.1	1	1.76	6.6	15	23.6	3	54.13
10041	172	0	0		12.57	1088	1	73			56.7	0	0	0	2	2	75.1	1	1.76	6.6	15	23.6	3	54.13
10042	172	0	0		13.07	1725	1	73			56.7	0	0	0	2	2	75.1	1	1.76	6.6	15	23.6	3	54.13
10043	172	0	0		13.64	1400	1	73			56.7	0	0	0	2	2	75.1	1	1.76	6.6	15	23.6	3	54.13
10044	172	0	0		14.64	899	1	73			56.7	0	0	0	2	2	75.1	1	1.76	6.6	15	23.6	3	54.13
10045	172	0	0		15.52	600	1	73			56.7	0	0	0	2	2	75.1	1	1.76	6.6	15	23.6	3	54.13
10046	172	0	0		17.44	359	1	73			56.7	0	0	0	2	2	75.1	1	1.76	6.6	15	23.6	3	54.13
10047	172	0	0		19.49	321	1	73			56.7	0	0	0	2	2	75.1	1	1.76	6.6	15	23.6	3	54.13
10048	172	0	0		21.5	259	1	73			56.7	0	0	0	2	2	75.1	1	1.76	6.6	15	23.6	3	54.13
10049	172	0	0		23.07	210	1	73			56.7	0	0	0	2	2	75.1	1	1.76	6.6	15	23.6	3	54.13

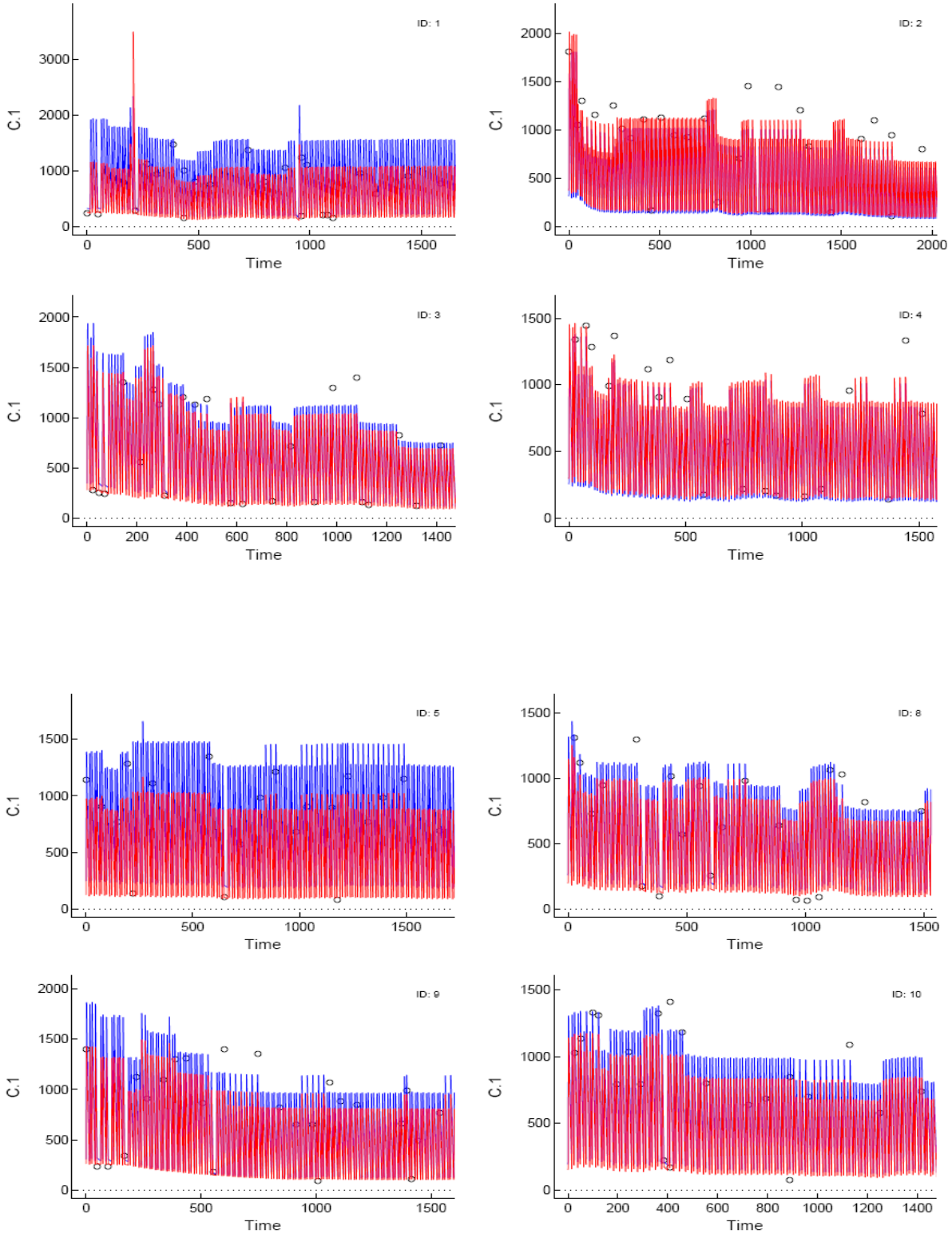
9.3 Control file for final model whole blood

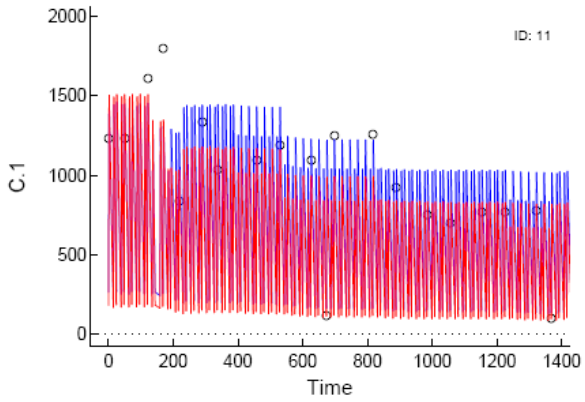
```
$PROBLEM IOV.

$DATA valider-iov.txt

$INPUT ID AMT RATE=DROP DAT1=DROP TIME C=DV VIST WT UREA=DROP SCR=DROP
CRCL MDV SS II CMT FLAG=DROP AGE GEN HGHT TXT STER BMI C3A5 LBM

$SUBROUTINE ADVAN4

$PK
   IF(C3A5.EQ.1) THEN
   TVCL=THETA(1)-(THETA(8)*CRCL)-(THETA(9)*AGE/56)
   ELSE
   TVCL=THETA(7)-(THETA(8)*CRCL)-(THETA(9)*AGE/56)
   ENDIF

   TVV1=THETA(2)*THETA(10)*BMI/THETA(11)*(AGE/56)

   TVQ=THETA(3)-THETA(12)*(1+STER/100)+THETA(13)*WT

   TVV2=THETA(4)

   TVKA=THETA(5)*THETA(14)**TXT/5*(THETA(15)*BMI)+THETA(16)*AGE/56

   TVALAG=THETA(6)

IOV = ETA(1)
IF (VIST.EQ.2) IOV = ETA(2)
IF (VIST.EQ.3) IOV = ETA(3)
IF (VIST.EQ.4) IOV = ETA(4)
IF (VIST.EQ.5) IOV = ETA(5)
IF (VIST.EQ.6) IOV = ETA(6)
IF (VIST.EQ.7) IOV = ETA(7)
IF (VIST.EQ.8) IOV = ETA(8)
IF (VIST.EQ.9) IOV = ETA(9)
IF (VIST.EQ.10) IOV = ETA(10)
IF (VIST.EQ.11) IOV = ETA(11)

   CL=TVCL*EXP(ETA(12))              ;Clearance (CL/F) L/hr
   V1=TVV1*EXP(ETA(13))              ;Central volume (V1/F), L
   Q=TVQ*EXP(ETA(14))                ;Intercompartmental clearance (Q/F)
   V2=TVV2*EXP(ETA(15)+IOV)          ;Peripheral volume (V2/F), L
   KA=TVKA*EXP(ETA(16))              ;Absorption rate constant, 1/hr
   ALAG1=TVALAG*EXP(ETA(17))         ;Absorption lag time, hr

   S2=V1

   K=CL/V1
   K23=Q/V1
   K32=Q/V2

$ERROR
   IPRED=F
   Y=F+F*ERR(1)+ERR(2)

$THETA (1,36.6)          ;THETA(1) is POPCL/F on C3A5
$THETA (15, 21.9, 40)    ;THETA(2) is POPV1/F
```

```
$THETA (1,18.6)          ;THETA(3) is POPQ/F
$THETA (1,1200)          ;THETA(4) is POPV2/
$THETA (0,0.821,1)       ;THETA(5) is POPKA
$THETA (0.1,0.452)       ;THETA(6) is POPLAGTIME
$THETA (1, 38)           ;THETA(7) is POPCL/F on C3A5
$THETA (0, 0.0047)       ;THETA(8) is CRCL on CL
$THETA (0, 10.6)         ;THETA(9) is AGE on CL
$THETA (0, 0.0001)       ;THETA(10)is BMI on V1
$THETA (0, 0.0012)       ;THETA(11)is AGE on V1
$THETA (0, 0.0001)       ;THETA(12)is STER on Q
$THETA (0, 0.001)        ;THETA(13)is WT on Q
$THETA (0, 0.006)        ;THETA(14)is TXT on ka
$THETA (0, 265)          ;THETA(15)is BMI on ka
$THETA (0, 2.1)          ;THETA(16)is AGE on ka

$OMEGA BLOCK(1) 0.1
$OMEGA BLOCK SAME
$OMEGA BLOCK SAME
$OMEGA BLOCK SAME
$OMEGA BLOCK SAME
$OMEGA BLOCK SAME
$OMEGA BLOCK SAME
$OMEGA BLOCK SAME
$OMEGA BLOCK SAME
$OMEGA BLOCK SAME
$OMEGA BLOCK SAME
$OMEGA 0.04         ;IIV CL
$OMEGA 0.01         ;BSVV1/F
$OMEGA 0.01         ;BSVQ/F
$OMEGA 2.77         ;BSVV2/F
$OMEGA 0.563        ;BSVKA
$OMEGA 0.006        ;BSVALAG1

$SIGMA 0.041     ;ERRCV, Proportional error (%)
$SIGMA 388       ;ERRSD, Additive error (ug/ml)

$ESTIMATION SIG=3 METHOD=1 INTER MAXEVAL=9999 PRINT=1 POSTHOC NOABORT

$TABLE ID TIME DV IPRED MDV
NOPRINT ONEHEADER FILE=table.txt

$TABLE ID CL V1 Q V2 KA ALAG1 WT CRCL AGE GEN HGHT TXT STER BMI C3A5 LBM
FIRSTONLY NOPRINT ONEHEADER NOAPPEND FILE=etatable.txt
```

9.4 Individual fitting made by R for final whole blood model

Circles: Observed concentrations; Red: Individual post hoc predicted concentrations; Blue: Population predicted concentrations.

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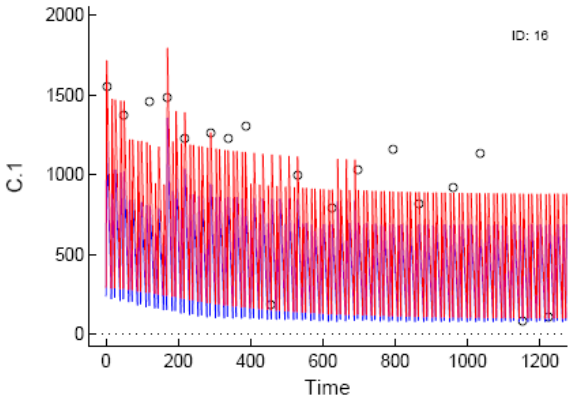
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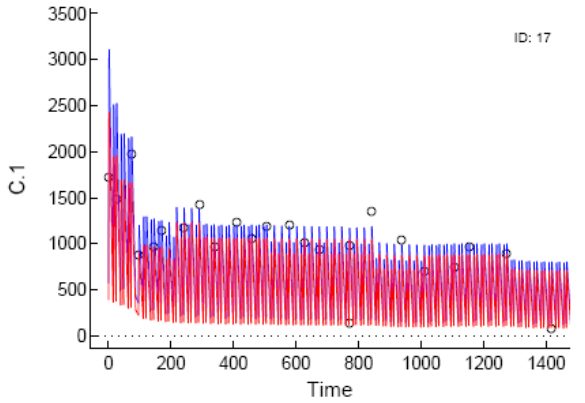
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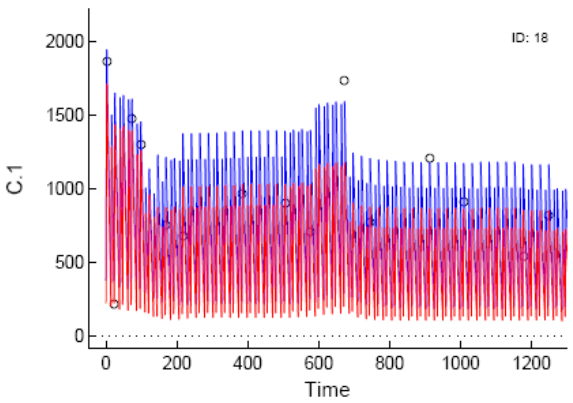
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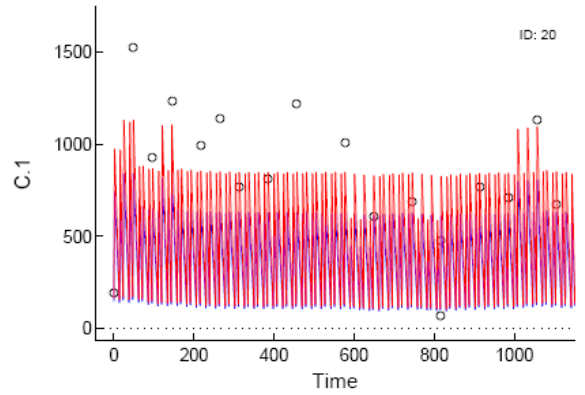
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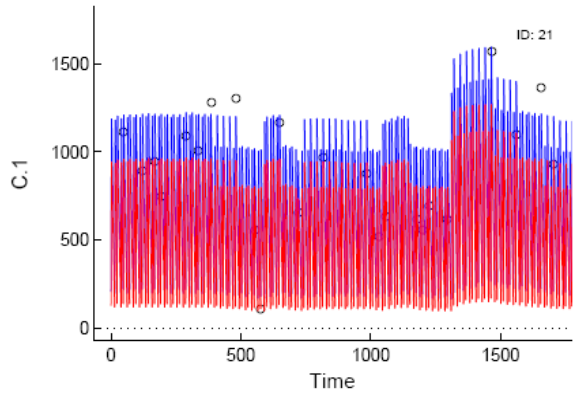
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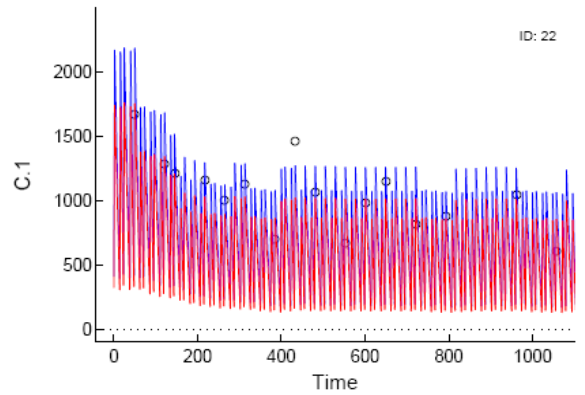
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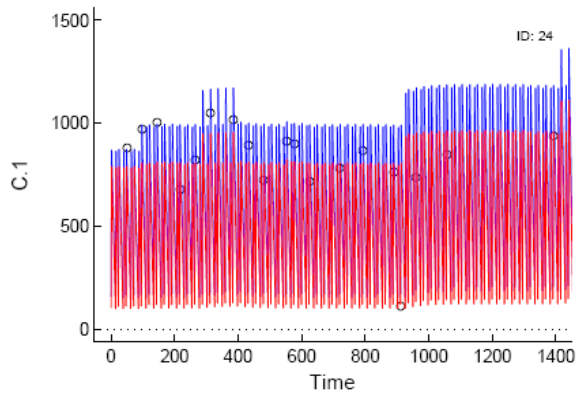
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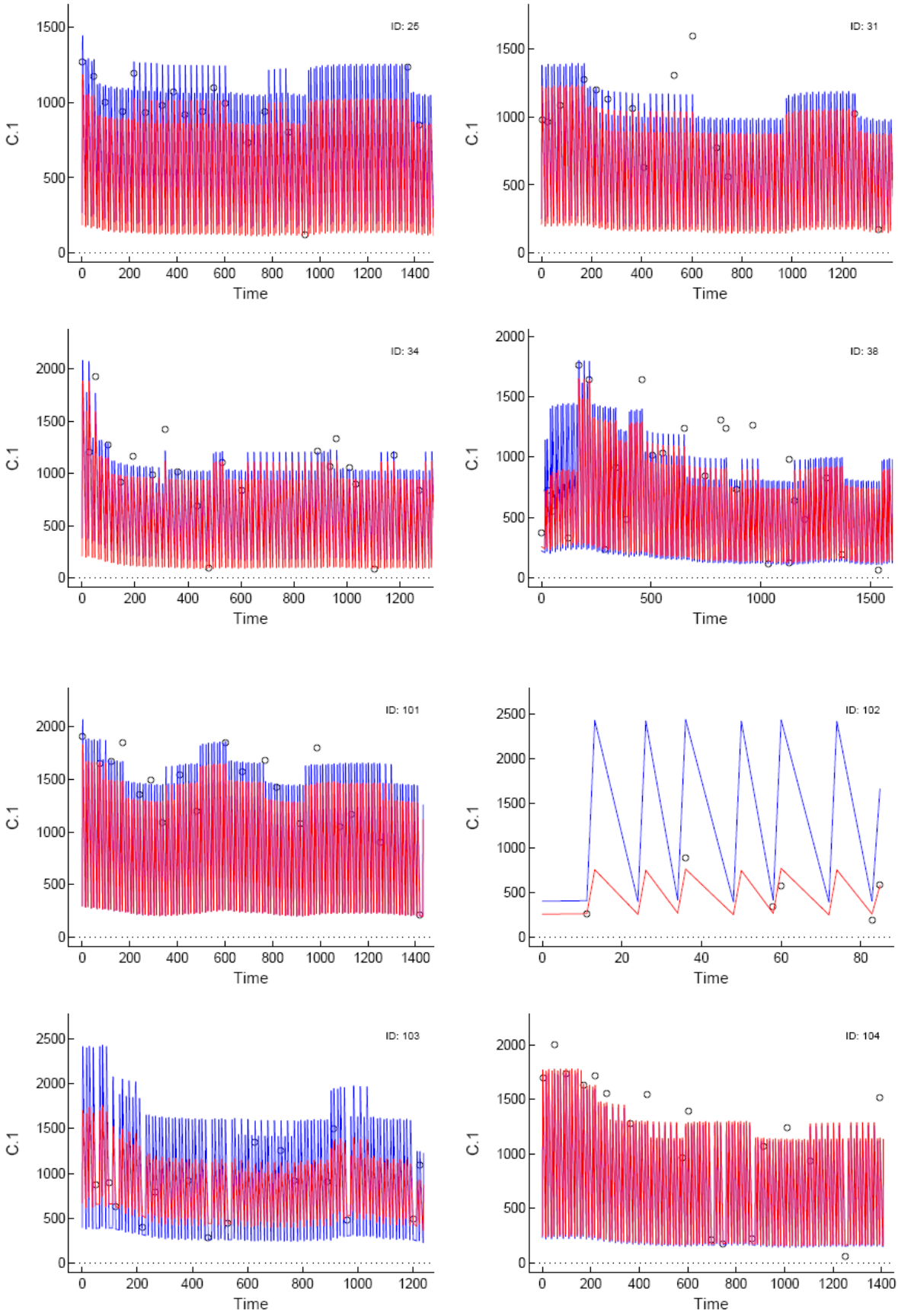
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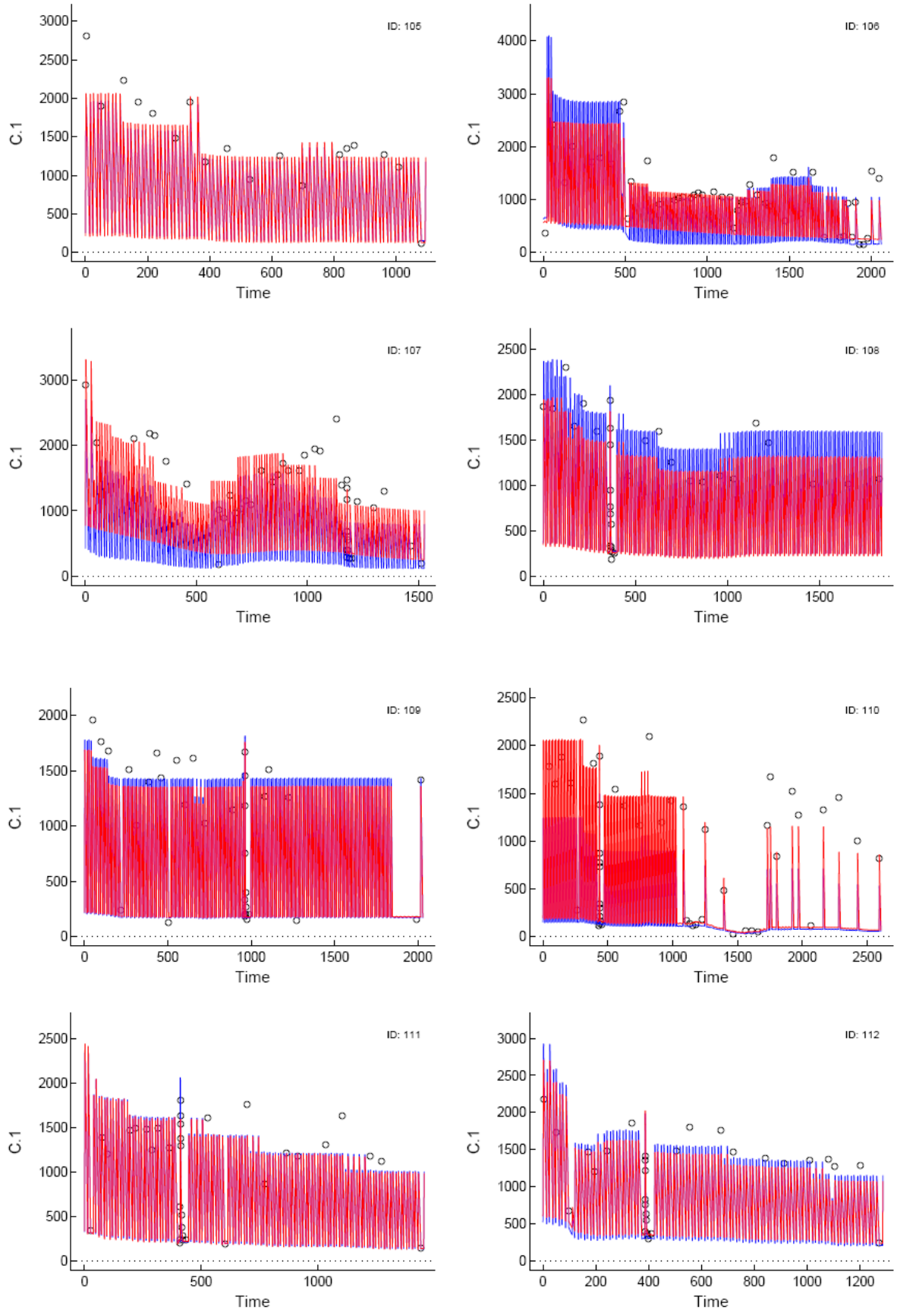
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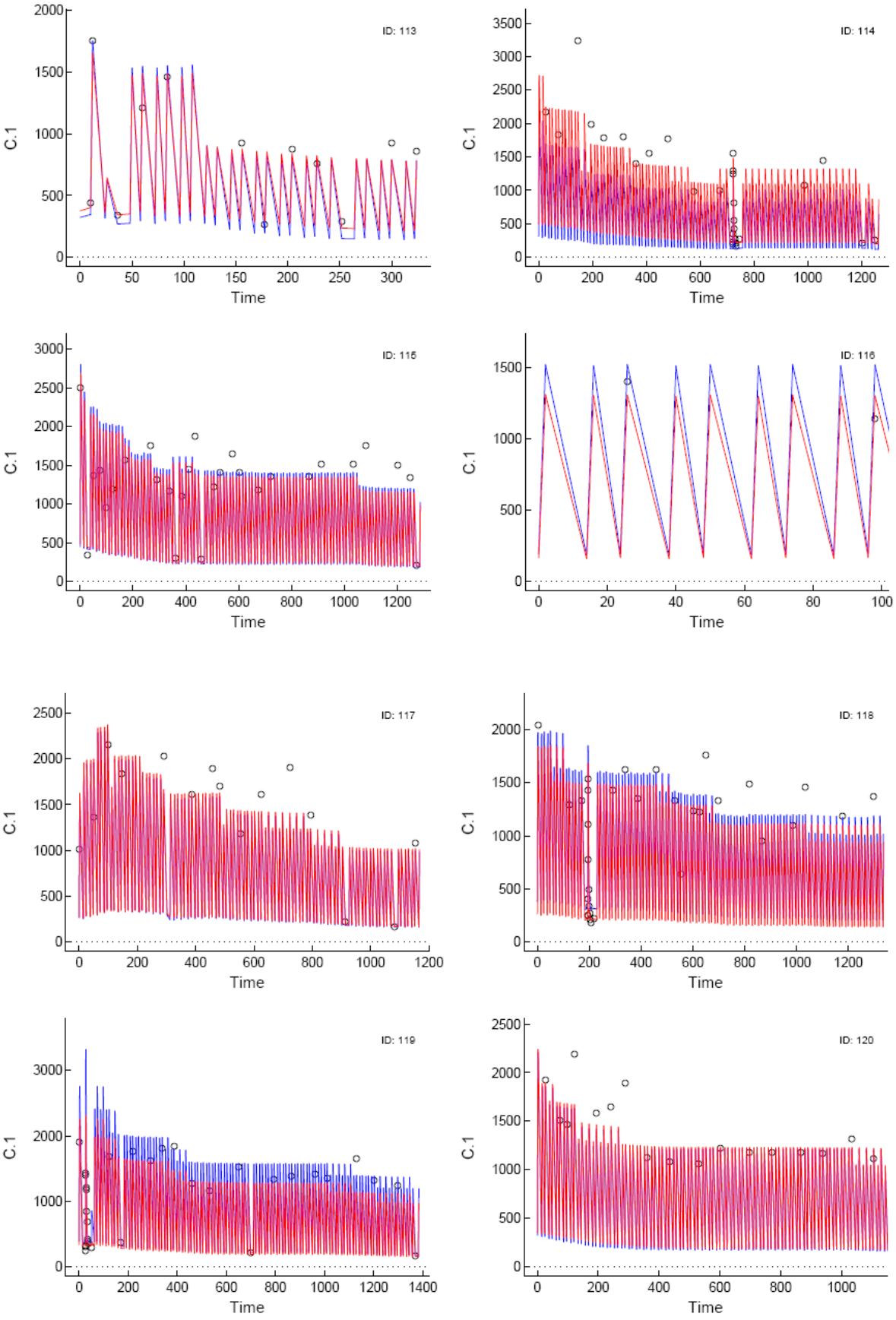
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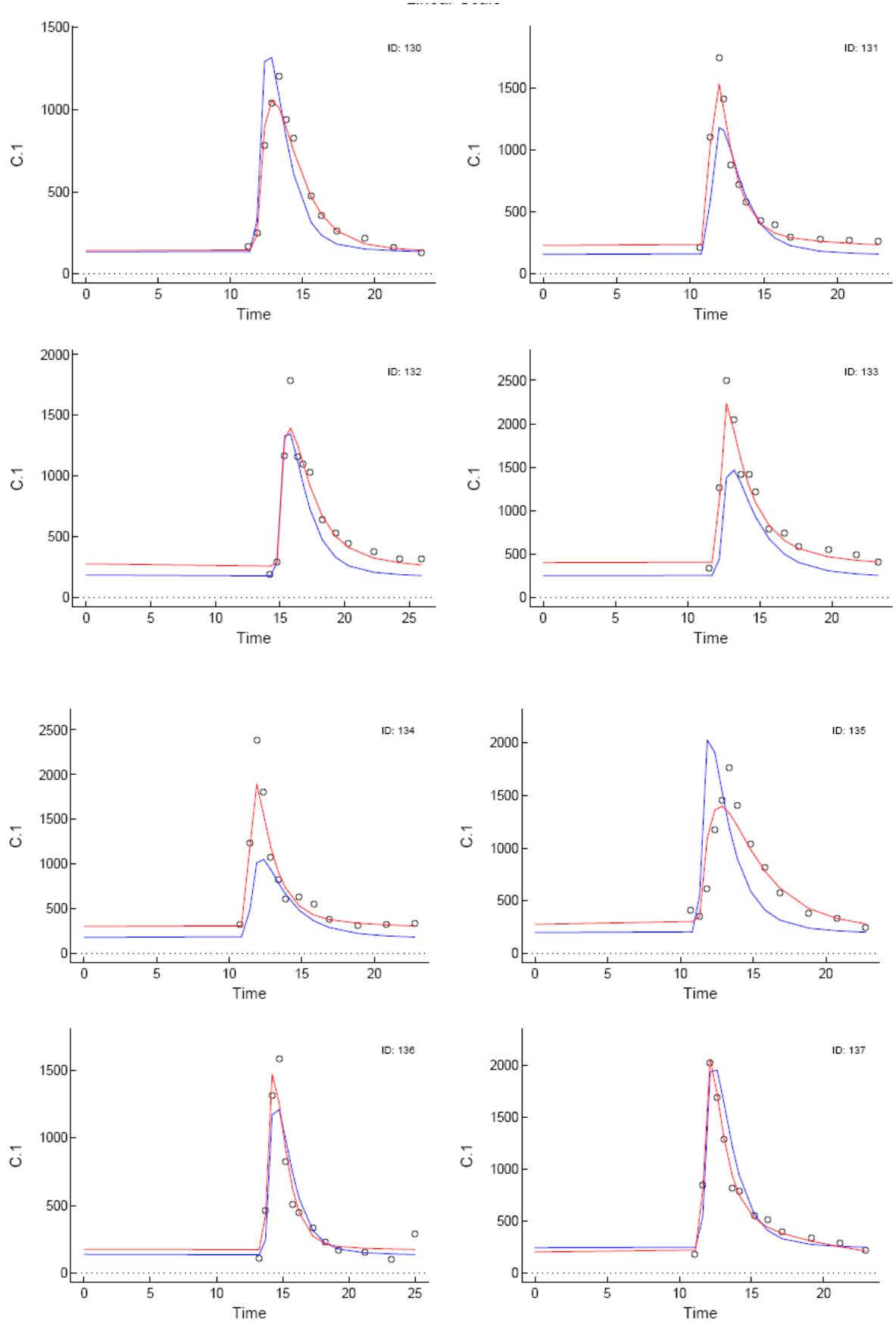
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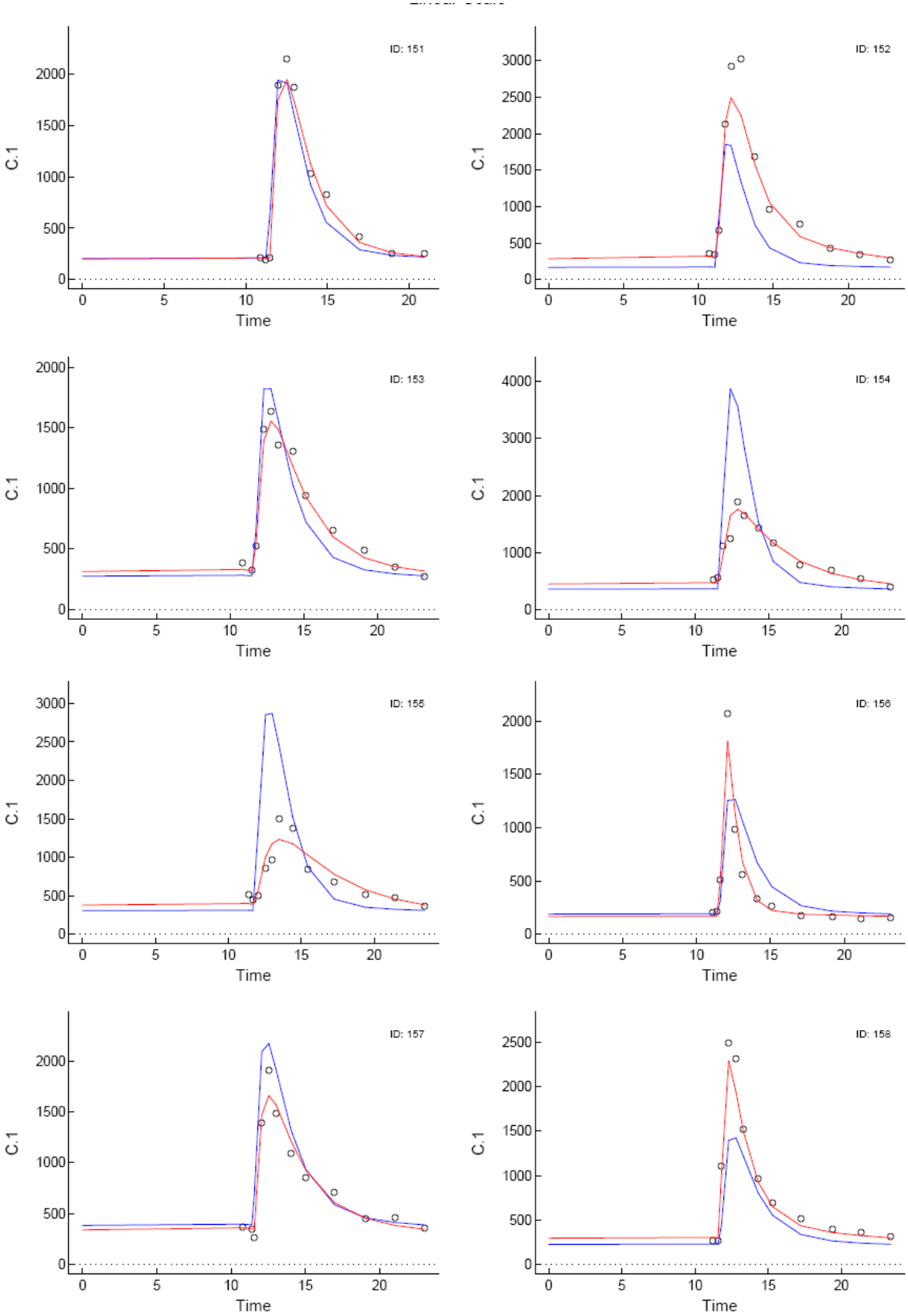
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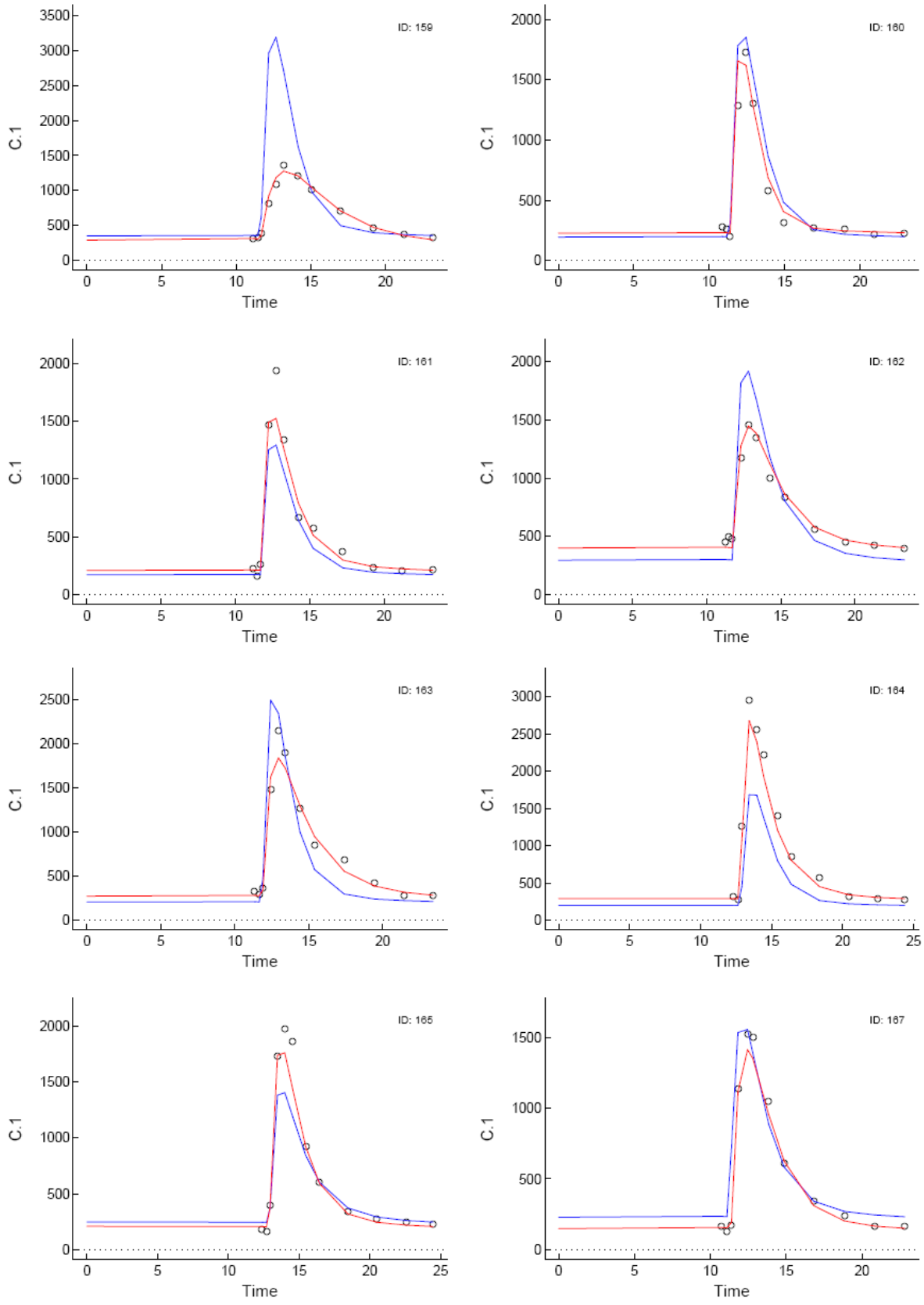
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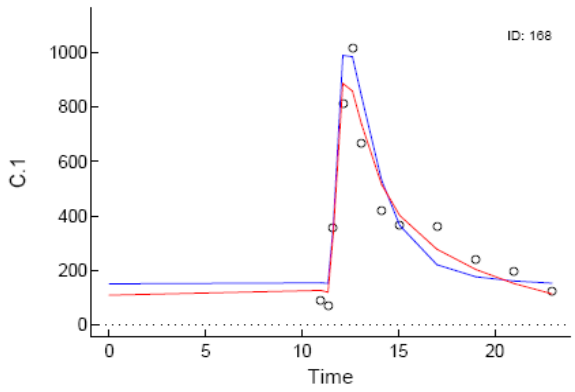
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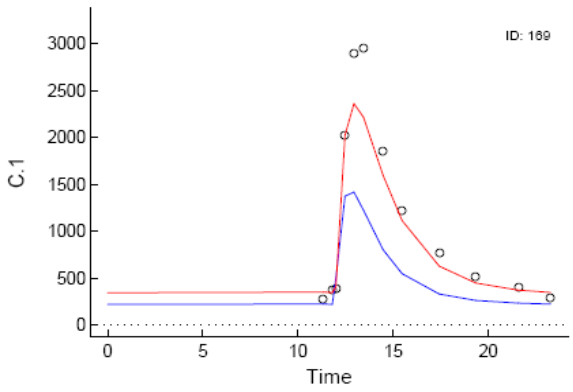
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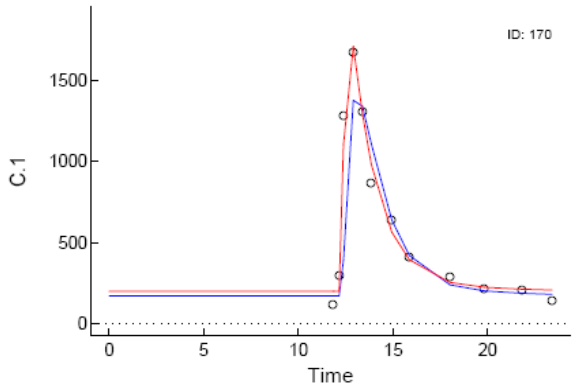
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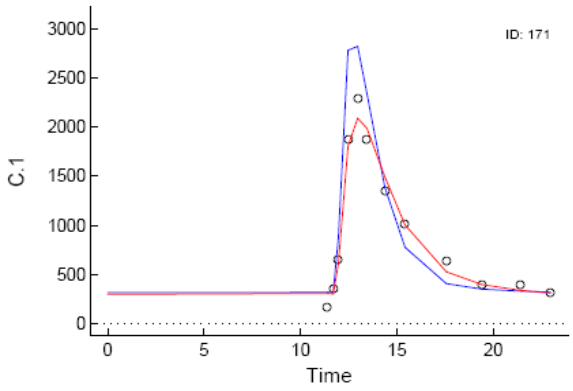
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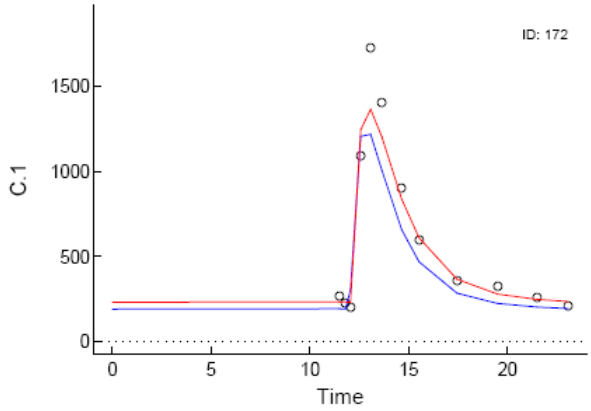
C.1
Time


ID: 172
C.1
Time

9.5 Partial input file for whole blood and intracellular model

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X
1	#ID	AMT	RATE	DAT1=DROP	TIME	LNDV=DV	EVID	WT	UREA	SCR	CRCL	SS	II	CMT	DVID	FLAG	AGE	GENDER	HEIGHT	TXT	STER	BMI	3A5	LBM
2	1	250000	0	24.06.2005	6	.	1	63.6	6.8	50	148.5	2	12	1	0	1	56	2	1.74	2	20	21	3	48.27
3	1	.	0	24.06.2005	8	7.552	0	63.6	6.8	50	148.5	0	0	2	1	1	56	2	1.74	2	20	21	3	48.27
4	1	.	0	24.06.2005	8	9.883	0	63.6	6.8	50	148.5	0	0	3	2	1	56	2	1.74	2	20	21	3	48.27
5	1	225000	0	24.06.2005	20	.	1	63.6	6.8	50	148.5	0	0	1	0	1	56	2	1.74	2	20	21	3	48.27
6	1	0	0	24.06.2005	22	0	2	63.6	6.8	50	148.5	0	0	2	0	1	56	2	1.74	2	20	21	3	48.27
7	1	225000	0	25.06.2005	6	.	1	63.6	6.8	50	148.5	0	0	1	0	1	56	2	1.74	2	20	21	3	48.27
8	1	0	0	25.06.2005	8	0	2	63.6	6.8	50	148.5	0	0	2	0	1	56	2	1.74	2	20	21	3	48.27
9	1	225000	0	25.06.2005	20	.	1	63.6	6.8	50	148.5	0	0	1	0	1	56	2	1.74	2	20	21	3	48.27
10	1	0	0	25.06.2005	22	0	2	63.6	6.8	50	148.5	0	0	2	0	1	56	2	1.74	2	20	21	3	48.27
11	1	225000	0	26.06.2005	6	.	1	63.6	6.8	50	148.5	0	0	1	0	1	56	2	1.74	2	20	21	3	48.27
12	1	0	0	26.06.2005	8	0	2	63.6	6.8	50	148.5	0	0	2	0	1	56	2	1.74	2	20	21	3	48.27
13	1	225000	0	26.06.2005	20	.	1	63.6	6.8	50	148.5	0	0	1	0	1	56	2	1.74	2	20	21	3	48.27
14	1	0	0	26.06.2005	22	0	2	63.6	6.8	50	148.5	0	0	2	0	1	56	2	1.74	2	20	21	3	48.27
15	1	225000	0	27.06.2005	6	.	1	63.1	8.3	45	162.5	0	0	1	0	1	56	2	1.74	2	20	20.8	3	48.12
16	1	.	0	27.06.2005	8	7.409	0	63.1	8.3	45	162.5	0	0	2	1	1	56	2	1.74	2	20	20.8	3	48.12
17	1	.	0	27.06.2005	8	9.885	0	63.1	8.3	45	162.5	0	0	3	2	1	56	2	1.74	2	20	20.8	3	48.12
18	1	200000	0	27.06.2005	20	.	1	63.1	8.3	45	162.5	0	0	1	0	1	56	2	1.74	2	20	20.8	3	48.12
19	1	0	0	27.06.2005	22	0	0	63.1	8.3	45	162.5	0	0	2	0	1	56	2	1.74	2	20	20.8	3	48.12
20	1	225000	0	28.06.2005	6	.	1	63.1	8.3	45	162.5	0	0	1	0	1	56	2	1.74	2	20	20.8	3	48.12
21	1	0	0	28.06.2005	8	0	2	63.1	8.3	45	162.5	0	0	2	0	1	56	2	1.74	2	20	20.8	3	48.12
22	1	200000	0	28.06.2005	20	.	1	63.1	8.3	45	162.5	0	0	1	0	1	56	2	1.74	2	20	20.8	3	48.12
23	1	0	0	28.06.2005	22	0	2	63.1	8.3	45	162.5	0	0	2	0	1	56	2	1.74	2	20	20.8	3	48.12
24	1	200000	0	29.06.2005	6	.	1	63.6	8.3	46	159.4	0	0	1	0	1	56	2	1.74	2	20	21	3	48.27
25	1	.	0	29.06.2005	8	7.418	0	63.6	8.3	46	159.4	0	0	2	1	1	56	2	1.74	2	20	21	3	48.27
26	1	.	0	29.06.2005	8	9.316	0	63.6	8.3	46	159.4	0	0	3	2	1	56	2	1.74	2	20	21	3	48.27
27	1	200000	0	29.06.2005	20	.	1	63.6	8.3	46	159.4	0	0	1	0	1	56	2	1.74	2	20	21	3	48.27
28	1	0	0	29.06.2005	22	0	2	63.6	8.3	46	159.4	0	0	2	0	1	56	2	1.74	2	20	21	3	48.27
29	1	200000	0	30.06.2005	6	.	1	63.6	8.3	46	159.4	0	0	1	0	1	56	2	1.74	2	20	21	3	48.27
30	1	0	0	30.06.2005	8	0	2	63.6	8.3	46	159.4	0	0	2	0	1	56	2	1.74	2	20	21	3	48.27
31	1	200000	0	30.06.2005	20	.	1	63.6	8.3	46	159.4	0	0	1	0	1	56	2	1.74	2	20	21	3	48.27
32	1	0	0	30.06.2005	22	0	2	63.6	8.3	46	159.4	0	0	2	0	1	56	2	1.74	2	20	21	3	48.27

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X
4278	19	350000	0	12.10.2005	6	.	1	79.5	12.6	145	62.5	2	12	1	0	2	52	1	1.76	2	20	25.7	3	56.27
4279	19	.	0	12.10.2005	8	7.550	0	79.5	12.6	145	62.5	0	0	2	1	2	52	1	1.76	2	20	25.7	3	56.27
4280	19	.	0	12.10.2005	8	10.373	0	79.5	12.6	145	62.5	0	0	3	2	2	52	1	1.76	2	20	25.7	3	56.27
4281	19	350000	0	12.10.2005	20	.	1	79.5	12.6	145	62.5	0	0	1	0	2	52	1	1.76	2	20	25.7	3	56.27
4282	19	.	0	13.10.2005	7.97	5.472	0	79.9	12.8	142	63.5	0	0	2	1	2	52	1	1.76	2	20	25.8	3	56.4
4283	19	.	0	13.10.2005	7.97	8.487	0	79.9	12.8	142	63.5	0	0	3	2	2	52	1	1.76	2	20	25.8	3	56.4
4284	19	350000	0	13.10.2005	8.08	.	1	79.9	12.8	142	63.5	0	0	1	0	2	52	1	1.76	2	20	25.8	3	56.4
4285	19	.	0	13.10.2005	8.08	8.487	0	79.9	12.8	142	63.5	0	0	3	2	2	52	1	1.76	2	20	25.8	3	56.4
4286	19	.	0	13.10.2005	8.32	5.775	0	79.9	12.8	142	63.5	0	0	2	1	2	52	1	1.76	2	20	25.8	3	56.4
4287	19	.	0	13.10.2005	8.32	9.310	0	79.9	12.8	142	63.5	0	0	3	2	2	52	1	1.76	2	20	25.8	3	56.4
4288	19	.	0	13.10.2005	8.57	5.710	0	79.9	12.8	142	63.5	0	0	2	1	2	52	1	1.76	2	20	25.8	3	56.4
4289	19	.	0	13.10.2005	8.57	10.335	0	79.9	12.8	142	63.5	0	0	3	2	2	52	1	1.76	2	20	25.8	3	56.4
4290	19	.	0	13.10.2005	9.17	7.243	0	79.9	12.8	142	63.5	0	0	2	1	2	52	1	1.76	2	20	25.8	3	56.4
4291	19	.	0	13.10.2005	9.17	11.026	0	79.9	12.8	142	63.5	0	0	3	2	2	52	1	1.76	2	20	25.8	3	56.4
4292	19	.	0	13.10.2005	9.63	7.265	0	79.9	12.8	142	63.5	0	0	2	1	2	52	1	1.76	2	20	25.8	3	56.4
4293	19	.	0	13.10.2005	9.63	10.534	0	79.9	12.8	142	63.5	0	0	3	2	2	52	1	1.76	2	20	25.8	3	56.4
4294	19	.	0	13.10.2005	10.13	7.098	0	79.9	12.8	142	63.5	0	0	2	1	2	52	1	1.76	2	20	25.8	3	56.4
4295	19	.	0	13.10.2005	10.13	10.599	0	79.9	12.8	142	63.5	0	0	3	2	2	52	1	1.76	2	20	25.8	3	56.4
4296	19	.	0	13.10.2005	11.12	7.062	0	79.9	12.8	142	63.5	0	0	2	1	2	52	1	1.76	2	20	25.8	3	56.4
4297	19	.	0	13.10.2005	11.12	9.967	0	79.9	12.8	142	63.5	0	0	3	2	2	52	1	1.76	2	20	25.8	3	56.4
4298	19	.	0	13.10.2005	12.1	6.732	0	79.9	12.8	142	63.5	0	0	2	1	2	52	1	1.76	2	20	25.8	3	56.4
4299	19	.	0	13.10.2005	12.1	9.346	0	79.9	12.8	142	63.5	0	0	3	2	2	52	1	1.76	2	20	25.8	3	56.4
4300	19	.	0	13.10.2005	14.07	6.534	0	79.9	12.8	142	63.5	0	0	2	1	2	52	1	1.76	2	20	25.8	3	56.4
4301	19	.	0	13.10.2005	14.07	9.560	0	79.9	12.8	142	63.5	0	0	3	2	2	52	1	1.76	2	20	25.8	3	56.4
4302	19	.	0	13.10.2005	16.18	6.009	0	79.9	12.8	142	63.5	0	0	2	1	2	52	1	1.76	2	20	25.8	3	56.4
4303	19	.	0	13.10.2005	16.18	9.702	0	79.9	12.8	142	63.5	0	0	3	2	2	52	1	1.76	2	20	25.8	3	56.4
4304	19	.	0	13.10.2005	18.18	5.948	0	79.9	12.8	142	63.5	0	0	2	1	2	52	1	1.76	2	20	25.8	3	56.4
4305	19	.	0	13.10.2005	18.18	9.776	0	79.9	12.8	142	63.5	0	0	3	2	2	52	1	1.76	2	20	25.8	3	56.4
4306	19	.	0	13.10.2005	19.87	5.881	0	79.9	12.8	142	63.5	0	0	2	1	2	52	1	1.76	2	20	25.8	3	56.4
4307	19	350000	0	13.10.2005	20.08	.	1	79.9	12.8	142	63.5	0	0	1	0	2	52	1	1.76	2	20	25.8	3	56.4
4308	19	.	0	14.10.2005	8.43	5.670	0	79.8	10.6	142	64.6	0	0	2	1	2	52	1	1.76	2	20	25.8	3	56.36
4309	19	350000	0	14.10.2005	8.5	.	1	79.8	10.6	142	64.6	0	0	1	0	2	52	1	1.76	2	20	25.8	3	56.36

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X
4739	20	125000	0	12.12.2005	20	.	1	68	10.2	102	78	0	0	1	0	1	56	2	1.7	8	10	23.5	3	47.9
4740	20	0	0	12.12.2005	22	0	2	68	10.2	102	78	0	0	2	1	1	56	2	1.7	8	10	23.5	3	47.9
4741	20	150000	0	13.12.2005	6	.	1	68	10.2	102	78	0	0	1	0	1	56	2	1.7	8	10	23.5	3	47.9
4742	20	0	0	13.12.2005	8	0	2	68	10.2	102	78	0	0	2	1	1	56	2	1.7	8	10	23.5	3	47.9
4743	20	125000	0	13.12.2005	20	.	1	68	10.2	102	78	0	0	1	0	1	56	2	1.7	8	10	23.5	3	47.9
4744	20	0	0	13.12.2005	22	0	2	68	10.2	102	78	0	0	2	1	1	56	2	1.7	8	10	23.5	3	47.9
4745	20	150000	0	14.12.2005	6	.	1	68	10.2	102	78	0	0	1	0	1	56	2	1.7	8	10	23.5	3	47.9
4746	20	0	0	14.12.2005	8	0	2	68	10.2	102	78	0	0	2	1	1	56	2	1.7	8	10	23.5	3	47.9
4747	20	125000	0	14.12.2005	20	.	1	68	10.2	102	78	0	0	1	0	1	56	2	1.7	8	10	23.5	3	47.9
4748	20	0	0	14.12.2005	22	0	2	68	10.2	102	78	0	0	2	1	1	56	2	1.7	8	10	23.5	3	47.9
4749	20	150000	0	15.12.2005	6	.	1	69	8.3	102	79.2	0	0	1	0	1	56	2	1.7	8	10	23.9	3	48.19
4750	20	.	0	15.12.2005	8	7.012	0	69	8.3	102	79.2	0	0	2	1	1	56	2	1.7	8	10	23.9	3	48.19
4751	20	.	0	15.12.2005	8	10.456	0	69	8.3	102	79.2	0	0	3	2	1	56	2	1.7	8	10	23.9	3	48.19
4752	20	125000	0	15.12.2005	20	.	1	69	8.3	102	79.2	0	0	1	0	1	56	2	1.7	8	10	23.9	3	48.19
4753	20	0	0	15.12.2005	22	0	2	69	8.3	102	79.2	0	0	2	1	1	56	2	1.7	8	10	23.9	3	48.19
4754	20	150000	0	16.12.2005	6	.	1	69	8.3	102	79.2	0	0	1	0	1	56	2	1.7	8	10	23.9	3	48.19
4755	20	0	0	16.12.2005	8	0	2	69	8.3	102	79.2	0	0	2	1	1	56	2	1.7	8	10	23.9	3	48.19
4756	20	125000	0	16.12.2005	20	.	1	69	8.3	102	79.2	0	0	1	0	1	56	2	1.7	8	10	23.9	3	48.19
4757	20	0	0	16.12.2005	22	0	2	69	8.3	102	79.2	0	0	2	1	1	56	2	1.7	8	10	23.9	3	48.19
4758	20	150000	0	17.12.2005	6	.	1	69	8.3	102	79.2	0	0	1	0	1	56	2	1.7	8	10	23.9	3	48.19
4759	20	0	0	17.12.2005	8	0	2	69	8.3	102	79.2	0	0	2	1	1	56	2	1.7	8	10	23.9	3	48.19
4760	20	125000	0	17.12.2005	20	.	1	69	8.3	102	79.2	0	0	1	0	1	56	2	1.7	8	10	23.9	3	48.19
4761	20	0	0	17.12.2005	22	0	2	69	8.3	102	79.2	0	0	2	1	1	56	2	1.7	8	10	23.9	3	48.19
4762	20	150000	0	18.12.2005	6	.	1	69	8.3	102	79.2	0	0	1	0	1	56	2	1.7	9	10	23.9	3	48.19
4763	20	0	0	18.12.2005	8	0	2	69	8.3	102	79.2	0	0	2	1	1	56	2	1.7	9	10	23.9	3	48.19
4764	20	125000	0	18.12.2005	20	.	1	69	8.3	102	79.2	0	0	1	0	1	56	2	1.7	9	10	23.9	3	48.19
4765	20	0	0	18.12.2005	22	0	2	69	8.3	102	79.2	0	0	2	1	1	56	2	1.7	9	10	23.9	3	48.19
4766	20	125000	0	19.12.2005	6	.	1	69	10.8	105	76.1	0	0	1	0	1	56	2	1.7	9	10	23.9	3	48.19
4767	20	0	0	19.12.2005	8	0	2	69	10.8	105	76.1	0	0	2	1	1	56	2	1.7	9	10	23.9	3	48.19
4768	20	.	0	19.12.2005	8	9.218	0	69	10.8	105	76.1	0	0	3	2	1	56	2	1.7	9	10	23.9	3	48.19
4769	20	125000	0	19.12.2005	20	.	1	69	10.8	105	76.1	0	0	1	0	1	56	2	1.7	9	10	23.9	3	48.19
4770	20	0	0	19.12.2005	22	0	2	69	10.8	105	76.1	0	0	2	1	1	56	2	1.7	9	10	23.9	3	48.19

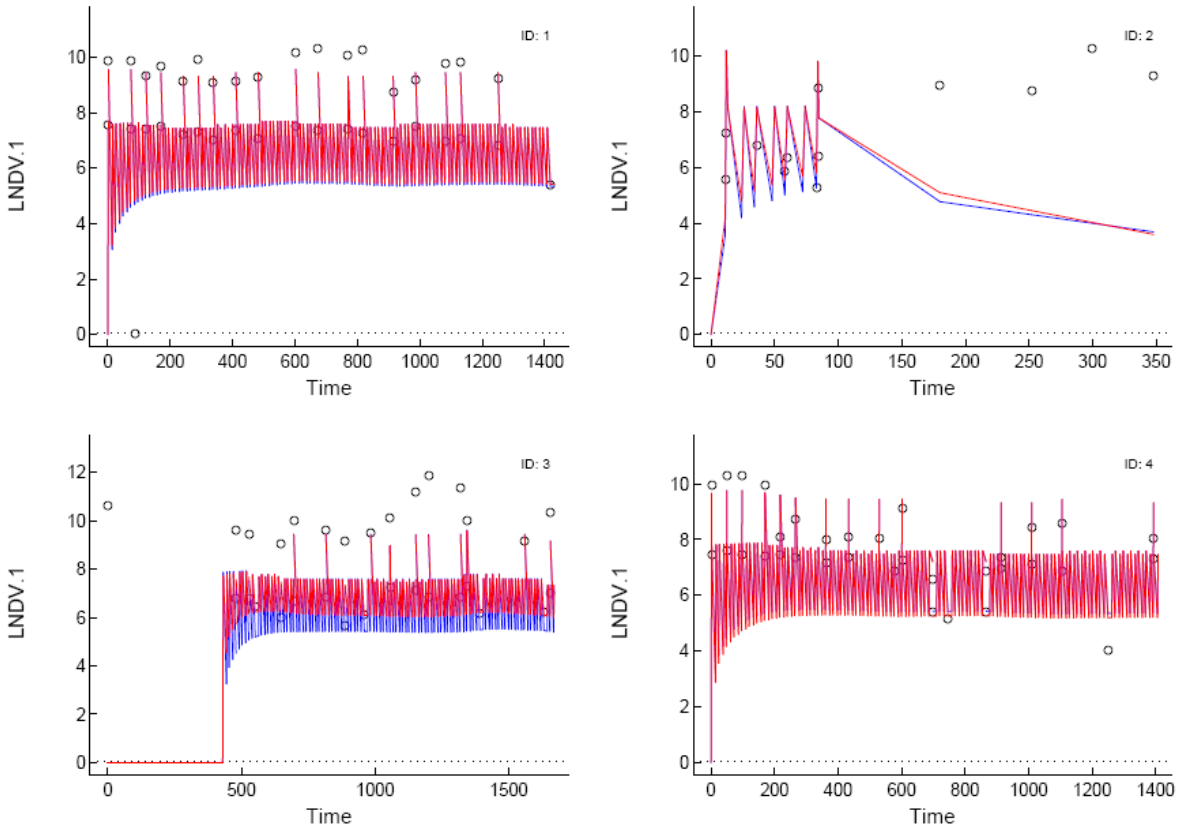
9.6 Control file for final model whole blood and intracellular concentrations

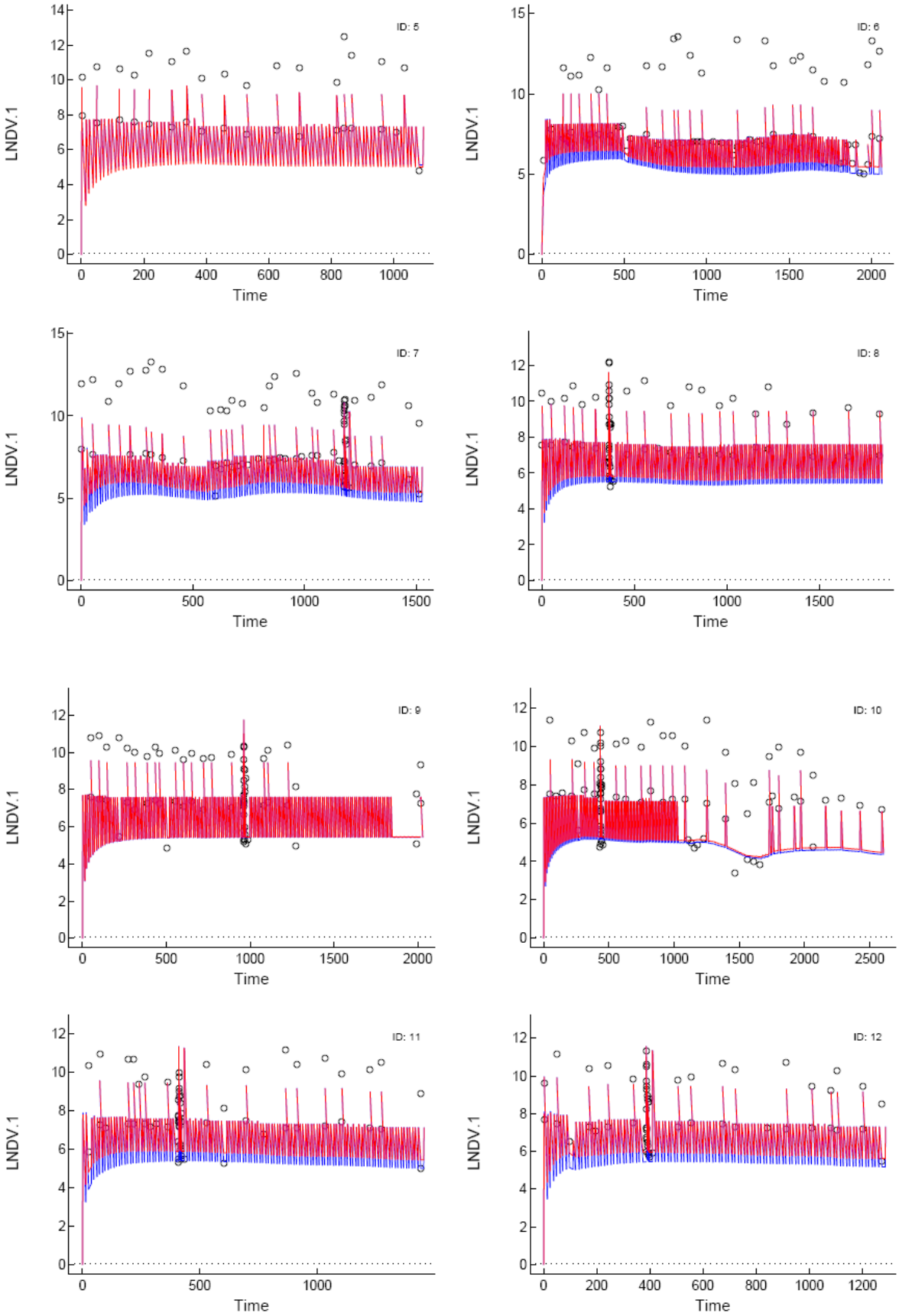
```
$PROBLEM EXAMPLE OF A THREE COMPARTMENT MODEL ABSORPTION INCLUDING
INTRACELLULAR VALUES AS METABOLITES

$INPUT ID AMT RATE=DROP DAT1=DROP TIME LNDV=DV EVID WT=DROP UREA=DROP
SCR=DROP CRCL=DROP SS=DROP II=DROP CMT DVID FLAG=DROP AGE=DROP GEN=DROP
HGHT=DROP TXT=DROP STER=DROP BMI=DROP C3A5=DROP LBM=DROP

$DATA ic.txt

$SUBROUTINES ADVAN6 TOL=4

$MODEL

COMP=(ABSORB)
COMP=(CENTRAL)
COMP=(INTRAC)
COMP=(PERIPH)

$PK
KA=THETA(1)*EXP(ETA(1))
CL2=THETA(2)
CL3=THETA(3)
CL4=THETA(4)*EXP(ETA(2))
V2=THETA(5)
V3=THETA(6)
V4=THETA(7)

K12=KA ; for matrix exponential solution
K20=CL2/V2
K23=CL3/V2
K32=CL3/V3
K24=CL4/V2
K42=CL4/V4

S2=V2
S3=V3
S4=V4

A_0(2)=0
A_0(3)=0
A_0(4)=0


$DES
DADT(1)= -KA*A(1)
DADT(2)= KA*A(1)-(A(2)*(K20+K23+K24))+A(3)*K32+A(4)*K42
DADT(3)= A(2)*K23-A(3)*K32
DADT(4)= A(2)*K24-A(4)*K42

$ERROR
PLASMA=A(2)/V2
INTRAC=A(3)/V3

IPRED=A(2)/V2
IF (DVID.EQ.2) INTRAC=A(3)/V3
```

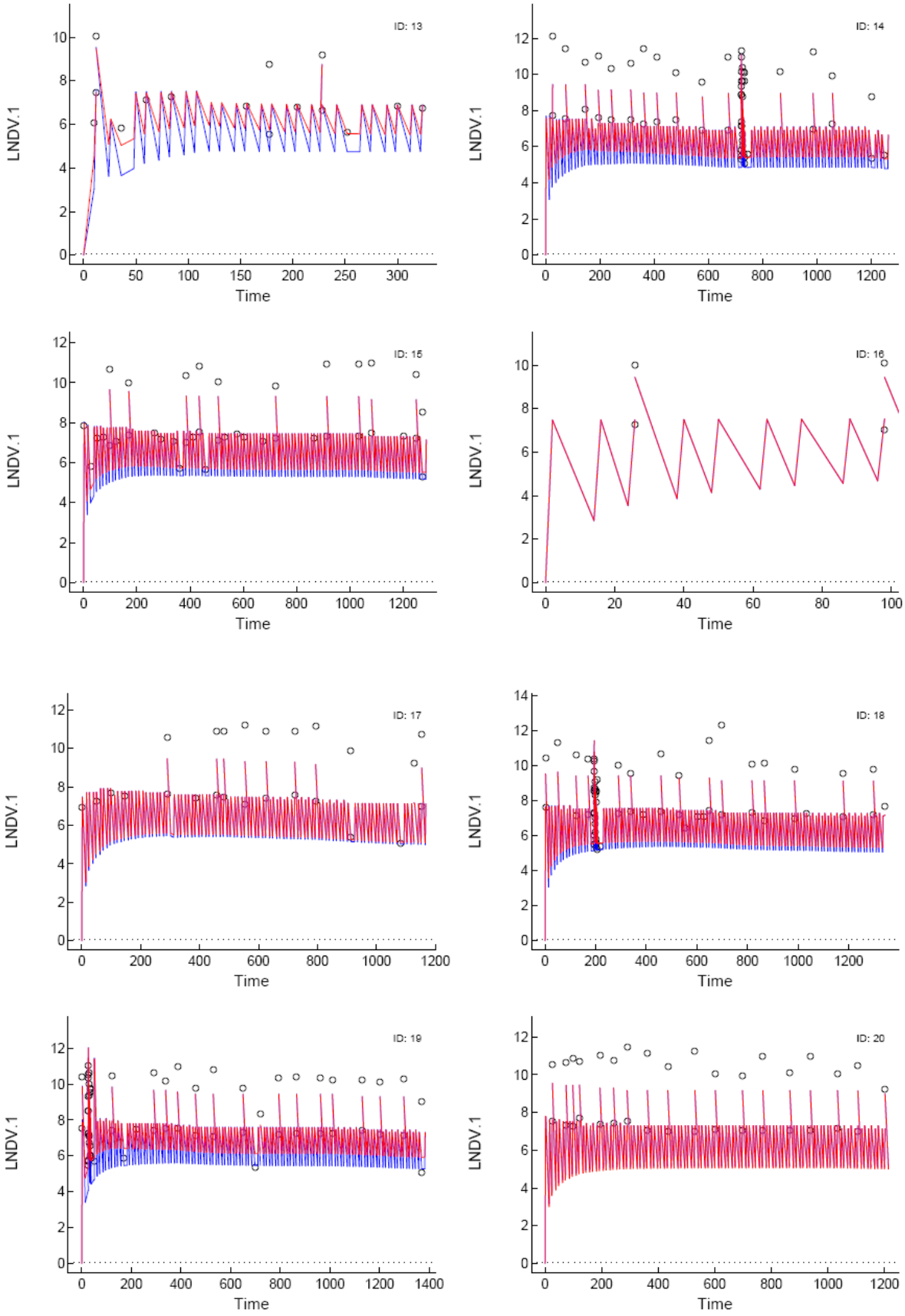
```
IPRED=0.001
IF(F.GT.0) IPRED=LOG(F)
W=SQRT(THETA(7)**2+THETA(8)**2/(F+0.001)**2)
IRES=DV-IPRED
IWRES = IRES/W
Y=IPRED+W*ERR(1)

$THETA
(0, 55)
(0, 3)
(0, 0.002)
(0, 0.9)
(0, 1.5)
(0, 0.006)
(0, 17.3)
(0.3)

$OMEGA
0.00136
1

$SIGMA
0.008

$ESTIMATION SIG=3 METHOD=1 INTER MAXEVAL=9999 PRINT=1 POSTHOC NOABORT

$TABLE ID TIME DV DVID IPRED MDV
NOPRINT ONEHEADER FILE=table.txt

$TABLE ID CL V1 V2 V3 KA ETA1 ETA2 ETA3 ETA4 ETA5 ETA6 ETA7 WT CRCL FLAG
AGE GEN HGHT TXT STER BMI C3A5 LBM FIRSTONLY NOPRINT ONEHEADER NOAPPEND
FILE=etatable.txt
```

9.7 Diagnostic plot made by R for whole blood and intracellular model

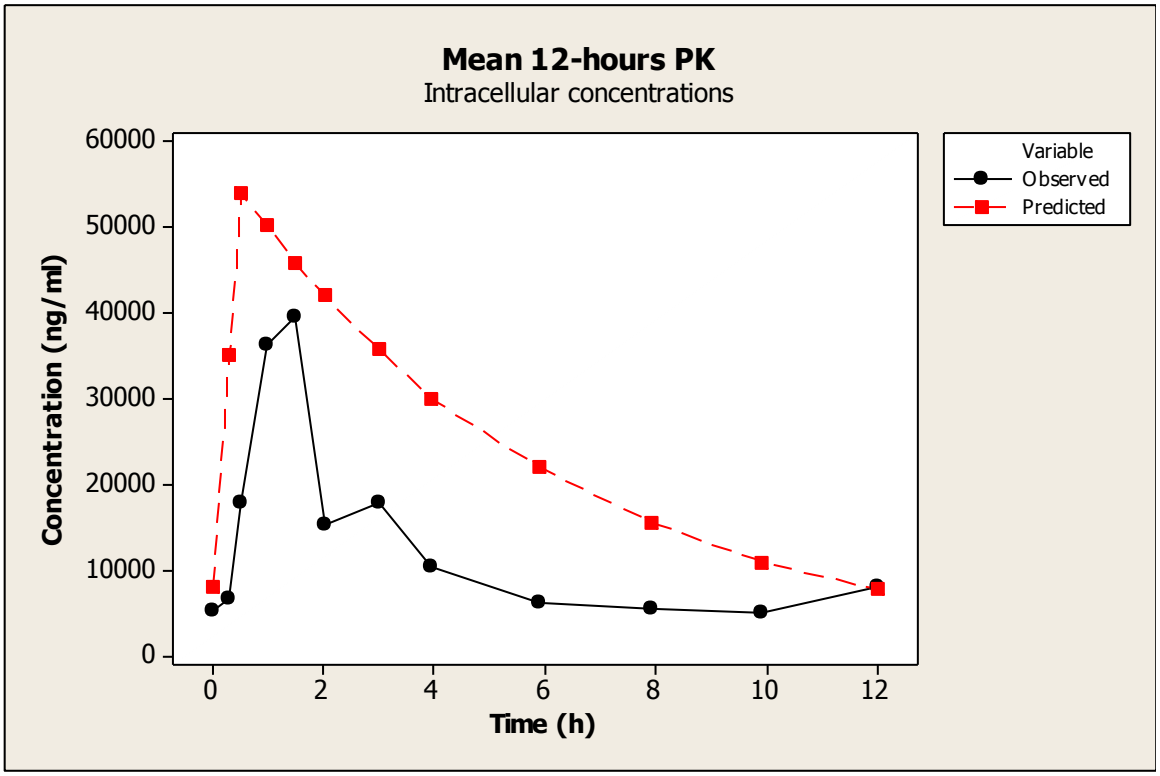
Circles: Observed concentrations; Red: Individual post hoc predicted concentrations; Blue: Population predicted concentrations.

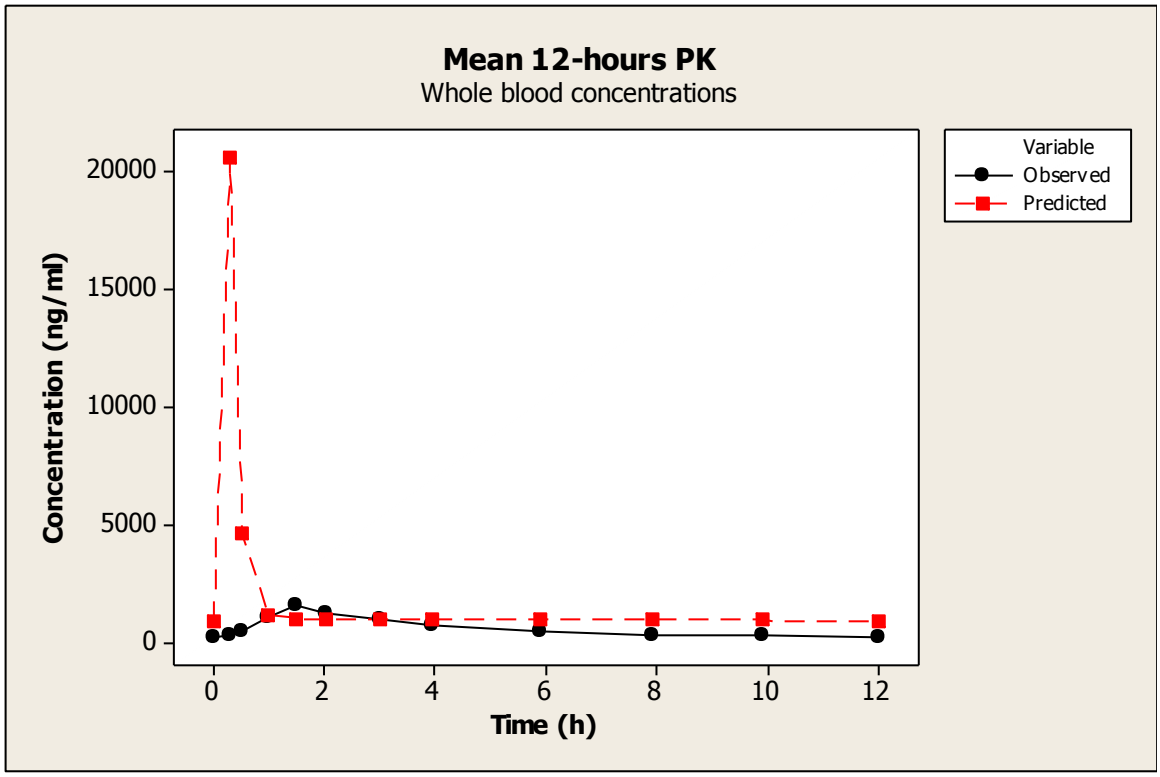


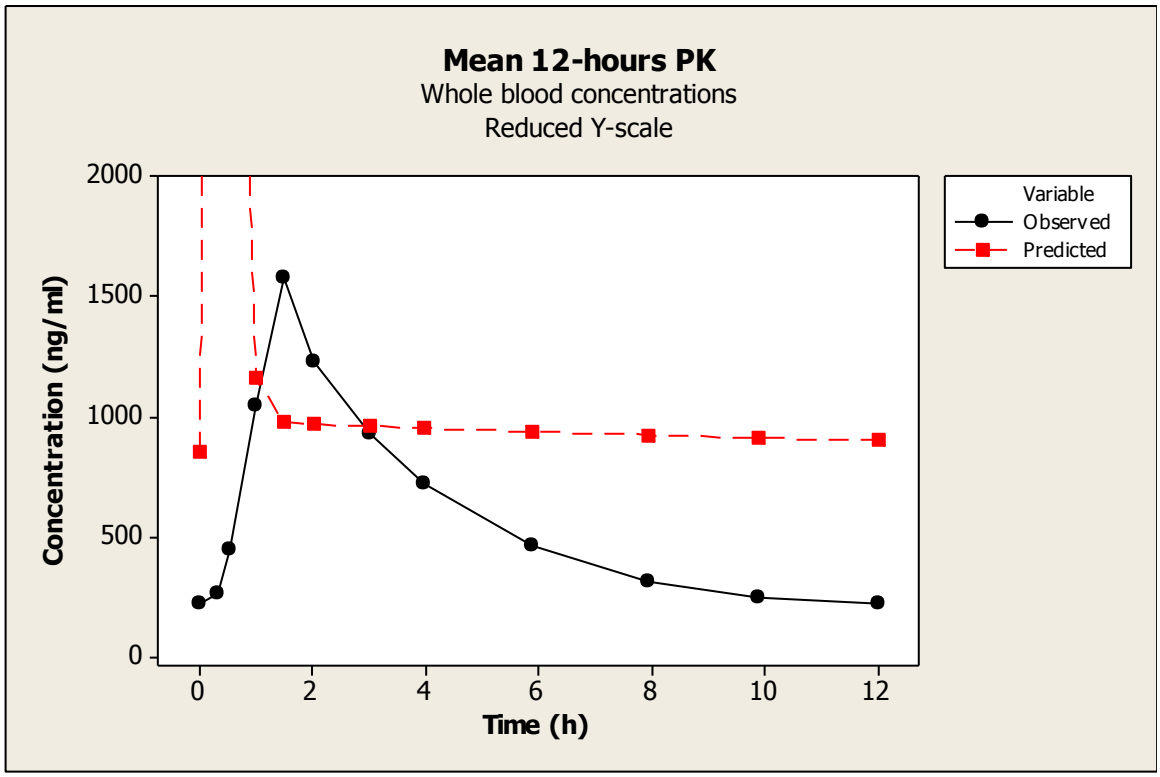

ID: 5
LNDV.1
Time
ID: 6
LNDV.1
Time
ID: 7
LNDV.1
Time
ID: 8
LNDV.1
Time
ID: 9
LNDV.1
Time
ID: 10
LNDV.1
Time
ID: 11
LNDV.1
Time
ID: 12
LNDV.1
Time

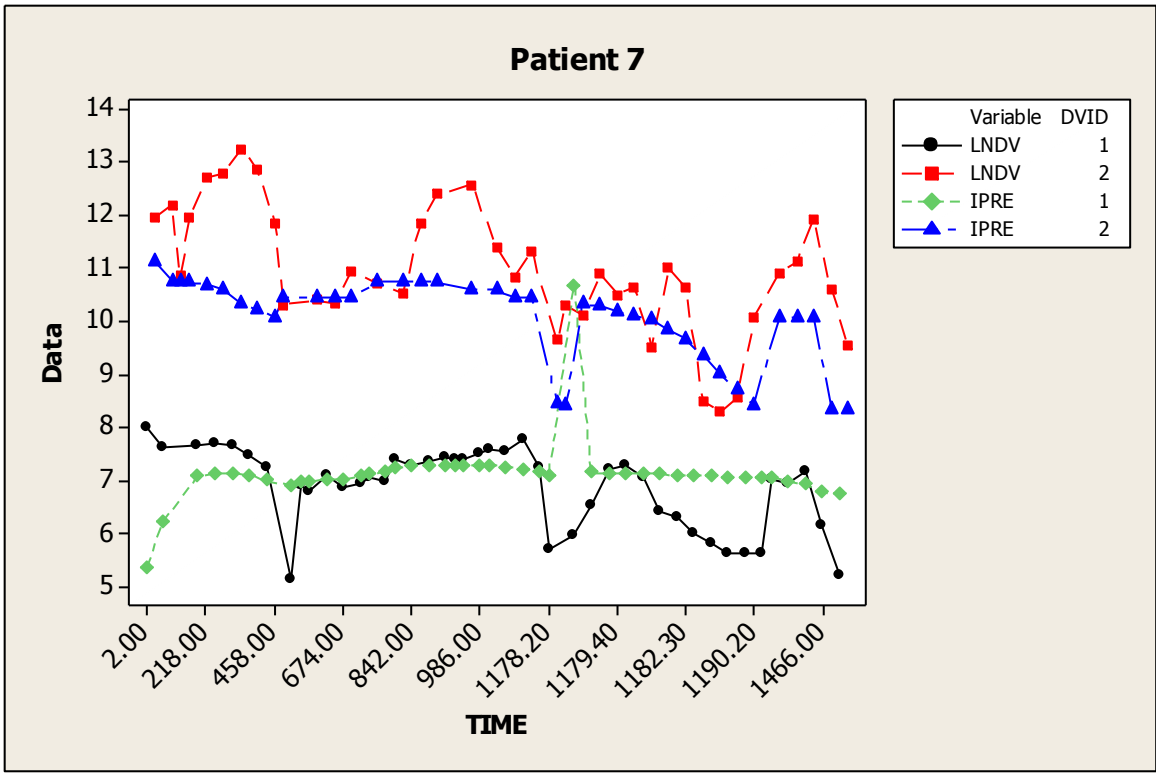

ID: 13
ID: 14
ID: 15
ID: 16
ID: 17
ID: 18
ID: 19
ID: 20
LNDV.1
Time

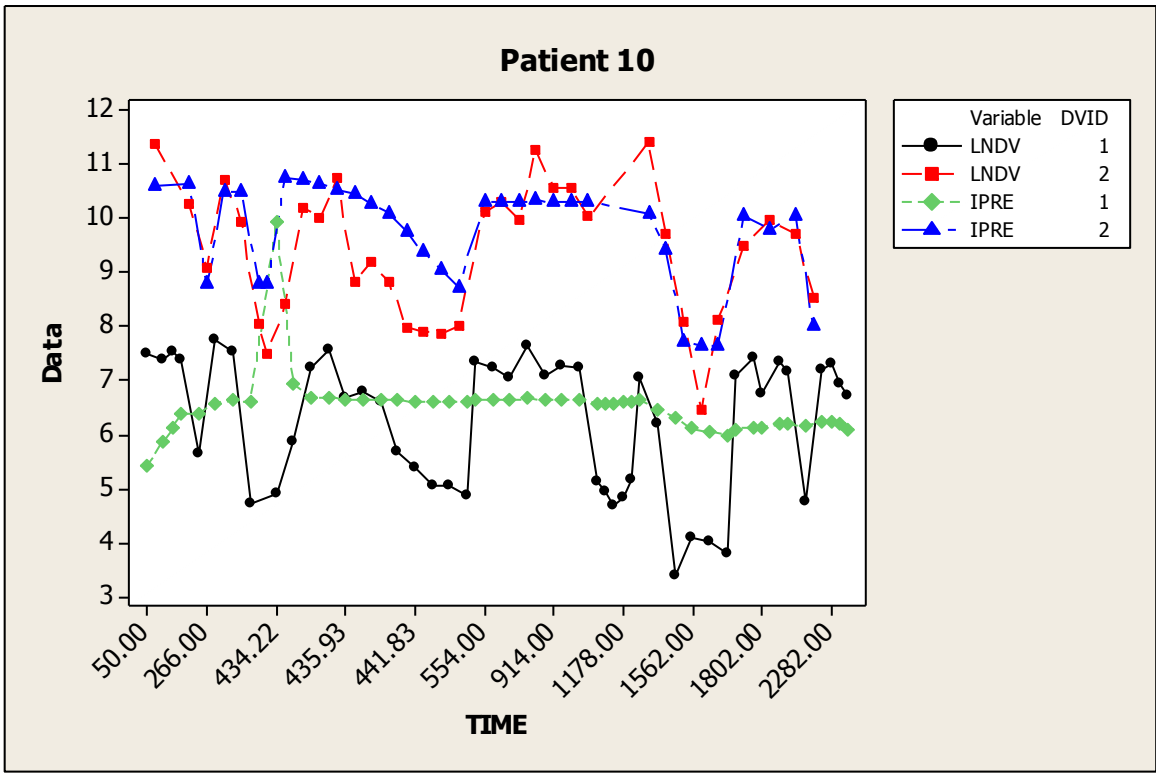
9.8 Diverse figures for whole blood and intracellular model with a lower Ka and OFV comparing with the final model

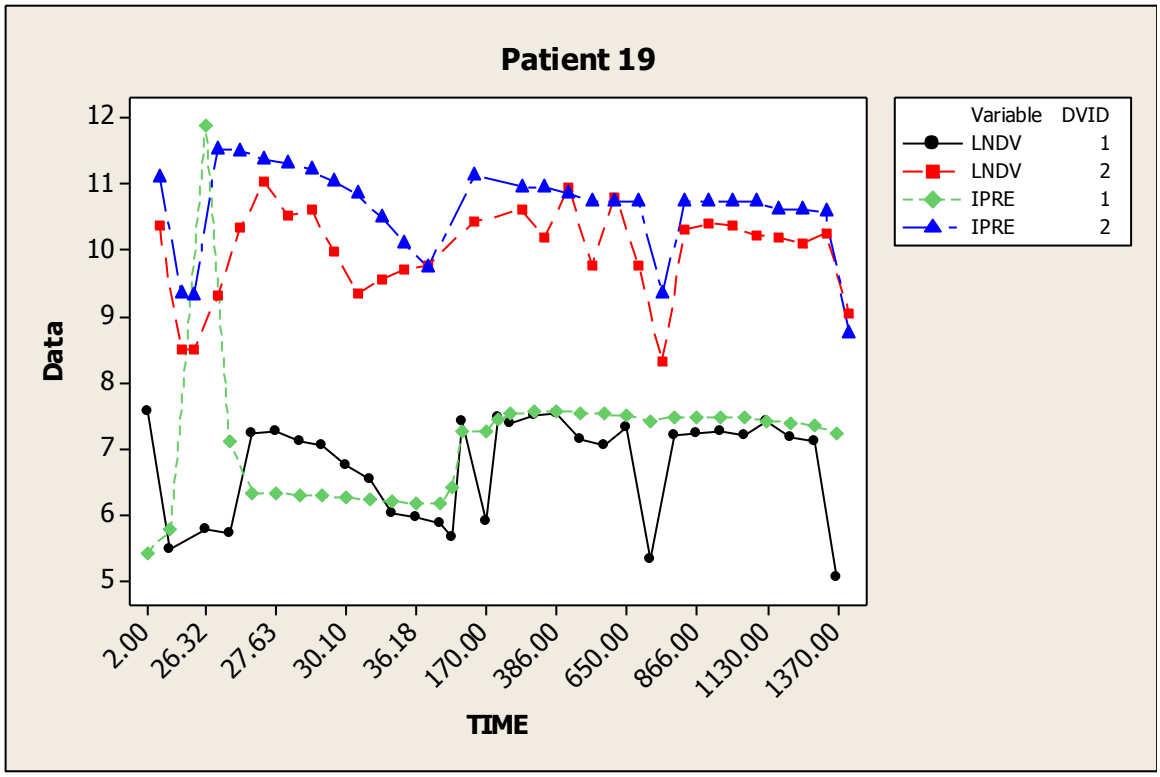



Mean 12-hours PK
Whole blood concentrations
Concentration (ng/ml)
20000
15000
10000
5000
0
0
2
4
6
8
10
12
Time (h)
Variable
Observed
Predicted


Mean 12-hours PK
Whole blood concentrations
Reduced Y-scale
Concentration (ng/ml)
2000
1500
1000
500
0
0
2
4
6
8
10
12
Time (h)
Variable
Observed
Predicted


Patient 7
Data
TIME
Variable DVID
LNDV 1
LNDV 2
IPRE 1
IPRE 2
14
13
12
11
10
9
8
7
6
5
2.00
218.00
458.00
674.00
842.00
986.00
1178.20
1179.40
1182.30
1190.20
1466.00


Patient 10
Data
TIME
Variable DVID
LNDV 1
LNDV 2
IPRE 1
IPRE 2
12
11
10
9
8
7
6
5
4
3
50.00
266.00
434.22
435.93
441.83
554.00
914.00
1178.00
1562.00
1802.00
2282.00


Patient 19
Data
TIME
12
11
10
9
8
7
6
5
2.00
26.32
27.63
30.10
36.18
170.00
386.00
650.00
866.00
1130.00
1370.00
Variable DVID
LNDV 1
LNDV 2
IPRE 1
IPRE 2