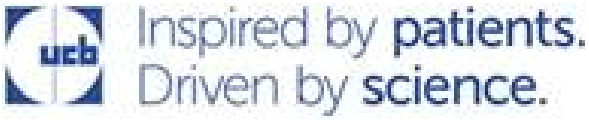

Development of a Combined Pharmacokinetic Model for Lacosamide and its Metabolite for Integrated Pharmacokinetic Modeling in Humans

INAUGURAL DISSERTATION

zur Erlangung des Doktorgrades

der Mathematisch-Naturwissenschaftlichen Fakultät

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vorgelegt von

Carina Schäfer

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I. ERKLÄRUNG ZUR DISSERTATION

Ich versichere an Eides statt, dass die Dissertation von mir selbstständig und ohne unzulässige fremde Hilfe unter Beachtung der "Grundsätze zur Sicherung guter wissenschaftlicher Praxis an der Heinrich-Heine-Universität Düsseldorf" erstellt worden ist. Die Dissertation wurde in der vorgelegten oder in ähnlicher Form noch bei keiner anderen Institution eingereicht. Ich habe bisher keine erfolglosen Promotionsversuche unternommen.

Düsseldorf den 12.10.2015 Carina Schäfer

II. DANKSAGUNG

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III. ZUSAMMENFASSUNG

Ziel der Pharmakokinetik ist es den zeitlichen Ablauf von Arzneistoffkonzentrationen im Körper zu beschreiben, um daraus optimale Dosierungen zu ermitteln. Pharmakokinetische Modelle in diesem Gebiet repräsentieren die Verbindung zwischen Arzneistoffkonzentrationsverläufen und der Arzneimitteldosis und bilden somit die fundamentale Basis für die Charakterisierung des optimalen Dosisschemas. In der vorliegenden Dissertation wurde herausgearbeitet, welche Schritte für die Entwicklung eines solchen pharmakokinetischen Modells nötig sind. Das Ziel war die Entwicklung eines definierten pharmakokinetischen Modells, welches an Studiendaten von gesunden Probanden und Probanden mit eingeschränkter Nierenfunktion unter der antiepileptischen Therapie von Lacosamide angewendet werden konnte.

Um zu bewerten, inwieweit Faktoren wie Alter und Geschlecht in der Entwicklung eines pharmakokinetischen Modells berücksichtigt werden mussten, wurde zunächst der Einfluss von Alter und Geschlecht auf die Pharmakokinetik von Lacosamide untersucht. Gegenstand der Fragstellung war eine post-hoc Analyse von pharmakokinetischen Daten oral appliziertem Lacosamides an gesunden männlichen und weiblichen Probanden verschiedenen Alters. Um zu evaluieren, inwieweit die Ergebnisse auf Patienten mit fokaler Epilepsie anzuwenden sind, wurde ein Vergleich der in der Dissertation erzielten Ergebnisse mit Literaturergebnissen einer populationskinetischen Auswertung von Laocsamide an Patienten mit fokaler Epilepsie durchgeführt. Dabei konnte gezeigt werden, dass Alter und Geschlecht keinen Einfluss auf die Kinetik von Lacosamide haben und somit Unterschiede in den Plasmakonzentrationen und pharmakokinetischen Parametern allein durch Skalierungsfaktoren, wie Körpergewicht, Körpergröße, approximiertem Verteilungsvolumen und fettfreier Körpermasse, erklärt werden konnten. Aus diesem Grund war es nicht notwendig diese Faktoren in der pharmakokinetischen Modellentwicklung zu berücksichtigen.

Mit diesen Erkenntnissen wurde ein pharmakokinetisches Modell entwickelt, welches die Kinetik eines Arzneistoffes und dessen Hauptmetaboliten im Plasma, sowie die Ausscheidungskinetik des unveränderten Arzneistoffes in den Urin beschreibt. Um letztendlich das entwickelte pharmakokinetische Modell an echten

Studiendaten anwenden zu können, war es nötig, die Prozesse innerhalb der ausgewählten Software an Hand einer fiktiv entwickelten Studienpopulation hinsichtlich ihrer Eignung für eine Modellierung zu validieren.

Da physiologische Einschränkungen, wie renale Dysfunktion, zu Änderungen in der Kinetik eines Arzneistoffes und damit zu Änderungen in Dosierungsregimen führen können, wurde eine Studie ausgewählt, die sowohl auf gesunden Probanden als auch auf Probanden mit eingeschränkter Nierenfunktion basierte. Dabei wurde gezeigt, dass das entwickelte Modell sowohl an gesunden, als auch an renal eingeschränkten Probanden Anwendung finden kann.

Zusammenfassend zeigt diese Dissertation die Entwicklung eines pharmakokinetischen Modells, welches die Kinetik eines Arzneistoffes und dessen Metaboliten im Plasma, als auch die Ausscheidungskinetik des Arzneistoffes in den Urin beschreibt und kombiniert. Dadurch konnte erklärt werden, dass die Summe der Gesamtelimination von Lacosamide aus renaler und metabolischer Elimination bestand. Das Modell wurde darüber hinaus zur Simulation von Plasmakonzentrationsverläufen auch bei renal eingeschränkten Probanden genutzt, und bietet somit den Grundstein für eine optimale Pharmakotherapie mit Lacosamide. Des Weiteren kann dieses Modell auch Anwendbarkeit an Studiendaten anderer Arzneistoffe finden, um das Verständnis des Zusammenspiels von Arzneistoffmetabolismus und renaler Elimination zu erweitern und es für eine gesicherte Pharmakotherapie vor allem in speziellen Populationen zu nutzen.

IV. SUMMARY

The main aim of pharmacokinetics is to describe the temporary process of a drug in the human body to investigate the optimal dose regimen. In this context, pharmacokinetic models represent the connection between drug concentration time profiles and drug dose and thus provide the fundamental basis for the characterisation of optimal drug dosing regimens. In the present dissertation, it was ascertained what steps were necessary to develop a pharmacokinetic model. The aim was to develop a defined pharmacokinetic model that could be applied to study data of healthy and renal impaired subjects under the therapy of the anti-epileptic drug lacosamide.

To investigate the extent to which age and gender have to be considered in developing a pharmacokinetic model, the influence of both factors on the pharmacokinetic of lacosamide was evaluated. The objective was a post-hoc analysis of pharmacokinetic data from orally administered lacosamide in healthy female and male subjects of different age. To evaluate the extent to which the results of healthy subjects were in line with those of patients, a comparison between the results obtained in the present thesis and those taken from a population pharmacokinetic analysis of patients with focal epilepsy receiving oral lacosamide was conducted. It could be shown that age and gender had no relevant effect on the pharmacokinetics of lacosamide, whereas differences in plasma concentrations and pharmacokinetic parameters could be explained by scaling factors such as body weight, height, approximated volume of distribution, fat-free mass and lean body weight. For this reason, it was not necessary to consider the factors age and gender during the pharmacokinetic model development.

By considering these findings, a new pharmacokinetic model was developed that included the model-dependent pharmacokinetic of unchanged drug and its main metabolite in plasma, as well as the pharmacokinetic of unchanged drug excreted in urine. To finally use the mathematical model for pharmacokinetic modeling with data of healthy and renal impaired subjects, it was necessary to validate the processes in the chosen software by using a generated fictive study population.

Since physiological restrictions such as renal impairment could lead to alterations in the pharmacokinetic profile of a drug and thus could lead to changes in

the dosing regimen, a study based upon healthy subjects as well as subjects with limited renal function was chosen. In the process, it was shown that the model could find implementation in healthy and renal impaired subjects.

In summary, the present thesis could demonstrate that the overall elimination of lacosamide comprised the sum of metabolism and renal elimination explainable through developing a pharmacokinetic model that combined the pharmacokinetic of unchanged drug and its main metabolite in plasma, as well as the excretion of unchanged drug in urine. The model could be used to stimulate plasma concentration time curves of healthy subjects and subjects with renal impairment and thus it represents the basis for an optimal pharmacotherapy with lacosamide. As a perspective, the model could be used for pharmacokinetic modeling with study data of other drugs to broaden the understanding of a drug's metabolism and its renal elimination. The final aim would always be to ensure safe pharmacotherapy, especially in populations with physiological restrictions such as renal impairment.

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ANOVA	Analysis of Variance
ADME	Absorption, Distribution, Metabolism, Elimination
ADR	Adverse Drug Reaction
$Ae_{\tau,ss}$	Amount excreted in urine
$AUC_{\tau,ss}$	Area under the plasma concentration time curve at steady state from time 0 of the last quantifiable concentration
AED	Anti-epileptic Drug
BH	Body Height
BMI	Body Mass Index
BW	Body Weight
Cl	Clearance
Cl_{CR}	Creatinine Clearance
CL/F	Total Body Clearance
CL_R	Renal Clearance
$C_{max,ss}$	Maximal Plasma Concentration
Conc	Concentration
Cp	Drug plasma concentration
CV	Coefficient of Variation
D	Drug
F	Relative bioavailability
FDA	Food and Drug Administration
FFM	Fat-Free Mass
f(t)	Time-dependent function
GFR	Glomerular Filtration Rate
GCP	Good Clinical Practice

ICH	International Conference of Harmonisation
inROA	Route of administration
IV	Intravenous
k_a	Rate constant of absorption
k_e	Rate constant of elimination
k_m	Rate constant of metabolism
k_{me}/k_{mu}	Rate constant of elimination of the metabolite
k_{other}	Other rate constants of elimination
k_{ren}	Renal rate constant of elimination
L	Litre
LBW	Lean Body Weight
LCM	Lacosamide
LLQ	Lower Limit of Quantification
LS	Least Squares
M	Metabolite
N	Number of Subjects
POS	Partial Onset Seizures
RMSE	Root Mean Square error
SAS	Statistical Analysis System
SD	Standard Deviation
t	time
$t_{1/2}$	Terminal half-life
t_{lag}	Absorption lag time
$t_{max,(ss)}$	time to maximum plasma concentration (at steady state)
TEAE	Treatment-emergent Adverse Event
U_d	Amount of unchanged drug in urine

U_m	Amount of metabolite in urine
V_d	Volume of Distribution
WHO	World Health Organization

1 CHAPTER 1: INTRODUCTION, MOTIVATION AND AIM OF THE THESIS

1.1 Introduction

In the area of drug development, pharmacokinetic modeling and simulation represent important tools for the integration of data to make rational decisions regarding drug use and drug development (Atkinson and Lalonde, 2007). The selection of dose and the choice of dosing regimens stress the importance of modeling and simulation in pharmacokinetics to achieve appropriate dosing schedules (Atkinson and Lalonde, 2007, Mould and Upton, 2012). It is necessary to develop and understand the mathematical context of a drug in different tissues and blood fluids to understand dose- and concentration-related adverse events such as toxic effects as well as therapeutic effective plasma levels (Dhillon and Gill, 2006).

When speaking of pharmacokinetics, it is necessary to differentiate between the pharmacokinetic compartmental, non-compartmental modeling and physiological based pharmacokinetic modeling, whereas all approaches are able to incorporate a pharmacodynamic component. The first pharmacokinetic studies were conducted by Widmark in 1919 (Widmark, 1919), using a single-compartment open model to characterise drug distribution, elimination and accumulation, whereas Teorell attempted a more physiological analysis of drug distribution with a two-compartment model in 1937 (Teorell, 1937). The non-compartmental analysis represents the preferred tool if the primary requirement is to determine pharmacokinetic parameters such as the degree of exposure expressed as the area under the concentration time curve, or the elimination half-life (Gabrielsson and Weiner, 2012). Furthermore, the non-compartment analysis requires fewer assumptions than model-based approaches and makes fewer assumptions about the underlying model (Atkinson and Lalonde, 2007, Gabrielsson and Weiner, 2012). Nevertheless, the building block of many pharmacokinetic models is the ‘compartment’, which describes a closed homogenous space for the transport processes of a drug and which could be the total blood volume (central compartment), the muscle or fat tissue (peripheral compartments), for example. However, physiological models represent an approach to conduct extrapolations across species and they are designed to execute simulations of

pharmacokinetic profiles under different physiological conditions (Espié, Tytgat et al., 2009).

Several pharmacokinetic textbooks exist describing the model-dependent pharmacokinetics of a drug in one tissue (e.g. drug in plasma or amount of drug excreted in urine). Given that most of the drugs are eliminated by pathways of excretion and metabolism, pharmacokinetic models that include parallel elimination pathways represent progress in developing new pharmacokinetic models. The main aim of the present thesis is to develop a pharmacokinetic model that combines the pharmacokinetics of a drug and its main metabolite in plasma as well as the profile of the cumulative amount of unchanged drug excreted in urine. This should lead to a better understanding of a drug's behaviour in populations characterised by physiological restrictions such as renal impairment, as well as in populations with different body compositions. To conduct a pharmacokinetic analysis, the chosen drug of the present thesis will be lacosamide, an anti-epileptic drug approved (in doses of up to 400 mg/day) for the treatment of focal seizures in adults as monotherapy (USA only) or adjunctive therapy (US, EU and other countries) (Cawello, Rosenkranz et al., 2013, UCB, 2014a, UCB, 2014b).

Developing a pharmacokinetic model that can provide a framework for the prediction of time courses of exposure and a response for different dosing regimens throughout a study population requires investigating how pharmacokinetic parameters of a drug are influenced by age and/or gender in healthy subjects, as well as adult subjects with focal epilepsy.

1.2 Motivations for the Subparts of the Thesis

1.2.1 Effect of Age and Gender on the Pharmacokinetic of Lacosamide

The objective of the first topic in this thesis (Chapter 2) is to investigate whether age and gender influence the pharmacokinetics of lacosamide. If age and gender have no relevant effect on the rate of absorption and elimination of lacosamide, numerical differences could be explained by scaling factors such as body weight, or volume of distribution. It is known that variability in drug metabolism, distribution or excretion with age limits the extrapolation of pharmacokinetic data from younger subjects to the elderly and thus underlines the importance of investigating age-related changes to ensure effective and safe utilisation of therapeutic agents across the age range (Italiano and Perucca, 2013, Perucca, Berlowitz et al., 2006). This objective should be evaluated by a post-hoc analysis of pharmacokinetic data taken at steady state from two phase I studies of oral lacosamide in healthy adult subjects compared to results of a population pharmacokinetic analysis by using data from two phase III studies of adjunctive oral lacosamide in adults with focal epilepsy taking 1-3 concomitant anti-epileptic drugs. The phase I data should be stratified by age (young subjects aged between 18-45 years and elderly subjects aged $\geq$ 65 years) and sex and normalised by body weight (lean body weight or fat-free mass), body height or volume of distribution. The analysis should be undertaken after non-compartmental methods whereas the population data stratified by sex were analysed by using a one-compartment model.

1.2.2 Integrated Pharmacokinetic Modeling

After evaluating in the second chapter whether the factors age and gender have to be considered in pharmacokinetic model development, a new pharmacokinetic model should be generated including the model-dependent pharmacokinetic of a drug and its metabolite in plasma as well as the amount of unchanged drug excreted in urine. The first objective of the third chapter is to develop a mathematical system of equations by using adequate mathematical methods. Furthermore, the chosen software tools should be validated with respect to their suitability for pharmacokinetic modeling by iterating values for pharmacokinetic parameters of a fictive study population based upon the model. If the software proves successful, the second objective is to evaluate the suitability of the developed system of equations with study

data of lacosamide in healthy subjects and subjects with mild to severe renal impairment. Physiological restrictions such as renal impairment could lead to alterations in the pharmacokinetic profile of a drug and its metabolites enforcing a change in the standard dosing regimens (Cawello, Fuhr et al., 2013). For lacosamide, it is known that metabolic clearance is a prominent component of total body clearance, whereby it can be assumed that renal and metabolic elimination represent the sum of total drug elimination. The main aim is to show a correlation between renal elimination of lacosamide and its metabolite on renal function following a compartmental analysis. By contrast, the rate constant of metabolism should be independent from renal function.

The results of this pharmacokinetic study should enforce progress in the understanding of lacosamide's behaviour in healthy populations as well as in populations characterised by different body compositions (Chapter 2) or physiological restrictions such as renal impairment (Chapter 3).

When applied to other drugs, this pharmacokinetic modeling approach may similarly enhance our present understanding of their behaviour in different patient populations with the aim of achieving optimal therapeutic dosing conditions.

1.3 Aim of the Thesis

The overall aim of the thesis was to analyse and evaluate the influences of age and gender-related differences in body compositions on the pharmacokinetic parameters of the anti-epileptic drug lacosamide to develop a new pharmacokinetic model capable of describing the pharmacokinetics of an unchanged drug and its main metabolite in plasma, as well as its cumulative amount excreted in urine in one equation.

In the first project of the thesis (Chapter 2), the extent to which known pharmacokinetic parameters of lacosamide were influenced by age and gender in healthy subjects was investigated. To ascertain how these results could be transferred to patients with focal epilepsy, a comparison was conducted between results of the present evaluation and those taken from publications of a population pharmacokinetic analysis. The explanation of the differences in plasma concentrations of lacosamide and its metabolite by chosen scaling factors form the basis to develop a new pharmacokinetic model that could be applied to study data of subjects characterised by different age and gender.

The second project (Chapter 3) was aimed to develop a new pharmacokinetic model that includes the model-dependent pharmacokinetic of a drug and its main metabolite in plasma, as well as the amount of unchanged drug excreted in urine. This project comprised the mathematical model development, the validation of used modeling software with a generated fictive study population and finally the pharmacokinetic modeling approach with real study data of healthy subjects and subjects with mild to severe renal impairment.

In the last chapter of the present thesis, the results of both projects were summarised in the light of scientific progress and a perspective of the conducted pharmacokinetic modeling approach was given.

2 CHAPTER 2: EFFECT OF AGE AND GENDER ON LACOSAMIDE PHARMACOKINETICS IN HEALTHY ADULT SUBJECTS AND ADULTS WITH FOCAL EPILEPSY

2.1 General Introduction

2.1.1 Epilepsy

Epilepsy is one of the most common chronic central nervous system disorders, affecting over 65 million people worldwide of all ages, of whom around 60% are diagnosed with partial onset seizures (POS) (Cawello, 2015, Cawello, Stockis et al., 2014, Mendhi, Suralkar et al., 2014). Treating epilepsy with anti-epileptic drugs (AEDs) aims to prevent the onset of new seizures or reduce the severity of seizures (Cawello, Stockis et al., 2014). Nevertheless, the quality of life should not be influenced by adverse drug reactions (ADR) or drug-drug interactions (Cawello, Stockis et al., 2014). The individual risk of epilepsy comprises the increased risk of premature death by about two or three times compared to the healthy population (Mendhi, Suralkar et al., 2014). The fact that epilepsy is a chronic disorder that requires a long-term therapy with anti-epileptic drugs makes it important to understand the drugs' metabolic pathway to avoid and understand drug-drug interactions across multiple drug classes (Cawello, Stockis et al., 2014). Furthermore, epilepsy can be found throughout all age classes, which makes an understanding of patient individual factors as well as the understanding of the drugs' pharmacokinetic properties inevitable (Cawello, Stockis et al., 2014).

Epilepsy occurs across the entire age range, although the incidence in the elderly population is high and increasing (Perucca, 2006, Stefan, May et al., 2014). It is evident that an understanding of possible age- and sex-related changes in the pharmacokinetic profile of a drug is the basic requirement for the therapy with anti-epileptic drugs (Italiano and Perucca, 2013). This information means an increasing challenge for physicians in view of the increasing number of anti-epileptic drugs that have entered the market in the last two decades (Italiano and Perucca, 2013).

2.1.2 Physiological Effects on Pharmacokinetics

Many factors exist that affect drug distribution and metabolism, including a subject's body mass index (BMI), body composition, plasma volume, tissue and plasma proteins and organ blood flow (Schaefer, Cawello et al., 2015). A sufficient number of studies exists in the target population during drug development, although much of the available pharmacokinetics data are generated in healthy subjects and typically younger adult males (Ghandi, Aweeka et al., 2004). Women and members of minority groups are generally less represented or excluded from participating in clinical studies, especially from phase I studies (Gleiter and Remy-Gundert, 1996). Variability in drug metabolism, distribution or excretion with age limits extrapolation of pharmacokinetic data from younger subjects to the elderly and highlights the importance of investigating age-related changes to aid effective and safe utilisation of therapeutic agents across the age range (Italiano and Perucca, 2013, Perucca, Berlowitz et al., 2006). Besides age, gender-related differences in pharmacokinetics have also frequently been discussed as potentially important determinants for the clinical effectiveness of drug therapy (Meibohm, Beierle et al., 2002). In the following, the potential effects of age and sex on the pharmacokinetic of drugs will be described.

The subsequent sections contain parts of the recent publication "Effect of Age and Sex on Lacosamide Pharmacokinetics in Healthy Adult Subjects and Adults with Focal Epilepsy" (Schaefer, Cawello et al., 2015).

2.1.3 Effect of Age on the Pharmacokinetics of Anti-epileptic Drugs

As defined by the World Health Organization (WHO), elderly patients are those aged over 65 years who might differ in their drug response when compared to younger subjects (Klotz, 2007). Geriatric patients represent a population that is vulnerable to drug interactions given that they often take many other medications for concurrent diseases (Perucca, Berlowitz et al., 2006). Problems often occurring in this special population comprise adverse drug reactions, which can be mostly related to dose-dependency (Klotz, 2007).

As body fat increases and total body water decreases over age, the apparent volume of distribution for hydrophilic drugs (V_d), the plasma volume and extracellular fluid decreases (Bossingham, Carnell et al., 2005, Klotz, 2009). With higher age, the ability to maintain water balance decreases, which leads to a decrease in total body water, associated with a loss of fat-free mass, a decrease in the sensation of thirst and alterations in plasma vasopressin, which can influence the kidneys' ability to concentrate urine (Bossingham, Carnell et al., 2005). This explains dehydration being one of the most common disorders of electrolytes in the elderly (Bossingham, Carnell et al., 2005). In one study, total body fat (% of total body weight) increased by 35% when subjects aged 65-80 years were compared with a younger population aged 20 years (Lackner, Cloyd et al., 1998). The change in body fat may be due to the body water percentage decreasing in elderly versus younger subjects (by about 17%), which may in turn influence the volume of distribution (Bossingham, Carnell et al., 2005, Lackner, Cloyd et al., 1998).

The metabolic clearance of drugs may also be affected by age. It is generally known that liver size and mass (20-30% decrease) and hepatic blood flow (20-50% decrease) decrease with increasing age (Klotz, 2009). This decrease could affect the elimination of drugs, especially those that are defined as high-clearance drugs (Klotz, 2009). There could be an impairment of drug clearance in the elderly by cytochrome P450 (CYP)-mediated phase I reactions as oxidation, reduction and hydrolysis (Shi, Mörike et al., 2008). For example, the antipyrine clearance has been reported to decrease by about 29% in subjects aged $\geq$ 70 compared with subjects aged 20-29 years (Leppik, 2008).

With increasing age, the kidney mass decreases by approximately 25-30%, whereas the renal blood flow declines about 1% per year after the age of 40 and the glomerular filtration rate (GFR) is reduced by 0.75-1.05 ml/min per year (Shi, Mörike et al., 2008). Unbound clearance of renal and metabolic eliminated anti-epileptic drugs can decrease by 20-40% as a result of age-related changes (Perucca, Berlowitz et al., 2006). Whether these changes in renal function will hold clinical relevance concerning the elimination of some drugs can only be stated after considering the extent that renal elimination contributes to total systemic elimination as well as the therapeutic index of the individual drug (Shi, Mörike et al., 2008).

2.1.4 Effect of Gender on the Pharmacokinetic of Anti-epileptic Drugs

Besides the factor age, there are a number of examples that explain pharmacokinetic differences between genders, which could affect the clinical effectiveness of drug therapy (Gleiter and Remy-Gundert, 1996, Meibohm, Beierle et al., 2002). Women and men differ in many physiological parameters such as body weight, body fat, muscle mass organ size and GFR (Nicolas, Espie et al., 2009). Men generally have a higher body weight than women, which is due to higher muscle mass, whereas women have a greater part of body fat of total body weight (25 vs. 16% in men)(Gleiter and Remy-Gundert, 1996, Nicolas, Espie et al., 2009). The higher amount of muscle mass in men - which mainly comprises water - could have an influence on the volume of distribution (Gleiter and Remy-Gundert, 1996). It might be necessary to adjust dose when treating women due to an increased volume of distribution for hydrophilic drugs resulting in a prolonged elimination half-life and a possible tissue accumulation over time (Nicolas, Espie et al., 2009). It is also mentioned that women have a lower plasma volume as well as a lower organ blood flow rate (Nicolas, Espie et al., 2009). There might be different fractions of unbound drugs between men and women, given that plasma protein binding is influenced by sex hormones (Ghandi, Aweeka et al., 2004).

The renal clearance of a drug is dependent on the GFR, the active tubular reabsorption and tubular secretion, whereas the GFR shows proportionality to body weight or surface (Nicolas, Espie et al., 2009). Lower GFR rates can be measured in women compared with men, due to a general lower body weight (Nicolas, Espie et al., 2009). Differences in GFR are also considered for women versus men in the Cockcroft-

Gault equation, which is generally used to calculate the creatinine clearance (Lackner, Cloyd et al., 1998).

2.2 Methods

2.2.1 Literature Review About the Anti-Epileptic Drug Lacosamide

Lacosamide ((*R*)-2-(Acetylamino)-*N*-benzyl-3-methoxypropanamid; Vimpat®, UCB Pharma, Brussels, Belgium) is a newer anti-epileptic drug indicated as monotherapy (only in USA) or adjunctive therapy in patients with POS with or without secondary generalisation (aged ≥ 17 years in the United States, ≥ 16 years in Europe (UCB, 2014a, UCB, 2014b)). It is approved in doses up to 400 mg/day and available for oral administration (as syrup or tablet) and as an intravenous infusion (Hoy, 2013). In multi-centre, randomised, placebo-controlled clinical trials, the efficacy and safety profile of lacosamide in a dose range from 200 mg (100 mg twice daily) to 600 mg/day (300 mg twice daily) has been established (Cawello, Stockis et al., 2014). Typical adverse events reported necessarily to be treated (incidence > 10% and greater than placebo) were dizziness, headache, nausea and diplopia (UCB, 2014a). Lacosamide is believed to enhance the slow inactivation (but has no apparent effect on the fast inactivation) of voltage-gated sodium channels (Hoy, 2013). Under conditions of slight prolonged depolarisation and repetitive neuronal activity, the sodium channel can go into the slow inactivated state by closing the pore from inside. This process happens on a second-to-minute time scale. Drugs can block the open channel (e.g. local anaesthetics) or enhance fast inactivation (classic anticonvulsants) or slow inactivation, as it is believed to be the mechanism of lacosamide.

2.2.1.1 Structural Formula of Lacosamide

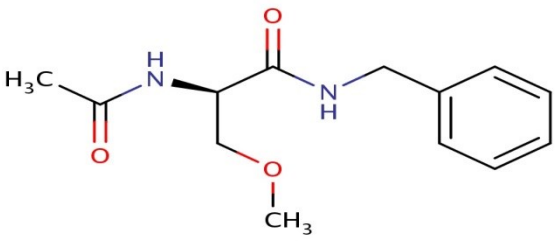


Figure 2-1 Structural Formula of Lacosamide

Lacosamide ((*R*)-2-acetamido-*N*-benzyl-3-methoxypropionamide)
(taken from http://images.ddccdn.com/img/mol/DB06218.mol.jpg)

2.2.1.2 Physiochemical Properties of Lacosamide

Lacosamide is a functionalised amino acid and an analogue of D-serine that has amphiphilic properties, whereby the molecule is soluble in water as well as being sufficiently lipophilic to transfer barriers such as the lipophilic blood brain barrier (Cawello, 2015).

2.2.1.3 Pharmacokinetic Profile of Lacosamide

The pharmacokinetic overview of lacosamide established through data from multiple clinical pharmacology studies in healthy subjects (aged 18-87 years), in patients with focal epilepsy (aged $\geq$ 16 years), in adults with POS and adults with renal or hepatic impairment (UCB, 2014a) indicated that lacosamide shows a high oral bioavailability, a linear pharmacokinetic profile, dose proportionality (100-800 mg) with low inter- and intra-individual variability and low potential for clinically relevant pharmacokinetic drug-drug interactions (Cawello, 2015).

The absorption of lacosamide from the gastrointestinal tract is rapid and complete, with negligible first-pass effect and a high absolute bioavailability (about 100%) (UCB, 2014a). Peak plasma concentrations (C_{max}) can be seen 0.5-4 hours post-dose in healthy subjects after oral administration whereas elimination half-life is about 13 hours (Cawello, Stockis et al., 2014, UCB, 2014b). Steady state conditions are achieved after 3 days with twice daily repeated administration (UCB, 2014a). The main metabolite of lacosamide is the *O*-desmethyl-metabolite, which achieves its time to

maximum plasma concentration (t_{max}) after 0.5 to 12 hours. Nevertheless, it has no known pharmacological activity (Cawello, 2015).

The V_d of lacosamide is about 0.6 L/kg and close to the volume of total body water, whereas the plasma protein binding is less than 15% (UCB, 2014a). The population mean of V_d was estimated for male and female subjects as 42.4 L and 35.5 L, respectively (Schiltmeyer, Cawello et al., 2005).

Lacosamide is primarily eliminated by the kidneys and through biotransformation to the *O*-desmethyl-metabolite (Cawello, Stockis et al., 2014). The elimination primarily occurs via the urine (97% and 94% for intravenously and for orally administered lacosamide, respectively) (Cawello, Boekens et al., 2012).

2.2.2 Data Extraction

Data were extracted post-hoc from two phase I clinical pharmacology studies in healthy subjects (SP599 (Cawello, Rosenkranz et al., 2013) and SP620 (Schiltmeyer, Cawello et al., 2005)) and separately from two phase III clinical studies of adjunctive lacosamide in adults with focal epilepsy taking 1-3 concomitant AEDs (SP754 (Chung, Sperling et al., 2010) and SP755 (Halász, Kälviäinen et al., 2009)). All studies were conducted in accordance with the relevant International Conference of Harmonisation (ICH), Good Clinical Practice (GCP) guidelines, the regulations of the German Drug Law and the principles described in the Declaration of Helsinki. Written informed consent was obtained prior to performing any trial eligibility assessment.

2.2.2.1 Study Designs and Populations

Study SP599

Study SP599 was an open-label, one-arm trial of approximately 90 days, which enrolled 40 pre-menopausal, non-pregnant, healthy Caucasian women aged 18-40 years (BMI 20-30 kg/m^2) ("younger female") (Cawello, Rosenkranz et al., 2013). Complete inclusion and exclusion criteria are detailed elsewhere (Cawello, Rosenkranz et al., 2013). Thirty-one subjects who completed SP599 were included in the analysis, as detailed in Table 2-1.

For pharmacokinetic assessment, subjects received oral lacosamide 400 mg/day (200 mg twice daily) plus an oral contraceptive once daily (containing 0.03 mg ethinylestradio and 0.15 levenorgestrel). Blood samples were drawn pre-dose (time 0) and post-dose after 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12 and 24 hours and were analysed at steady state (Cawello, Boekens et al., 2012).

Study SP620

Study SP620 was a double-blind, placebo-controlled, parallel group study of single and multiple doses of oral lacosamide administered to healthy subjects of different age and gender. Healthy male or female subjects with BMI 19-30 kg/m^2 were enrolled and stratified by age $\geq$ 65 years ("elderly male or female") and 18-45 years ("younger male"). Exclusion criteria included: ECG abnormality, including QTc interval > 470 msec (female), or > 450 msec (male) and PR interval > 200 msec; any significant renal or hepatic disorder; known drug sensitivity or clinically relevant allergy, or any

other acute or chronic medical condition considered clinically relevant by the investigator on screening. Fifty subjects were randomised to three groups. Subjects received single dose lacosamide 100 mg or placebo on day 1. On days 4 to 7, subjects received lacosamide 100 mg or placebo twice daily, followed by single dose lacosamide 100 mg or placebo on day 8 in the morning. Overall, 35/36 lacosamide treated subjects completed the study and were included in the present post-hoc analysis (12 young males, 12 elderly females, 11 elderly males). Lacosamide data are used herein. Blood samples were drawn pre-dose (time 0) and post-dose after 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, 48 and 72 hours and analysed at steady state. Urine samples were taken pre-dose and post-dose on day 1, post-dose on day 8 and collected at the following intervals: 0-6, 6-12 and 12-24 hours.

From these two study populations, 66 healthy subjects were included and stratified by age and gender (31 young females, 11 young males, 12 elderly females, 12 elderly males) (Table 2-1). All subjects completed the respective studies and had sufficient samples collected to enable pharmacokinetic parameters.

Table 2-1 Summary of Baseline Characteristics for Study Populations

(Table taken from Schaefer et al.(Schaefer, Cawello et al., 2015))

Group	*n*	Age (years)	Height (cm)	Weight (kg)	BMI (kg/m^2)	Dose normalised by body weight (mg/kg)
Healthy subjects						
Elderly females[a]	12	69.7 (4.1)	161.5 (7.3)	66.0 (10.4)	25.2 (2.7)	1.55 (0.23)
Elderly males[a]	12	70.9 (6.9)	171.1 (8.1)	76.6 (11.7)	26.1 (2.7)	1.34 0.22)
Young females[a]	31	30.1 (5.0)	169.5 (6.9)	63.8 (7.1)	22.2 (1.8)	3.17 (0.34)
Young males[a]	11	36.8 (6.4)	178.4 (9.0)	80.5 (11.8)	25.2 (2.5)	1.27 (0.17)
Adults (aged 16–71) with focal epilepsy						
Female[b]	278	37.6 (12.2)	164 (8)	71.4 (18.5)	26.8 (6.1)	
Male[b]	287	37.4 (12.3)	177 (9)	85.5 (19.1)	27.5 (6.0)	

Data are arithmetic mean (standard deviation)

[a] Young females were 18–40, young males were 18–45 years, elderly males and females were aged ≥65 years

[b] Patients were not sub-categorised by age

2.2.2.2 Bioanalytical Methods in the Studies

The information of the present section and section 2.2.2.3 should provide a brief overview of the bioanalytical methods and methods of pharmacokinetic parameter calculation used during the studies SP599 and SP620 and taken from literature (Cawello, Rosenkranz et al., 2013, Schiltmeyer, Cawello et al., 2005).

Lacosamide and its main metabolite - *O*-desmethyl lacosamide - were analysed in plasma and urine. In the phase I studies, blood samples were separately stored in lithium heparin tubes at each collection point. Within 30 minutes after collection, samples were centrifuged (10 minutes at 4°C) and stored at -20°C until analysed. Lacosamide and the internal standard (lacosamide-D^7) were separated from plasma by liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) in turbo ion spray mode. The MS/MS system was focused in the multiple reaction-monitoring model to monitor the following ion transitions: 251.3-> 108.2 for lacosamide and 258.3-> 115.2 for lacosamide-D^7. The assay was conducted using a 0.2 mL sampling volume of human plasma or urine and the validated lower limit of quantification (LLQ) of the method to determine lacosamide was 0.1 µg/mL for plasma samples and 5.0 µg/mL for urine samples. Typical precision [% coefficient of variation (CV)] of quality control and standard samples ranged from 2.1% to 3.7% and the typical estimated accuracy (% bias) ranged from -7.3% to 3.9% (Cawello, Nickel et al., 2010). The calibration range was 0.1-20 µg/mL for plasma samples and 5-500 µg/mL for urine samples. For O-desmethyl lacosamide, LLQ was 0.02 µg/ml for plasma and 1.0 µg/mL for urine samples. The corresponding calibration range was 0.02-4 µg/mL for plasma and 1-100 µg/mL for urine samples.

2.2.2.3 Determination of Pharmacokinetic Parameters in Healthy Subjects

For the phase I studies, pharmacokinetic parameters were determined by non-compartmental analysis and reported in the clinical trial reports (Gruber, 2002, Waitzinger and Pabst, 2001). The area under the plasma concentration time curve at steady state from time 0 to the time of the last quantifiable concentration ($AUC_{\tau,ss}$, calculated using the log-trapezoidal rule), the maximal plasma concentration at steady state ($C_{max,ss}$, observed from the data), the amount excreted ($Ae_{\tau,ss}$ observed from the data) and the total body clearance (CL/F, dose divided by $AUC_{\tau,ss}$) were examined. In study SP620, $Ae_{\tau,ss}$ and renal clearance (CL_R) were calculated from urine samples.

2.2.2.4 Normalisation Process of Plasma Concentrations and Pharmacokinetic Parameters

To evaluate which factors were responsible for the differences in plasma concentrations and in pharmacokinetic parameters of subjects with different age and gender the plasma concentrations and pharmacokinetic parameters $AUC_{\tau,ss}$ and $C_{max,ss}$ were normalised by body weight and body height (BH) through multiplication. Fat-free mass and lean body weight (LBW) were used for normalisation of $AUC_{\tau,ss}$ and $C_{max,ss}$.

The plasma concentrations of lacosamide and its main metabolite were plotted with and without normalisation by body weight in Exel (Figure 2-3 and 2-4).

LBW as well as FFM are often used as size descriptors, which can be found in the pharmacokinetic literature (Janmahasatian, Duffull et al., 2005). $AUC_{\tau,ss}$ and $C_{max,ss}$ were normalised by V_d through an equation for approximation of total body water as adequate surrogate for the V_d of lacosamide (Jorquera, Almar et al., 1995). FFM calculates the difference between body weight and the sum of body fat, lymph- and bone mass (Janmahasatian, Duffull et al., 2005). In contrast to FFM, lipids are included in LBW as a small fraction of total body weight (about 3% in males and 5% in females) (Janmahasatian, Duffull et al., 2005). The same pharmacokinetic parameters were determined for *O*-desmethyl lacosamide.

Equations used to calculate FFM, LBW and V_d (Janmahasatian, Duffull et al., 2005, Jorquera, Almar et al., 1995) are described by:

1) $FFM\ [kg](male) = \frac{9.27 \cdot 10^3 \cdot BW}{6.68 \cdot 10^3 + 216 \cdot BMI}$

2) $FFM\ [kg](female) = \frac{9.27 \cdot 10^3 \cdot BW}{8.78 \cdot 10^3 + 244 \cdot BMI}$

3) $LBW\ [kg](male) = 1.10 \cdot BW - 0.0128 \cdot BMI \cdot BW$

4) $LBW\ [kg](female) = 1.07 \cdot BW - 0.0148 \cdot BMI \cdot BW$

5) $V_d\ [L](male) = 0.3625 \cdot BW + 0.2239 \cdot BH - 0.1387 \cdot Age - 14.47$

6) $V_d\ [L](female) = 0.2363 \cdot BW + 0.1962 \cdot BH - 0.0272 \cdot Age - 10.26$

Body weight has the unit kg, body height the unit cm and BMI the unit kg/m^2.

Data extraction and the normalisation of plasma concentrations and pharmacokinetic parameters were conducted using SAS version 9.3 (SAS Institute Inc., Cary, NC, USA).

2.2.3 Statistical Evaluation

Statistical analyses were performed using SAS version 9.1. Demographic parameters such as age, height, weight, BMI and dose normalised by body weight were summarised by descriptive statistics (number of subjects (n), arithmetic mean, standard deviation (SD), coefficient of variation (CV), range and median). The pharmacokinetic parameters $AUC_{\tau,ss}$ and $C_{max,ss}$, CL_R and CL/F were characterised by geometric mean, geometric CV, range and median. $Ae_{\tau,ss}$ was described by arithmetic mean, SD, median and range. For $t_{max,ss}$, the median and range were selected as appropriate statistical methods. Given that different dosing regimens were applied in both phase I studies (100 mg twice daily in SP620 and 200 mg twice daily in SP599), the pharmacokinetic parameters from the SP599 study had to be normalised to the same dose schedule (100 mg twice daily) by dividing the SP599 data by two. This was possible due to the dose proportionality of lacosamide (Cawello, Stockis et al., 2014).

For $AUC_{\tau,ss}$ and $C_{max,ss}$, the parametric point estimate for the ratios group 1 versus group 2 and the 90% CI were calculated using the least squares (LS) means and the root mean square of error (RMSE) from the analysis of variance

(ANOVA) of the log-transformed data with subsequent exponential transformation. The intra-group variability was estimated from the ANOVA. The ANOVA model included sex and age group (group 1 and 2) and nested age and sex (group 1-4) as fixed effects. Equivalent relative bioavailability was accepted of the 90% CI of the point estimates for $AUC_{\tau,ss}$ and $C_{max,ss}$ were located within the 80-125% range for bioequivalence. Table 2-2 shows the codes used for the ANOVA in SAS.

Table 2-2 Codes Used for ANOVA of Log-Transformed Data

Effect	Group code	Group
Sex	1	Male
	2	Female
Age	1	Elderly
	2	Young
Sex*Age	1	Elderly male
	2	Young male
	3	Elderly female
	4	Young female

All SAS programs written for data extraction, the normalisation of plasma concentrations and pharmacokinetic parameters as well as the statistical analysis and the combination of studies SP599 and SP620 can be seen in Appendices 1, 2 and 3. The program for the analysis of variance can be seen in Appendix 4.

2.2.4 Application of Results to Patients with Focal Epilepsy

To evaluate the extent to which the results of the present evaluations in healthy subjects could be compared to results of studies with patients, the results of a population pharmacokinetic analysis of lacosamide in adults with focal epilepsy were taken. All following information was taken from literature and should serve as an information tool to understand the following comparison analysis between subjects and patients. The population analysis used data derived from two phase III, double-blind, randomised, parallel group, placebo-controlled trials, where adults (16–70 years old) received lacosamide (maintenance dose 200, 400, or 600 mg/day) in addition to 1–3 concomitant AEDs for 18–21 weeks, followed by 2–3 weeks transition or taper phase (Chung, Sperling et al., 2010, Halász, Kälviäinen et al., 2009). The population pharmacokinetic evaluation was conducted with 2,370 plasma sample records obtained on 7–8 occasions (1–2 samples in a dosing interval) from 565 subjects (287 male; 278 female; aged 16–71 years; BMI 14.2–66.5 kg/m^2) (Nickel, Zisowski et al., 2008). Age, sex, weight, height, BMI, ethnicity, creatinine clearance and concomitant AEDs were examined as possible covariates to interpret inter-individual variability and pharmacokinetic parameters. Pharmacokinetic parameters were generated using a one-compartmental model (Nickel, Zisowski et al., 2008). Lacosamide concentration data were modelled by non-linear mixed-effect modeling using NONMEM version 6 (ICON plc, Dublin, Ireland) with the first-order conditional estimation method (Nickel, Zisowski et al., 2008). Monte Carlo simulations with n=1000 were performed to assess the significance of covariates included in the model of population pharmacokinetics (Nickel, Zisowski et al., 2008).

2.2.5 Safety Assessment

Subjects were monitored for treatment-emergent adverse events (TEAEs) during each trial. TEAEs were coded according to the WHO-ART dictionary for SP620 and SP599 (Cawello, Rosenkranz et al., 2013). In the phase III, clinical studies used for population pharmacokinetic analyses, TEAEs were coded according to MedDRA preferred terms and are described elsewhere (Chung, Sperling et al., 2010, Halász, Kälviäinen et al., 2009).

2.3 Results

2.3.1 Demographics

A total of 66 healthy subjects (12 elderly females, 12 elderly males, 31 young females and 11 young males) were used for evaluation. Elderly females were aged about 69.7 years with a SD of 4.1 years, whereas young females had a mean age of 30.1 years with a SD of 5.0 years. Elderly males were aged around 70.9 years with a SD of 6.9 years and young males had a mean age of 36.8 years with a SD of 6.4 years. In the patient population, 278 females with a mean age of 37.6 years with a SD of 12.2 years and 287 males with a mean age of 37.4 years and a SD of 12.3 years were used for pharmacokinetic assessment (Table 2-1).

2.3.2 Influence of Age or Gender on Pharmacokinetic Parameters of Lacosamide in Healthy Subjects

Figure 2-2 shows the plasma concentration time curve of lacosamide in healthy subjects of different age and gender. Figure 2-2a shows the concentration time curve without normalisation, whereas Figure 2-2b shows the plasma concentration time curve after normalising the concentration levels with body weight. Comparing young subjects with elderly subjects, younger subjects generally had lower maximal plasma concentrations and exposure than their older counterparts. Furthermore, the clearance was higher in younger subjects (Figure 2-2, Table 2-3). Young males had 24% lower exposure compared with elderly males. Nevertheless, when parameters were normalised by body weight, the difference reduced to 21%. FFM and LBW did not change parameters, whereas normalisation by V_d reduced difference in exposure between young and elderly males to 10% (Table 2-3). For young versus elderly females, a decrease in exposure of 10% was found without adjustment. Following weight normalisation, the same comparison showed a decrease of 12%, whereas normalisation by FFM and LBW, differences were 6% and 8%, respectively. When normalising with parameter V_d, the effect was very small with 4% for young versus elderly females (Table 2-3). In a comparison of elderly females with young males, exposure differed by about 33%. When normalising with body weight, the difference was only 19%, whereas normalisation by V_d resulted in a difference in exposure for elderly females versus young males of only 6%. With a normalisation by LBW the difference was minimal, namely 0.1% (Table 2-3).

Prior to normalisation, females from both age groups generally had numerically higher maximal mean plasma concentration and exposure compared with their male counterparts (Figure 2-2a, Table 2-3). Regarding the exposure parameters, a low variability was noted within each group, as could be seen in a CV of $\leq$ 23%. After multiple dose administration, plasma concentrations rapidly increased from (mean $\pm$ SD) 3.71 $\pm$ 0.86 µg/mL for young females and 2.60 $\pm$ 0.33 µg/mL for young males, to maximum mean plasma concentration of 6.62 $\pm$ 1.26 µg/mL and 4.39 $\pm$ 0.59 µg/mL, respectively (Table 2-4). Subsequently, the concentration of lacosamide decreased to 3.23 $\pm$ 0.65 µg/mL in young females and 2.44 $\pm$ 0.42 µg/mL in young males, respectively (Table 2-4). A similar relationship could be observed between the lacosamide plasma concentration time profiles of elderly females and elderly males. Elderly females had mean maximal plasma concentrations of 6.92 $\pm$ 1.19 µg/mL, which decreased to 2.14 $\pm$ 0.53 µg/mL. By contrast, elderly males had mean maximal plasma concentrations of 6.92 $\pm$ 1.19 µg/mL, which decreased to 2.14 $\pm$ 0.53 µg/mL. Nevertheless, elderly females had higher mean plasma concentrations at the time of the last administration (4.01 $\pm$ 0.83 µg/mL) than their male counterparts (3.82 $\pm$ 1.06 µg/mL) (Table 2-4).

For individual parameters, young females showed 36% higher exposure compared with young males, whereas by weight normalisation of $AUC_{\tau,ss}$ this increase in young females was only 8% and by LBW normalisation only 3% (Table 2-3). Comparing maximal mean plasma concentrations of young females with those of young males, the values were 42% higher in females. When normalising by body weight, the difference was only 13% (Table 2-3). For the elderly cohort, females had a 13% higher exposure than their male counterparts before normalisation. After normalisation by body weight and V_d exposure was 2% and 4% lower, respectively. Normalisation by FFM or LBW caused no change in exposure between elderly females and elderly males (Table 2-3).

Table 2-3 Pharmacokinetic Parameters of Lacosamide at Steady State in Healthy Subjects Normalised to a Dose of 100 mg

(Table taken from Schaefer et al. (Schaefer, Cawello et al., 2015))

Parameter	Norm	Units	Elderly female	Elderly male	Young female	Young male
N			12	12	31	12
$AUC_{\tau,ss}$	None	[µg/ml·h]	62.0 (14.0)	54.7 (23.0)	56.0 (16.8)	41.3 (13.6)
	Height	[µg/ml·h·m]	100 (13.6)	93.5 (21.9)	94.9 (16.7)	73.5 (12.4)
	Weight	[µg/ml·h·kg]	4042.8 (20.6)	4141.6 (26.4)	3552.8 (18.1)	3287.0 (17.3)
	FFM	[µg/ml·h·kg]	2512.3 (17.5)	3122.3 (28.8)	2322.2 (17.5)	2515.4 (14.7)
	LBW	[µg/ml·h·kg]	2813.1 (16.8)	3171.4 (23.9)	2634.1 (17.5)	2553.1 (14.8)
	V_d	[µg/ml·h·L]	2165.0 (16.1)	2263.9 (23.6)	2078.6 (17.0)	2030.5 (14.4)
$C_{max,ss}$	None	[µg/ml]	7.4 (12.0)	6.2 (20.0)	6.8 (15.6)	4.8 (10.1)
	Height	[µg/ml·m]	11.9 (11.8)	10.6 (18.5)	11.6 (15.1)	8.6 (8.4)
	Weight	[µg/ml·kg]	480.2 (17.2)	469.7 (22.6)	432.6 (16.9)	384.0 (12.5)
	FFM	[µg/ml·kg]	298.4 (14.7)	354.1 (20.0)	282.7 (16.0)	293.3 (10.1)
	LBW	[µg/ml·kg]	334.2 (14.2)	359.7 (20.1)	320.7 (15.9)	298.3 (10.14)
	V_d	[µg/ml·L]	257.2 (13.6)	256.8 (19.9)	253.1 (15.4)	237.2 (10.4)
CL/F		[L/h]	1.61 (14.0)	1.84 (23.0)	1.79 (16.8)	2.42 (13.6)
$t_{max,ss}$		[h]	0.8 / 0.5–2.0[a]	0.5 / 0.5–2.0	1.1 / 0.7–1.5	1.0 / 0.5–3
$t_{1/2}$		[h]	13.8 (22.0)	16.7 (22.0)	15.3 / 13.3–17.3[a]	14.2 (11)
$Ae_{\tau,ss}$		[mg]	41.5 (54.0)[b]	34.5 (37.0)	NA[c]	33.0 (31)
CL_R		[L/h]	10.2 (32.0)[b]	9.7 (57.0)	NA[c]	12.0 (68.0)

Data are geometric mean & geometric CV (%), unless otherwise indicated. [a] Median and range, [b] arithmetic mean CV (%), [c] No urine samples taken

Norm, normalised; $AUC_{\tau,ss}$, area under concentration time curve at steady state over the dosing interval; $C_{max,ss}$, maximum plasma concentration at steady state; $Ae_{\tau,ss}$, excretion; CL/F, total body clearance; CL_R, renal clearance; FFM, fat-free mass; LBW, lean body weight; NA, not available; norm., normalised; $t_{1/2}$, terminal half-life;
$t_{max,ss}$, time to reach $C_{max,ss}$; V_d, volume of distribution

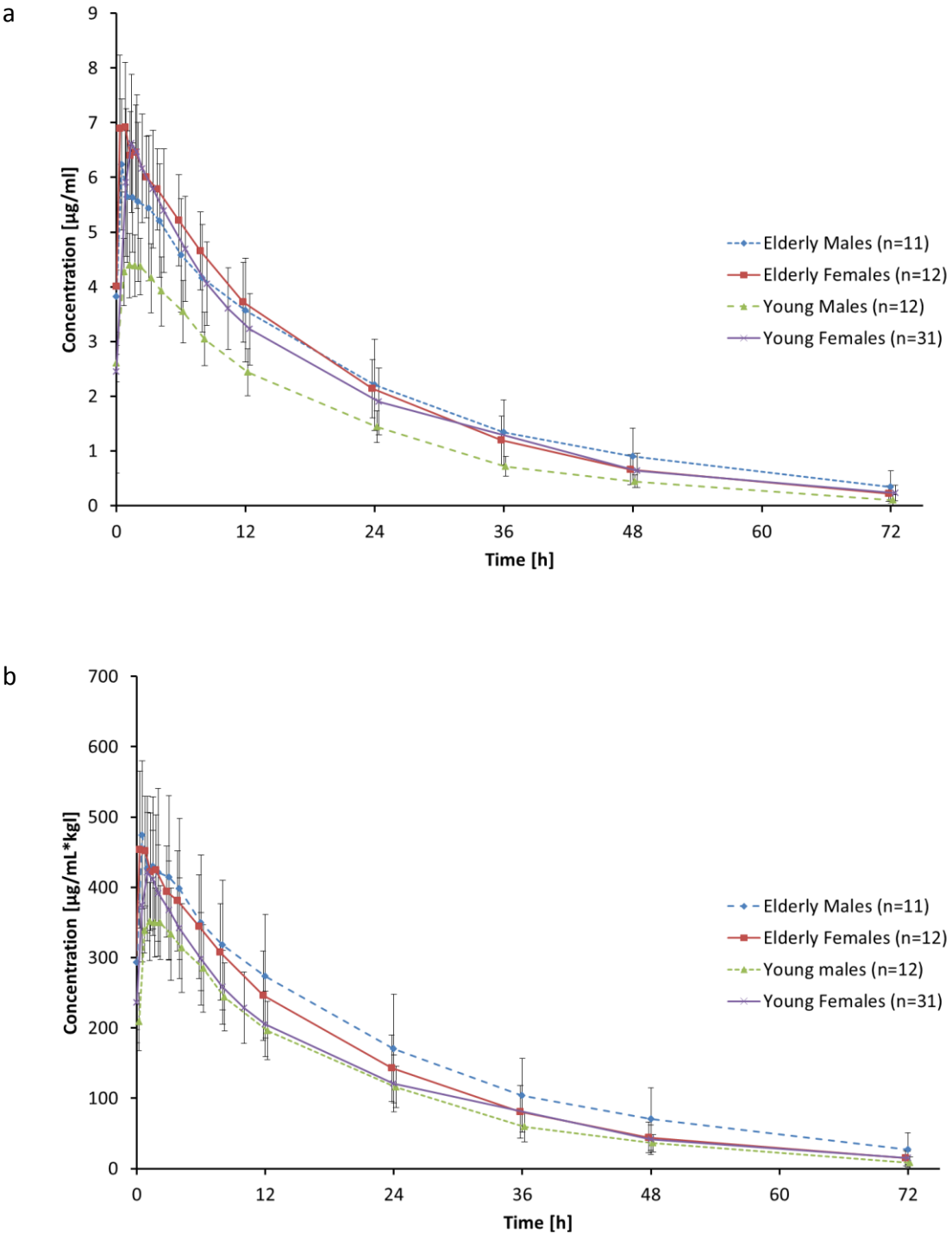


Figure 2-2 Plasma Concentration Time Profile of Lacosamide

(Figure taken from Schaefer et al. (Schaefer, Cawello et al., 2015))

Plasma Concentration Time Profile of Lacosamide at steady state (arithmetic mean ± SD) in healthy subjects a) without normalisation and b) after normalisation by body weight

Table 2-4 Plasma Concentration Time Profile of Lacosamide in Subjects of Different Age and Gender (arithmetic mean ± SD)

	Elderly Females (n=12)		Elderly Males (n=12)		Young females (n=31)		Young males (n=12)	
Time	**Conc [µg/mL]**	**SD**	**Conc [µg/mL]**	**SD**	**Conc [µg/mL]**	**SD**	**Conc [µg/mL]**	**SD**
0	4.01	0.83	3.82	1.06	3.71	0.86	2.60	0.33
0.5	6.89	1.34	6.24	1.20	5.91	1.35	4.27	0.61
1	6.92	1.19	5.65	1.21	6.62	1.26	4.39	0.59
1.5	6.41	0.86	5.63	1.00	6.47	1.03	4.38	0.57
2	6.46	0.86	5.55	1.45	6.16	0.99	4.37	0.52
3	6.01	0.75	5.44	1.33	5.79	1.08	4.16	0.63
4	5.78	0.74	5.21	1.04	5.39	1.13	3.92	0.63
6	5.22	0.83	4.57	1.04	4.69	0.96	3.54	0.57
8	4.66	0.71	4.16	0.98	4.06	0.77	3.05	0.49
12	3.72	0.73	3.57	0.95	3.60	0.75	2.44	0.42
24	2.14	0.53	2.20	0.84	3.23	0.65	1.44	0.29
36	1.19	0.45	1.34	0.59	1.90	0.61	0.72	0.18
48	0.66	0.28	0.90	0.51	0.64	0.31	0.44	0.12
72	0.21	0.14	0.34	0.29	0.23	0.14	0.10	0.09

Table 2-5 Plasma Concentration Time Profile of Lacosamide in Subjects of Different Age and Gender after Normalisation by Body Weight (arithmetic mean ± SD)

	Elderly Females (n=12)		Elderly Males (n=12)		Young females (n=31)		Young males (n=12)	
Time	**Conc [µg/mL]**	**SD**	**Conc [µg/mL]**	**SD**	**Conc [µg/mL]**	**SD**	**Conc [µg/mL]**	**SD**
0	266.44	75.86	293.23	95.77	236.08	56.94	209.03	40.98
0.5	453.69	111.75	474.43	105.67	374.27	79.89	338.62	32.07
1	451.56	78.26	426.86	102.73	420.89	85.49	350.98	55.54
1.5	422.17	83.42	429.87	98.55	410.84	69.86	349.82	48.77
2	424.71	78.38	421.54	119.53	391.56	68.52	349.85	52.68
3	394.05	64.70	413.95	116.14	367.19	70.47	333.56	65.73
4	380.70	71.36	397.76	100.32	341.89	71.63	313.62	63.23
6	344.15	73.92	349.73	96.53	298.30	65.66	284.51	62.34
8	308.03	68.80	318.16	92.10	257.92	52.49	244.12	48.40
12	246.24	63.60	273.80	87.75	228.77	50.74	196.05	41.48
24	142.69	47.30	170.52	77.17	205.50	46.40	116.05	29.23
36	80.49	37.49	103.81	52.49	121.11	40.48	58.99	20.81
48	44.43	21.55	70.40	44.40	41.07	20.54	35.96	12.29
72	14.82	10.86	26.72	24.50	15.03	9.57	8.57	7.98

The analysis of the relative bioavailability for the pharmacokinetic parameters $AUC_{\tau,ss}$ and $C_{max,ss}$ concerning the age effect the 90% CI of the ratio (male/female) for $AUC_{\tau,ss}$ and $C_{max,ss}$ fell outside the 80-125% range accepted for bioequivalence (Figure 2-3a). Normalisation of the 90% CI of the ratio (male/female) for relative bioavailability for $C_{max,ss}$ by height, weight, FFM, LBW and V_d resulted in the 90% CIs of the ratio to fall within the range of 89-125%. Regarding the normalisation of the 90% CI of the ratio (male/female) for relative bioavailability for $AUC_{\tau,ss}$, the 90% CI interval fell within the range of 80-125% when normalised by body height, FFM, LBW or V_d.

Regarding the gender effect, normalisation of the 90% CI of the ratio (elderly/younger) for relative bioavailability $C_{max,ss}$ by weight, FFM, LBW and V_d resulted in the 90% CIs to fall within the range of 80-125% for bioequivalence (Figure 2-3b). The same was valid for the normalisation of the 90% CI of the ratio (elderly/younger) for relative bioavailability $AUC_{\tau,ss}$ (Figure 2-3b).

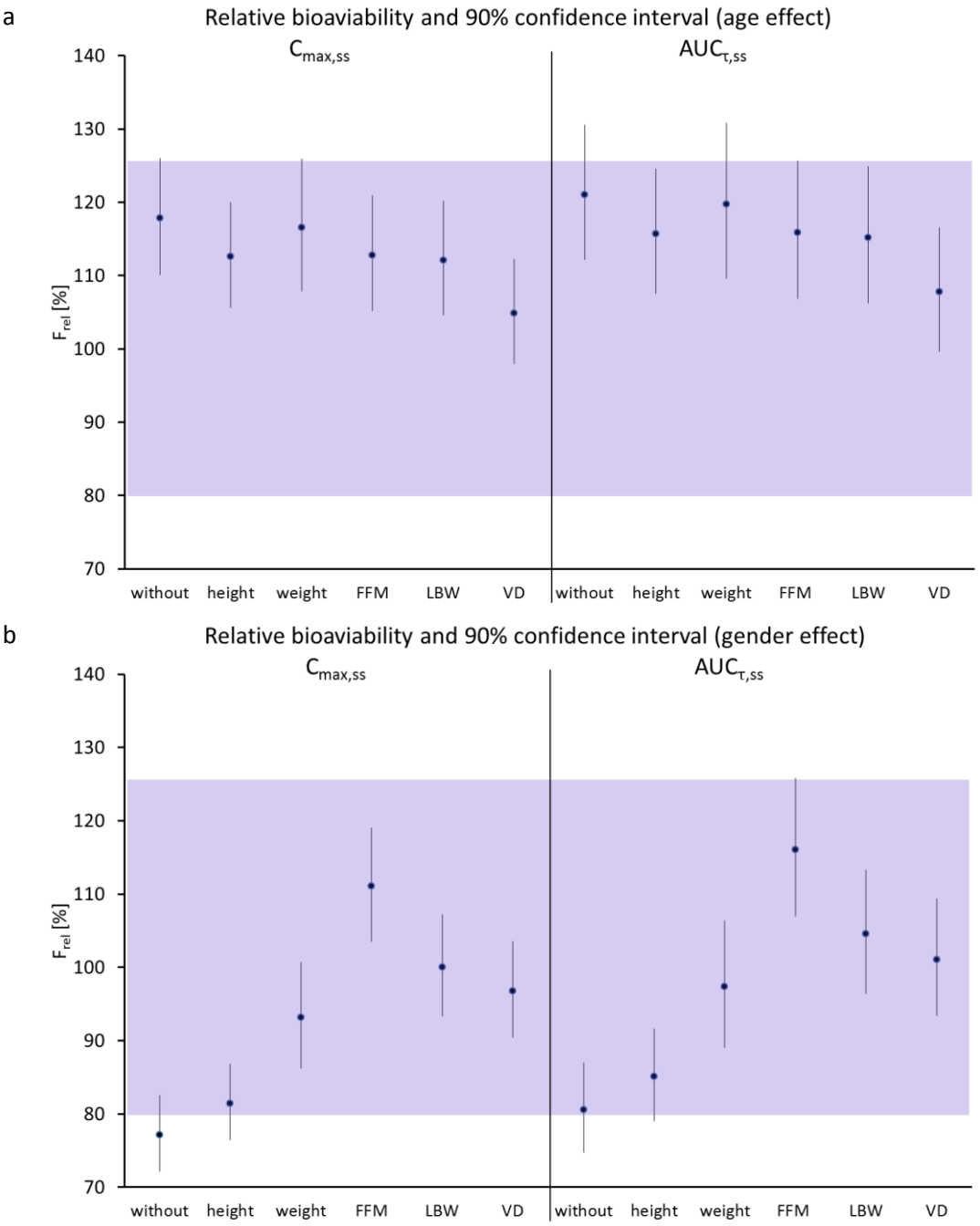


Figure 2-3 Relative Bioavailability of Lacosamide

(Figure taken from Schaefer et al. (Schaefer, Cawello et al., 2015))

Relative bioavailability (90% confidence interval) of lacosamide by a) age (healthy young versus elderly subjects) and b) sex (healthy male versus female subjects)

Figure a) and b) show the values of relative bioavailability for $AUC_{\tau,ss}$ and $C_{max,ss}$ with and without normalisation by body height, weight, FFM, LBW and V_d concerning the age and gender effect.

Shaded area represents the 80-125% range accepted for bioequivalence (FDA, 2014)

2.3.3 Influence of Age and Gender on the Pharmacokinetic Properties of *O*-Desmethyl-Lacosamide in Healthy Subjects

Figure 2-4 shows the plasma concentration time profile of *O*-desmethyl-lacosamide in healthy young males, elderly males and elderly females. Data were not available for young females. Comparing younger males with elderly subjects, young males generally had lower plasma concentrations and exposure than the elderly subjects. Furthermore, young subjects had a higher clearance than their older counterparts (Figure 2-4, Table 2-5). Elderly males had a 19% lower exposure ($AUC_{\tau,ss}$) compared with elderly females. Normalisation by LBW caused a 4% difference in exposure for elderly males vs. elderly females, whereas after normalisation by body height elderly females had a 14% higher exposure than elderly males. When comparing young males with elderly females, young males had 21% lower exposure before normalisation. Following normalisation by body weight, the decrease in exposure for elderly females versus young males was only 2%, whereas with normalisation by body height the decrease in exposure was 12%. (Table 2-6).

After multiple dose administration of lacosamide, plasma concentrations of the *O*-desmethyl metabolite increased from (mean+ SD) 0.51 $\pm$ 0.23 µg/mL for elderly males and 0.59 $\pm$ 0.24 µg/mL in elderly females, to maximum mean plasma concentrations of 0.54 $\pm$ 0.21 µg/mL and 0.60 $\pm$ 0.22 µg/mL, respectively (Table 2-6). Thereafter, the concentration of the metabolite decreased to 0.47 $\pm$ 0.21 µg/mL in elderly males and 0.43 $\pm$ 0.18 µg/mL in elderly females. In young males, the concentration of the metabolite increased from 0.49 $\pm$ 0.16 µg/mL to maximum mean plasma concentrations of 0.50 $\pm$ 0.16 µg/mL and subsequently decreased to 0.31 $\pm$ 0.08 µg/mL (Table 2-7). Normalisation also resulted in a decrease in the difference between maximal plasma concentrations, in line with observations for lacosamide.

Table 2-6 Pharmacokinetic Parameters of *O*-Desmethyl-Lacosamide at Steady State in Healthy Subjects Normalised to a Dose of 100 mg

(Table taken from Schaefer et al. (Schaefer, Cawello et al., 2015))

Parameter	Normalisation	Units	Elderly female	Elderly male	Young male
N			12	12	12
$AUC_{\tau,ss}$	None	[µg/mL·h]	6.8 (43.9)	5.5 (59.9)	5.4 (30.0)
	Height norm	[µg/mL·h·m]	10.9 (43.9)	9.4 (61.4)	9.7 (32.3)
	Weight norm	[µg/mL·h·cm]	440.9 (46.8)	417.1 (61.7)	432.9 (34.2)
	FFM norm	[µg/mL·kg]	274.0 (45.2)	314.5 (62.16)	331.3 (34.2)
	LBW norm	[µg/mL·kg]	306.8 (44.8)	319.4 (62.2)	336.3 (34.1)
$C_{max,ss}$	None	[µg/mL]	0.7 (41.8)	0.5 (56.1)	0.6 (31.0)
	height norm	[µg/mL·m]	1.1 (41.5)	0.91 (57.2)	1.0 (32.9)
	weight norm	[µg/mL·kg]	42.8 (45.1)	40.5 (56.5)	44.5 (34.8)
	FFM norm	[µg/mL·kg]	26.6 (43.3)	30.5 (57.3)	34.1 (34.7)
	LBW norm	[µg/mL·kg]	29.8 (42.9)	31.0 (57.2)	34.6 (34.6)
$t_{max,ss}$		[h]	7.0 (0-12)[a]	3.0 (0-24)	1.55 (0-6)
$t_{1/2}$		[h]	16.8 (30)	21.3 (19)	18.8 (20)
$Ae_{\tau,ss}$		[mg]	19.8 (42)[b]	19.0 (53)	26.3 (40)
CL_R		[L/h]	44.6 (25)[b]	43.6 (54)	74.2 (31)

Data are geometric mean & geometric CV (%) unless otherwise indicated

[a] Median and ranges

[b] Arithmetic mean & CV (%)

$AUC_{\tau,ss}$, area under concentration time curve at steady state over the dosing interval; $C_{max,ss}$, maximum plasma concentration at steady state; $Ae_{\tau,ss}$, excretion; CL_R, renal clearance; FFM, fat-free mass; LBW, lean body weight; NA, not available; norm., normalised; $t_{1/2}$, terminal half-life; $t_{max,ss}$, time to reach $C_{max,ss}$

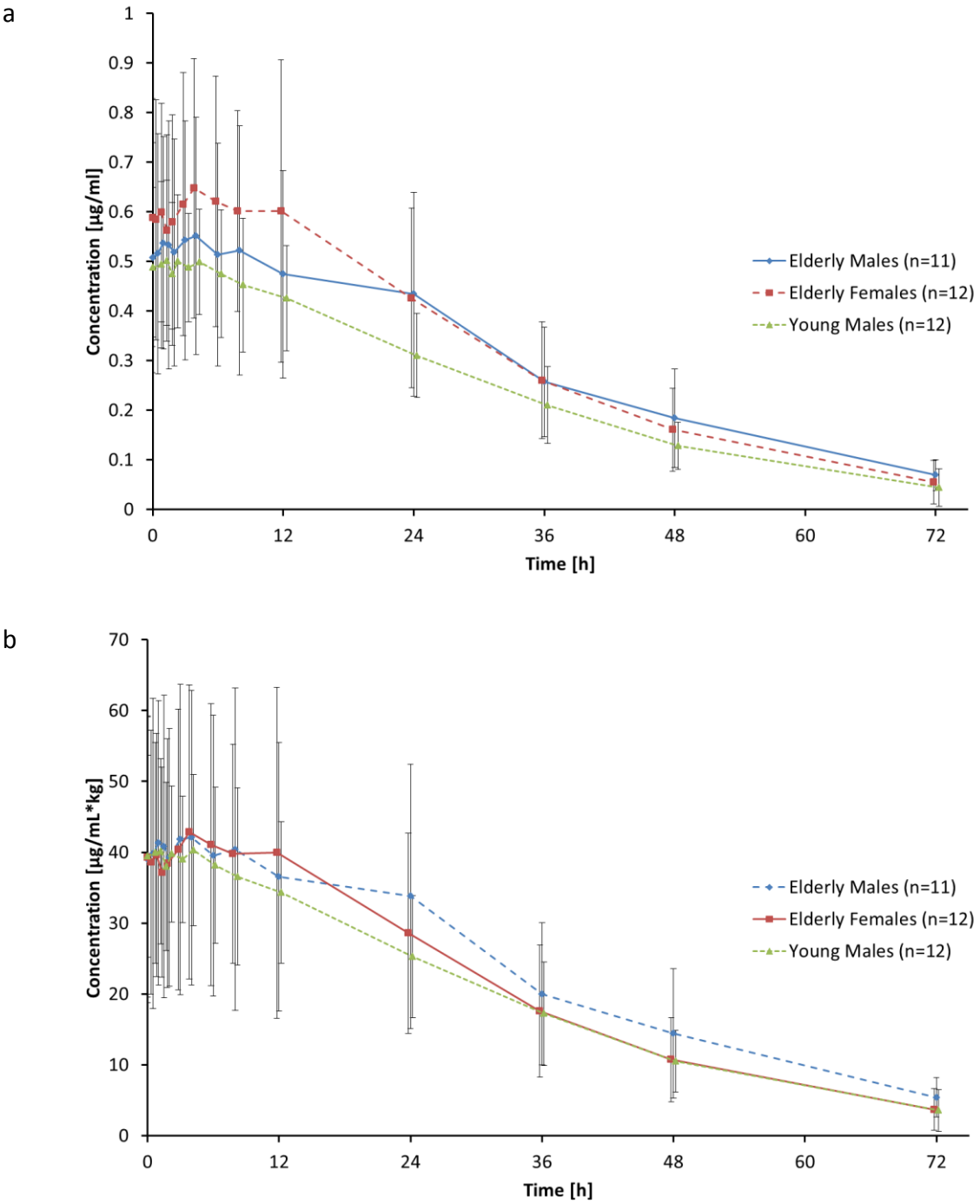


Figure 2-4 Plasma Concentration Time Profile of *O*-Desmethyl-Lacosamide

(Figure taken from Schaefer et al. (Schaefer, Cawello et al., 2015))

Plasma concentration time profile of *O*-desmethyl-lacosamide at steady state (arithmetic mean ± SD) in healthy subjects a) without normalisation and b) after normalisation by body weight

Table 2-7 Plasma Concentration Time Profile of *O*-Desmethyl-Lacosamide in Subjects of Different Age and Gender (arithmetic mean ± SD)

	Elderly Females		Elderly Males		Young Males	
Time	**Conc [µg/mL]**	**SD**	**Conc [µg/mL]**	**SD**	**Conc [µg/mL]**	**SD**
0	0.59	0.24	0.51	0.23	0.49	0.16
0.5	0.58	0.24	0.52	0.24	0.49	0.17
1	0.60	0.22	0.54	0.21	0.50	0.16
1.5	0.56	0.19	0.53	0.25	0.47	0.14
2	0.58	0.22	0.52	0.23	0.50	0.13
3	0.61	0.27	0.54	0.24	0.49	0.11
4	0.65	0.26	0.55	0.24	0.50	0.11
6	0.62	0.25	0.51	0.22	0.48	0.13
8	0.60	0.20	0.52	0.25	0.45	0.13
12	0.60	0.30	0.47	0.21	0.43	0.11
24	0.43	0.18	0.43	0.21	0.31	0.08
36	0.26	0.12	0.26	0.11	0.21	0.08
48	0.16	0.08	0.18	0.10	0.13	0.05
72	0.06	0.04	0.07	0.03	0.04	0.04

Table 2-8 Plasma Concentration Time Profile of *O*-Desmethyl-Lacosamide in Subjects of Different Age and Gender after Normalisation by Body Weight (arithmetic mean ± SD)

	Elderly Females		Elderly Males		Young Males	
Time	**Conc [µg/mL]**	**SD**	**Conc [µg/mL]**	**SD**	**Conc [µg/mL]**	**SD**
0	39.31	19.75	39.02	20.20	39.42	14.26
0.5	38.57	18.60	39.82	21.90	39.93	15.59
1	39.62	17.19	41.34	20.03	40.13	13.05
1.5	37.17	14.81	40.81	21.37	38.01	11.84
2	38.43	17.54	39.26	18.19	39.76	9.61
3	40.40	19.78	41.80	21.94	39.01	8.92
4	42.87	20.74	42.06	20.80	40.28	10.67
6	41.07	19.86	39.51	19.81	38.18	10.99
8	39.77	15.45	40.43	22.75	36.56	12.50
12	39.96	23.35	36.56	18.98	34.31	9.99
24	28.57	14.15	33.79	18.65	25.28	8.62
36	17.60	9.31	20.02	10.01	17.21	7.27
48	10.73	5.92	14.43	9.11	10.49	4.34
72	3.71	2.95	5.42	2.80	3.58	2.94

2.4 Discussion

Present post-hoc evaluation of the effect of age and sex on the pharmacokinetic parameters of lacosamide revealed higher exposure ($AUC_{\tau,ss}$) and maximal plasma concentration ($C_{max,ss}$) in females compared with males in both age groups (older and younger). These differences could largely be explained by the lower body weight and a lower V_d in females compared with males. Furthermore, lacosamide had a broadly similar pharmacokinetic profile in adults with focal epilepsy, which could be compared to the pharmacokinetics in healthy subjects, although direct comparison was not possible. Adults with focal epilepsy received 200, 400, or 600 mg lacosamide per day in addition to 1-3 concomitant anti-epileptic drugs. In summary, the present observations may lead to the assumption that lacosamide represents an anti-epileptic drug with predictable exposure when administered twice daily regardless of an individual's age or sex.

In a recent analysis, Markoula et al. investigated the effects of dose, age, gender and hepatic enzyme-inducing anti-epileptic drugs on the pharmacokinetics of lacosamide assessed by steady state serum lacosamide concentrations (Markoula, Teotonio et al., 2014). They noted that serum lacosamide concentrations increased dose-dependently, were not dependent on age and were higher in women compared with men across the entire age range (44-66 years). It has to be considered that in this analysis no stratification of measured concentration by dose nor a normalisation of measured concentration by body weight were conducted (Markoula, Teotonio et al., 2014). The current evaluation was based upon whole concentration-over-time profiles in healthy subjects stratified in groups of different age and gender (young females aged between 18-40 years, young males aged between 18-45 years, elderly males and females aged $\geq$ 65 years). By contrast, Markoula's analysis was based upon single plasma samples collected from adults patients aged between 19-66 years with the diagnosis of simple or complex POS, with or without secondary generalisation at not defined points of time (Markoula, Teotonio et al., 2014). Furthermore, a median lacosamide dose of 300 mg in a range of 50-600 mg was prescribed to patients in Markoula's study. By contrast, the current evaluation was based upon concentration and parameter values of a consistent dose level of 100 mg per day in each group as the pharmacokinetic parameters of young females were normalised from a dose of 200 mg

to a dose of 100 mg. Nevertheless, overall observations were broadly consistent with present data before normalising maximum plasma concentration by body weight, height, FFM, LBW and V_d. It could be observed that the difference in exposure between young males and young females could be related to different body compositions such as body fat being part of total body weight, due to the reason that following a normalisation approach by body weight or V_d no differences could be observed between young males and females. The same could be observed when the lacosamide exposure of elderly males and females was normalised by body weight or V_d. Therefore, almost all of the numerical differences observed in lacosamide exposure or maximal plasma concentration at steady state between genders, irrespective of age, could be explained by body weight and V_d.

These observations could be supported by the results of the ANOVA. The ANOVA was used to compare the relative bioavailability for overall exposure and maximal plasma concentration by sex after normalisation by body weight or by V_d. It could be seen that after normalising exposure and maximal plasma concentration the 90% confidence interval fell within the 80-125% range accepted for bioequivalence. Without normalisation, the 90% confidence interval fell outside of this accepted range. The range for bioequivalence generally is used to determine whether two medicinal products containing the same active substance show similar bioavailability (rate and extent) after administration in the same molar doses within acceptable predefined limits (EMEA, 2010). The results of plasma concentration time curves are generally used to assess the rate and extent of absorption (EMEA, 2010). AUC - the area under the concentration time curve, reflecting the extent of exposure - and C_{max} - the maximum plasma concentration - are parameters that reflect the extent and rate of exposure. Selected pharmacokinetic parameters and present acceptance limits allow the final decision concerning bioequivalence (EMEA, 2010). In the current evaluation the range for bioequivalence was used to assess whether AUC and C_{max} values of the different age and gender groups were 'bioequivalent' when normalising values by scaling factors.

When regarding the age effect, one might assume that older age would lead to higher mean lacosamide plasma concentration due to the lower percentage of total body water in the elderly, as well as to changes in renal clearance and metabolic

function, found with advancing age (Bossingham, Carnell et al., 2005, Klotz, 2009). Accordingly, it was noted that the healthy elderly cohorts aged between 65 and 71 years had numerically higher exposure and maximal plasma concentration compared with healthy younger cohorts. Nevertheless, differences were reduced following normalisation by body weight or V_d. When assessing the age effect on relative bioavailability, normalisation by lean body weight or V_d resulted in equivalent values for exposure and maximal plasma concentration, as the 90% confidence interval of the ratio (older/younger) fell within the 80-125% range accepted for bioequivalence. The present observations with lacosamide were supported by what is already known about the compound namely its high water solubility (>20 mg/mL) and the changes in renal function/clearance, metabolism, total body water and extracellular fluid, which could be found with advancing age (Beyreuther, Freitag et al., 2007, Bossingham, Carnell et al., 2005, Klotz, 2009, UCB, 2014b).

When trying to obtain actual available information on pharmacokinetic characteristics of anti-epileptic drugs, most of them were based upon healthy subjects of male gender and with an age under 65 years (Ghandi, Aweeka et al., 2004). The current analysis was intended to broaden this spectrum through analysing lacosamide pharmacokinetics in adults with focal epilepsy by age and gender, whereas those patient results were taken from the analysis of Schaefer et al. and other literature reported in section 2.2.4. The results in adults with focal epilepsy were comparable to those reported herein for healthy subjects. Patients with focal epilepsy had similar overall age, height, weight and BMI characteristics compared with those reported for healthy subjects from studies SP620 and SP599. Age was reported to be no covariate in the analysis of patients with focal epilepsy. Total body clearance of lacosamide was higher in male (2.06 L/h) compared with female (1.88 L/h) adults with focal epilepsy. However, numerical differences in plasma concentrations for subjects of different age, sex and body weight contributed less than 20%. As the differences in exposure and maximal plasma concentrations in healthy subjects of present evaluation could mostly be explained by differences in body weight and V_d, the same could be determined in patients with focal epilepsy. The differences between male and female patients with focal epilepsy reported in literature could mostly be explained by differences in total body water. Furthermore, simulations of the population with focal epilepsy were

conducted by using extremes in body weight versus average body weight suggested < 15% variation in plasma concentrations, which again was not considered as clinically relevant. The overall observations taken from the population pharmacokinetic evaluation from 565 adults with focal epilepsy (details see section 2.2.4) suggested that lacosamide exposure was predictable, regardless of age. Nevertheless, it could be shown that special populations existed for whom dose adjustment was required. Cawello et al. have shown that in patients with severe renal impairment with a creatinine clearance of below 30 mL/min, lacosamide exposure was increased by approximately 60% compared to subjects with normal renal function (Cawello, Fuhr et al., 2013).

Nevertheless, some limitations should be considered when interpreting the results of the present *post-hoc* analysis. The datasets were pooled from two different studies of healthy subjects and two different trials of adjunctive lacosamide for adults with focal epilepsy. For this reason, the healthy and focal epilepsy cohorts could not be directly compared to each other and thus observations should be considered in a descriptive way. The pharmacokinetic evaluation in healthy subjects used a non-compartmental analysis, whereas the population pharmacokinetic evaluation of adults with focal epilepsy used a compartmental analysis. Both standard methods of evaluations had total body clearance (CL/F) as one of the main pharmacokinetic parameters, providing a point of reference between the two approaches. Indeed, despite different methodologies, observations remained broadly consistent. Concerning the urine data in the SP599 study, no urine data were taken from healthy young women and thus no renal clearance data were available. Moreover, healthy young females could have been up to 5 years younger than their male counterparts as the maximum enrolment age for women was 40 years in the SP599 study (Cawello, Rosenkranz et al., 2013).

2.5 Conclusion

In the present work, it is shown that age and gender had no effect on the rate of absorption and rate of elimination of lacosamide. Numerical differences in concentration dependent pharmacokinetic parameters such as AUC and C_{max} between cohorts are explained by the main scaling factor of body weight or V_d. Following normalisation by either body weight or V_d, pharmacokinetic parameters irrespective of age or sex were similar to those typically expected for lacosamide. Lacosamide had a broadly similar pharmacokinetic profile in adults with focal epilepsy as seen in healthy subjects, although direct comparison was not possible. Therefore, lacosamide represented an AED with predictable exposure when administered twice daily in individuals, regardless of age or gender.

3 CHAPTER 3: COMBINED PHARMACOKINETIC MODEL FOR LACOSAMIDE AND ITS METABOLITE FOR INTEGRATED PHARMACOKINETIC MODELING IN HUMANS

3.1 General Introduction

The introductory part should provide an insight into the necessary background information on pharmacokinetic modeling by providing a short overview of the general methods of pharmacokinetic model development as well as the handling with special populations such as renal impaired subjects.

3.1.1.1 Pharmacokinetic Models and Pharmacokinetic Modeling

To develop pharmacokinetic models, it was necessary to consider age- and gender-related differences in subjects. In Chapter 2 it was investigated the extent to which known pharmacokinetic parameters of lacosamide were influenced by age and gender in healthy subjects. It could be shown that both factors had no effect on absorption and elimination processes of lacosamide whereby it was not necessary to consider them during pharmacokinetic model development.

Although several pharmacokinetic textbooks describe the model-dependent pharmacokinetics of a drug in one tissue (e.g. drug in plasma or its amount excreted in urine), to understand the complex mechanisms of these transport processes it is important to develop new models that include the model-dependent pharmacokinetics of a drug and its metabolites, including the amount of unchanged drug excreted into urine. Given that most drugs are eliminated by excretion and metabolism, pharmacokinetic models that include parallel elimination pathways represent a progress in developing new pharmacokinetic models describing the kinetic behaviour of one drug and its metabolite in different tissues.

Pharmacokinetic models represent hypothetical structures that are used to describe the fate of drug and its metabolites in a biological system following its administration and have numerous uses in clinical applications and drug design (Dhillon and Gill, 2006, Gerlowski and Jain, 1983). A model generally has to match itself with real conditions to be confirmed or neglected (Bozler, Heinzel et al., 1977). Two approaches exist based upon classical and physiological models, whereby the present evaluation is based upon the classical approach, which uses a compartmental system

and fits exponential functions to time-dependent plasma concentration data (Gerlowski and Jain, 1983). Models generally describe certain aspects of reality by mathematical means in a simplified way (Meibohm, Beierle et al., 2002). Pharmacokinetic models are very useful to summarise data from a pool of subjects or patients (Bourne, 2013, P.296).The most common method to describe the pharmacokinetic character of a drug is to present the human body as a system of compartments (Gibaldi and Perrier, 1975, P.1). A compartment describes a closed homogenous space for the transport processes of a drug and could be total blood volume (described as the central compartment) or muscle or fat tissue (described as peripheral compartments) (Brett, Weimann et al., 2003, P.7). The simplest model is the one-compartment model, which mirrors the body as a homogenous unit (Gibaldi and Perrier, 1975, P.1). This model is often used when analysing blood, plasma or serum concentrations and urinary excretion data for drugs which are marked by a rapid distribution between plasma and other body fluids upon entry into the systemic circulation (Gibaldi and Perrier, 1975, P.1). The criterion to use a one-compartment model is that the elimination of a drug occurs from the body in a first-order fashion, meaning that the rate of elimination of a drug from the body at any time is proportional to the amount of drug in the body at that time (Gibaldi and Perrier, 1975, P.1). Whereas the first part of the present dissertation is based upon a non-compartmental analysis, the present part is based upon a compartmental analysis.

The following figure illustrates the compartment models for the transport processes in pharmacokinetics.

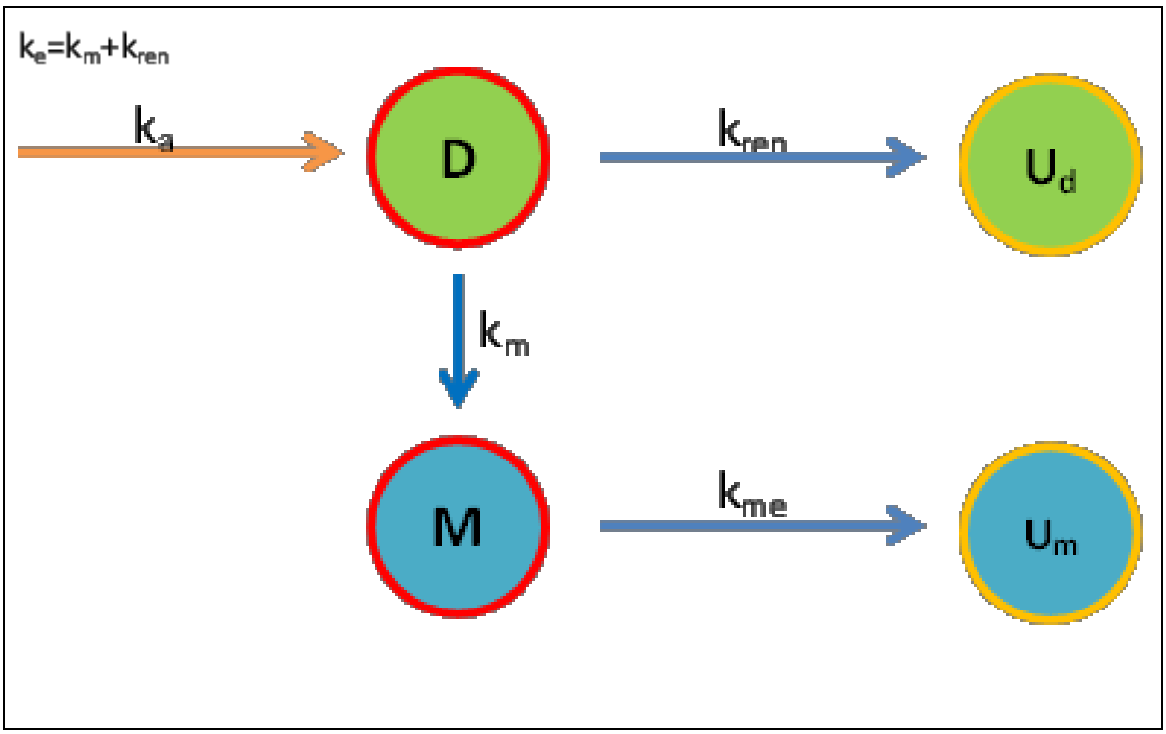


Figure 3-1 Compartment Model

Illustration of the drug (D) and its metabolite (M) in central compartment as well as the drug absorption, metabolism, renal elimination and renal elimination of the metabolite. U_d and U_m represent the amount of unchanged drug and metabolite in urine. k_a represents the rate constant of absorption of drug, k_e represents the overall rate constant of elimination and should be the sum of renal excretion, characterised by rate constant k_{ren}, metabolism and other elimination processes. k_{me} represents the rate constant of elimination of the metabolite and k_m the rate constant of metabolisation (Bourne, 2010).

Pharmacokinetic modeling represents an important tool in drug development and for the integration of data to make rational decisions regarding drug use and development (Mould and Upton, 2012). If an appropriate model is developed, it can provide a framework for the prediction of time courses of exposure and response for different dose regimens (Mould and Upton, 2012). The basis of pharmacokinetic models represent differential equations whereas a good model should be able to fit the data to simulate and predict different case scenarios with a certain degree of comfort (Ruiz-Garcia, Bermejo et al., 2008). Simulation approaches allow the consequent application of all earned information to predictions and offer the opportunity of correction when testing the acquisition to reality of the model (Bozler, Heinzel et al., 1977). After developing pharmacokinetic models, it is possible to observe correlations between pharmacokinetic parameter values and clinical measures such as measures of renal, hepatic, cardiac or other patient characteristics such as body height or volume of distribution (Bourne, 2013, P. 297). If an adequate pharmacokinetic model is built up, it is possible to predict the parameters of a pharmacokinetic model with given subsequent blood level determinations whereby dosage can be designed to produce therapeutically desirable drug plasma concentration levels (Sheiner, Rosenberg et al., 1972).

3.1.2 Pharmacokinetic Model Development

Several pharmacokinetic text books include the description of the model-dependent pharmacokinetics of a drug in a certain tissue like blood or plasma or the amount of unchanged drug in urine. The AGAH working group developed a collection of terms, symbols, equations and explanations of common pharmacokinetic and pharmacodynamic parameters and some statistical functions to provide an overview of existing pharmacokinetic models in a one- and two-compartment model (AGAH, 2004). As previously mentioned, pharmacokinetic models are described as equations including a dependent variable (y variable) expressed as a function of independent variable(s) (x variable) with various constants and/or parameters (Bourne, 2013, P.301). When developing a pharmacokinetic model after a one-compartment pharmacokinetic model it can be represented schematic or as a differential equation as for the one-compartment model after an intravenous (IV) bolus injection (Equation 1) (Bourne, 2013). The following differential equation (Equation 7) describes the rate of change of drug plasma concentration (Cp) versus time (t), which is proportional to the concentration remaining to be eliminated. The slope of this line - the proportionality constant - can be defined as k_e, the rate constant of elimination. The rate constant of elimination can also be expressed through the quotient of clearance (CL) and V_d.

7) $$\frac{dCp}{dt} = -k_e \cdot Cp = -\frac{CL}{V_d} \cdot Cp$$

Nevertheless, it can be difficult to use a differential equation described before when trying to determine k_e or CL. For this reason, an integrated form of Equation 1 can be more useful. A method to transform a differential function into an integrated form is the Laplace transformation. The Laplace transformation and its inversion belong to the functional transformations or integral transformations and represent methods to solve linear differential equations (Gibaldi and Perrier, 1975, P.267).

3.1.2.1 Laplace Transformation

Laplace transformation replaces the time domain of a rate expression by the complex domain of the Laplace operators. Through algebraic techniques complex rate expressions can be reconstructed if the time variable is replaced by the Laplace operator. The transformed expression can be rearranged whereby it results in a form

that is included in the table of Laplace transforms. When transforming back into the time domain the differential equation is completely solved (Gibaldi and Perrier, 1975, P.267).

The Laplace integral Lf(t) defined by

8) $Lf(t) = \int_0^\infty e^{-st} f(t)\, dt$

is used to transform a time-dependent expression into the s domain, whereas f(t) represents the time-dependent function. f(t) is multiplied by e^{-st} and evaluated by integration from time zero (0) to infinity (∞) (Equations 9 and 10). To facilitate the implementation of the Laplace transformation, repeatedly used transformed functions can be found in a table of transforms, which can be referred to for the conversion of the desired time-dependent expression (Gibaldi and Perrier, 1975, P.268).

Example:

9) $L(A) = \int_0^\infty e^{-st} A\, dt$

Integrated:

10) $L(A) = A\left(-\frac{1}{s}\right) e^{-st} \cdot \Big|_0^\infty$

Evaluated between the limits of time zero and infinity yields:

11) $L(A) = \frac{A}{s}$

Therefore, for any constant, the form $L(A) = \frac{A}{s}$ can be taken. When the transforms are known, integration will not be necessary (Gibaldi and Perrier, 1975, P.268).

The Laplace equations in the present thesis were developed via the convolution method and their back-transformation, which will be described in the methods part.

3.1.3 Pharmacokinetic in Special Populations with and without Epilepsy

Many factors influence the choice of an AED, including the efficacy, the tolerability, the indication and the toxicity (Anderson and Hakimian, 2014). It is important to know that the effect of renal and hepatic impairment on the dosing regimen will be dependent on the AED fraction eliminated by hepatic and/or renal excretion and other processes (Anderson and Hakimian, 2014). Physiological restrictions such as renal impairment could lead to alterations in the pharmacokinetic profile of a drug and their metabolite(s) if it has path of renal elimination (Cawello, Fuhr et al., 2013). This could result in changes of absorption, hepatic metabolism, plasma protein binding, or distribution which could lead to justify changes in the dosing regimen (Cawello, Fuhr et al., 2013). For this reason, it is important to assess the potential effect of renal impairment on drug pharmacokinetics to evaluate safety assessment (Cawello, Fuhr et al., 2013). Renal impairment results in a lower capacity of the kidney to eliminate drugs via compromised glomerular filtration and/or tubular secretion (Gibaldi and Perrier, 1975, P.254). The result is an accumulation of potentially toxic drugs and metabolites which are dependent on elimination via the kidneys (Brater, 2009, Gibaldi and Perrier, 1975, P.254). It has to be considered that in people who are aged over 65 years, the pharmacokinetics of a drug is influenced more by the loss of kidney function than by the ageing process of any other organ (Aymanns, keller et al., 2010). Especially among the elderly population, developing seizures and epilepsy increases, which enforces the evaluation of the pharmacokinetics of AEDs in subjects with renal impairment (Brodie, Elder et al., 2009, Cawello, Fuhr et al., 2013). Treating seizures in patients with renal failure represents a frequently encountered challenge, supported by the fact that limited data exists for the new AEDs, which makes an understanding of how the drug is affected by kidney disease much more difficult (Diaz, Deliz et al., 2012).

3.1.4 Impact of Renal Impairment on the Pharmacokinetics of Lacosamide

Lacosamide - which is approved for the adjunctive treatment of POS in adults - is mainly excreted via the kidneys. Further information on the AED lacosamide can be found in Chapter 2, section 2.2.1 of the present dissertation. The non-compartmental evaluation from Cawello et al. could show that the pharmacokinetic profile of lacosamide and the *O*-desmethyl-metabolite were altered when renal function was impaired (Cawello, Fuhr et al., 2013). The fact that lacosamide is metabolised by the CYP enzyme system, mainly CYP2C19, underlined the results of Cawello et al., namely that lacosamide metabolism plays an important role in the total body clearance of lacosamide. Nevertheless, the main outcome from the evaluation of Cawello et al. was that the dose of lacosamide does not need to be adjusted for patients with mild-to-moderate renal impairment, but rather for those with severe renal impairment (Cawello, Fuhr et al., 2013). It could also be shown that there was no correlation between metabolic clearance and renal function but rather between renal clearance and renal function (Cawello, Fuhr et al., 2013).

3.1.5 Objective

The objective of the prior part of the present dissertation was to develop a pharmacokinetic model that included the model-dependent pharmacokinetics of a drug and its main metabolite in plasma as well as its unchanged cumulative amount excreted in urine. The software used for pharmacokinetic modeling should be validated with respect to its suitability for pharmacokinetic modeling by iterating values for pharmacokinetic parameters based upon the model. The suitability of the developed system of equations should be evaluated with study data of the AED lacosamide in healthy and mild to severe renal impaired subjects to evaluate whether the results of pharmacokinetic parameters were in line with present understanding of lacosamides' behaviour in plasma and the dependence of lacosamides' metabolism and its renal excretion on renal function.

3.2 Methods

3.2.1 General Methods in Developing Pharmacokinetic Models

The following paragraph will provide a general overview of the methods of pharmacokinetic model development, statistical methods of model evaluation and the software programs used.

3.2.1.1 The Hierarchy of Pharmacokinetic Models

When discussing pharmacokinetic models, it is important to know their hierarchy. At the lowest level there is the empirical model, which is often described by a sum of exponential terms and describes the plasma concentration time-curve of a drug (Aarons, 2005). They are used to derive pharmacokinetic parameters such as clearance and terminal half-life (Aarons, 2005). When adopting a compartmental approach, it is possible to relate the pharmacokinetic parameters to physiological processes such as clearance that can be related to renal function. This can be represented through the pharmacokinetic parameter renal rate constant of elimination and the effect of renal function on the concentration time profile of a drug (Aarons, 2005). Whereas many pharmacokinetic models are based upon physiological considerations - which would be a step further than the compartment methods – the present evaluations are based upon compartmental pharmacokinetic evaluations resting upon empirical equations.

3.2.1.2 Pharmacokinetic Model Development

As mentioned in the introduction, a pharmacokinetic model after a one-compartment pharmacokinetic model can be represented schematically or as a differential equation. The convolution method described by Benet (Benet, 1972) can be used to develop Laplace equations for the amount of drug in the central compartment by a simple multiplication of the input fraction and the disposition function (Equation 12). The input function is used to describe the route of administration (in_{ROA}), whereas the disposition function describes the first-order distribution and elimination processes (Bourne, 2010).

The basis of the equation is:

12) $$\overline{X}_i = in_{roa} \cdot disposition_i$$

A general pharmacokinetic model is shown below with elimination via excretion into urine (k_e), metabolism (k_m) or other processes (k_{other}) (Bourne, 2010).

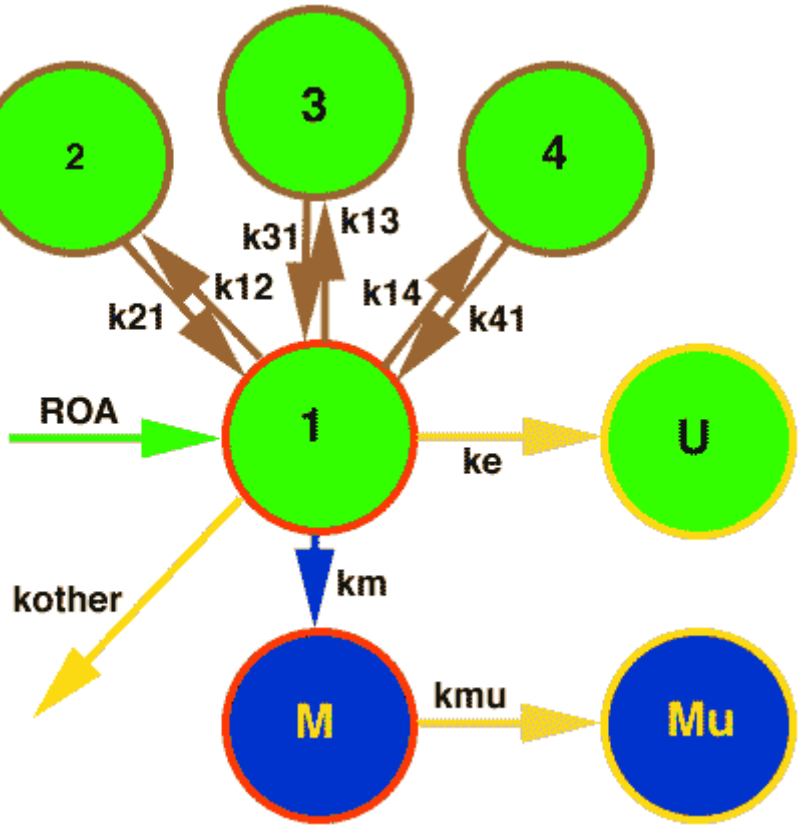


Figure 3-2 General Pharmacokinetic Model

The central compartment is red; the tissue compartments are brown whereas the urine component of the model is represented by an orange border. The drug is shown by the filled green circles and metabolite by the blue circle. k_e represents the overall elimination rate constant and is the sum of all renal excretion, metabolism and other elimination processes (Bourne, 2010). k_{mu} represents the metabolic elimination rate constant

Table 3-1 Input Route of Administration:

Route of Administration		Input Function
13)	IV Bolus	Dose
14)	IV Infusion-Continuous	$\frac{k_0}{s}$
15)	IV Infusion[1]	$\frac{k_0 \cdot (e^{-a \cdot s} - e^{-z \cdot s})}{s}$
16)	Oral[2]	$\frac{F \cdot Dose \cdot k_a}{(s + k_a)}$

a=time when the infusion is started, z=time when infusion is stopped, F=bioavailability

If a=0 and z=∞ it is simplified to $\frac{k_0}{s}$

Table 3-2 Laplace Disposition Functions

Number of Compartments		Disposition Function
17)	One	$\frac{1}{(s + k_e)}$
18)	Two[1]	$\frac{(s + k_{21})}{(s + \alpha) \cdot (s + \beta)}$
19)	Three[2]	$\frac{(s + k_{21}) \cdot (s + k_{31})}{(s + \alpha) \cdot (s + \beta) \cdot (s + \gamma)}$

[1] $\boldsymbol{\alpha + \beta = k_e + k_{12} + k_{21}}$ ***and*** $\boldsymbol{\alpha \cdot \beta = k_e \cdot k_{21}}$

[2] $\boldsymbol{\alpha + \beta + \gamma = k_e + k_{12} + k_{21} + k_{13} + k_{31}}$,

$\boldsymbol{\alpha \cdot \beta \cdot + \alpha \cdot \gamma + \beta \cdot \gamma = k_e \cdot k_{21} + k_e \cdot k_{31} + k_{13} \cdot k_{21} + k_{12} \cdot k_{31} + k_{21} \cdot k_{31}}$ ***and***

$\boldsymbol{\alpha \cdot \beta \cdot \gamma = k_e \cdot k_{21} \cdot k_{31}}$

Table 3-3 Sample Site Function

Sample Site		Function
20)	Drug in Central Compartment	1
21)	Drug in Peripheral Compartment[1]	$\frac{k_{1x}}{(s + k_{1x})}$
22)	Drug in Urine	$\frac{k_e}{s}$
23)	Metabolite in Central Compartment	$\frac{k_m}{(s + k_{me})}$
24)	Metabolite in Urine	$\frac{k_m \cdot k_{me}}{s \cdot (s + k_{me})}$

[1]x refers to the 2nd, 3rd or 4th, peripheral, compartment as shown in Figure 3-2

The fingerprint method represents a method for the back-transformation of many of the Laplace equations that can be found in pharmacokinetics. The methods are derived from the explanation of the general partial fraction method presented by Benet and Turi (Benet and Turi, 1971, Bourne).

Limitations/Requirements

- The degree - in s - of the polynomial in the denominator has to be higher than the polynomial in the numerator
- No repeating terms in the denominator

General Procedure

- To check the limitations described above
- Determine the roots in the denominator; when the amount of concentration of interest was solved for the Laplace, the denominator should be the form:

25) $s \cdot (s + a) \cdot (s + b) \ldots$

To find the root(s) of the polynomial in the denominator each of the factors can be set to zero and used to find a value (or root) for s.

Therefore, from Equation 25, the Equations 26, 27, and 28 can be formulated and the roots for the denominator are s=0, s=-a, s=-b, etc.

26) s=0

27) s+a=0

28) s+b=0

There can be one more root to the denominator, whereas the next step is to cover the part corresponding to each root in turn and replace all instances of ‘s’ in the remaining equation with the current root. Subsequently, the term is multiplied by $e^{root \cdot t}$. The sum of all the terms from each root represents the result that can be simplified as much as necessary.

The implementation of the convolution method and the method of back-transformation in the present evaluation will be described later.

3.2.1.3 Pharmacokinetic Parameters

Once the pharmacokinetic model has been written the pharmacokinetic parameters have to be specified and/or estimated. In the following a brief description of pharmacokinetic parameters generally used in pharmacokinetic models will be given.

Total Body Clearance

The total body clearance (CL/F) is a pharmacokinetic disposition parameter which describes how quickly a drug is eliminated from the body and represents a useful parameter to describe drug disposition (Benet, 1984, Bourne, 2013, P.36, Gibaldi and Koup, 1981). Clearance is often defined as the volume of blood or plasma which is completely cleared of the drug per time. It represents a measure of the efficiency and rate by which a drug is eliminated from the body via all routes and thus determines the plasma drug concentration (Brett, Weimann et al., 2003, P.81). It has to be considered that clearance can be described in the context of one eliminating organ or the sum of those organs (Brett, Weimann et al., 2003, P.81).

Apparent Volume of Distribution

To calculate a plasma concentration it is necessary to know the volume of distribution (V_d) which represents a mathematical factor which relates to the amount of drug in the body or in the measured compartment (usually plasma, serum or blood) (Bourne, 2013, P.38). As for the main substance, a volume of distribution exists for the metabolite (V_{dm}).

Elimination Rate Constant

The overall elimination rate constant (k_e) represents the first-order rate constant describing drug elimination from the body (Bourne, 2013, P.41). The fact that it is an overall elimination rate constant means that it includes all elimination processes such as excretion and metabolism, whereas metabolites have their own elimination rate constants (k_{me}) due to other chemical properties (Bourne, 2013, P.41). If a drug is eliminated we will speak of renal rate constant of elimination (k_{ren}).

Rate Constant of Absorption

The rate constant of absorption represents the first-order absorption constant (k_a) and it can also describe the creation of the metabolite, albeit with an own parameter, namely k_m. After drug administration by the oral routes, some time passes until the drug appears in the central circulation. This time is named 'lag'-time (t_{lag}) and it reflects the time required for disintegration and dissolution of the drug and the time until the drug reaches the absorbing surface of the small intestine (Atkinson, Daniels et al., 2001, P.44)

3.2.1.4 Software for Pharmacokinetic Modeling and Parameter Estimation

Several software programs exist for pharmacokinetics and pharmacodynamic modeling. In a recent work of the AGAH working group for pharmacokinetic/pharmacodynamic modeling "Pharmacokinetic modeling using different software packages", different software items were compared and evaluated. The software used was Topfit, a PC-based pharmacokinetic/pharmacodynamics data analysis program, Kinetica 2000, NONMEN 5.1, a modeling software that can estimate parameters in mixed-effects models based upon maximum-likelihood or Bayesian techniques that take stochastic or gradient estimation models and WinNonlin Version 3 (Keizer, Karlsson et al., 2013, Tanswell and Koup, 1993). SAS being also one of the software programs that were used for pharmacokinetic modeling is explained more in detail due to the fact being the chosen software tool for the present evaluations.

SAS is a software suite developed by SAS Institute for advance analytics, business intelligence, data management and predictive analytics and is able to mine, alter, manage and retrieve data from a variety of sources and perform statistical analysis (Yang and Wang, 2010). SAS version 9.3 for Windows was used for the present evaluation.

Several digital computer programs are used in pharmacokinetics for the non-linear LS estimation of pharmacokinetic parameters and also for the simulation of concentration time courses of drugs in pharmacokinetic systems (Kinetica, Winnonlin etc.). Programs such as NLIN or NONLIN can be used for the non-linear squares regression analysis of pharmacokinetic systems (Gibaldi and Perrier, 1975, P.307). The

procedure *nlin* which was used in present analysis in SAS is explained in detail in section 3.2.4.2.

All statistical evaluation and pharmacokinetic modelling was executed in SAS. The datasets for the validation of SAS program as well as the datasets of the SP641 study were transferred into SAS datasets for further evaluations.

A SAS script comprises two main steps, namely the DATA and the PROC step.

DATA Step

The DATA step is a group of SAS language statements that begins with a DATA statement and contains programming statements that create SAS data sets from a raw data files or manipulate existing SAS data sets (BAILII, 2010). DATA step statements may be either executable or declarative statements. Executable statements result in some action during individual iterations of the DATA step. The declarative statements supply information to SAS and take effect when the system compiles program statements (BAILII, 2010).

PROC Step

The PROC step is a SAS language statement that begins with a PROC statement. The PROCs are software tools that are written by SAS Institute to perform a wide variety of particular types of data analysis and reporting. Through the PROC statement, it is possible to process and analyse data in SAS datasets to procedure statistics, tables, reports, charts, plots, etc. (BAILII, 2010).

Once a software program has been chosen, it has to be validated regarding its goodness of fit and thus its suitability for pharmacokinetic modeling with selected mathematical model. In the following paragraph, the validation process including the procedure used in SAS will be described based upon the developed pharmacokinetic models. Furthermore, the process of pharmacokinetic modeling with lacosamide based upon study data of SP641 will be described including the structure of the chosen study.

3.2.1.5 Validation Methods

A validation process in analytical procedures includes all of the procedures that show that a particular method used for quantitative measurement of analytes in a given biological matrix such as blood, urine etc., is reliable and reproducible for the intended use (FDA, 2001). Several parameters exist that must be determined for the validation process, such as accuracy, precision, selectivity, sensitivity, reproducibility and stability, although the present validation process is based upon accuracy and precision as desired statistical measures for evaluation. It has to be considered that the present validation did not represent the classical analytical procedure of taking samples, although the analytical method to evaluate the extent to which the procedure in the used software was able to find the correct pharmacokinetic parameters through iteration processes.

Accuracy

"The accuracy of an analytical method describes the closeness of mean test results obtained by the method to the true value (concentration) of the analyte. Accuracy is determined by replicate analysis of samples containing known amounts of the analyte" (FDA, 2001).

Precision

"The precision of an analytical method describes the closeness of individual measures of an analyte when the procedure is applied repeatedly to multiple aliquots of a single homogenous volume of biological matrix" (FDA, 2001).

A detailed insight in the method of calculating precision and accuracy in the present evaluation can be seen in section 3.2.4.4.

3.2.2 Development of Pharmacokinetic Models

To validate the used tools in SAS a total of three combined pharmacokinetic models were chosen and developed. The system of pharmacokinetic models developed was designed to predict the behaviour of 1) unchanged drug in plasma, 2) main metabolite in plasma and 3) unchanged drug excreted in urine. As described above, the mathematical pharmacokinetic models were developed by using the convolution method. Figure 3-1 illustrates the relationship between amounts of drug (D) and its metabolite (M) in the central compartment showing the drug absorption, metabolism and renal elimination, as well as renal elimination of the metabolite.

While the mathematical model for the pharmacokinetic of a drug after oral administration was represented by the Bateman function, Laplace equations were generated for the plasma concentration of the metabolite and the amount of unchanged drug excreted in urine. The fingerprint method, as explained in section 3.2.1.2, was used for the back-transformation of generated Laplace equations. The volume of distribution of unchanged drug and of the metabolite served as scaling factors to connect the concentrations of unchanged drug and metabolite in plasma. Both were included in the mathematical models to enable the calculation of concentrations rather than amounts of drug or metabolite in plasma.

In the validation process, the equations for the model-dependent pharmacokinetic of unchanged drug in plasma and its amount excreted in urine, as well as the model-dependent pharmacokinetic of the main metabolite in plasma were connected to one system of equations to involve the pharmacokinetic parameters into each of the aforementioned pharmacokinetic system of subunits. The model was applied to plasma and urine data generated for a fictive study population. The source data (pharmacokinetic parameters) and methods of data generation for this fictive study population are described in section 3.2.4.

The final model, namely the combination of all three models, was finally used for the pharmacokinetic modeling process based upon plasma and urine data of lacosamide and its metabolite of study SP641. The data of renal elimination of the main metabolite were not considered.

3.2.3 Software

The plasma and urine data of a fictive study population for the pharmacokinetic model of unchanged drug in plasma and its amount excreted in urine as well as the model metabolite in plasma were generated in SAS.

Further information on SAS can be found in section 3.2.1.4.

3.2.4 Validation Process

3.2.4.1 Data Generation and Generation of Plasma and Urine Datasets

The generation of plasma and urine data of twenty fictive subjects with individual pharmacokinetic parameters was executed in SAS. The values of pharmacokinetic parameters for each subject were randomised with a normal distributed variability to imitate inter-individual variability. The procedure for obtaining randomised normal distributed values is conducted through the command *rannor* in SAS.

Based upon the random values of the set of pharmacokinetic parameters of all twenty subjects and the mathematical models, data sets of the concentration time profiles or the amount excreted in urine at defined points of time were calculated by inserting individual pharmacokinetic parameters at each point of time t. Calculated plasma concentrations and amounts excreted in urine were assigned a proportional error to imitate the case of typical errors occurring through analysing processes for drug concentrations or amounts in urine. For this step, procedure *rannor* was used to generate random numbers following a normal distribution around zero with a SD of 0.1.

In the following table, the values for pharmacokinetic parameters and chosen sampling points are presented (Table 3-4). These values represent the reference values for all pharmacokinetic parameters.

The SAS programs for the data generation and generation of plasma and urine data sets can be seen in Appendix 5. The source data - namely the plasma and urine data sets generated for 20 fictive subjects of a study population - can be seen in Appendix 6.

Table 3-4 Pharmacokinetic Parameters of 20 Fictive Subjects

Parameter	Mean	SD
k_a	2/h	0.2/h
k_{me}	0.04/h	0.005/h
k_m	0.07/h	0.01/h
k_{ren}	0.05/h	0.005/h
V_d	50 L	10 L
V_{dm}	100 L	30 L

The sampling time points were 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 12, 16, 24 and 48 hours for the unchanged drug in plasma and same time points plus 36, 72, 96 and 120 hours for the metabolite in plasma. For urine data, the time intervals for sampling were 0–2, 2–4, 4–6, 6–8, 8–12, 12–16, 16–24, 24–36, 36–48 and 48–72 hours.

3.2.4.2 Pharmacokinetic Modeling

To estimate individual pharmacokinetic parameters of each subject from a given set of concentration data values, the non-linear regression model in SAS was used. The *nlin* procedure generally works with LS or weighted least squares estimates of the parameters of a non-linear model (not to confound with non-linear pharmacokinetic models) that are produced (SAS Instiute Inc., 1999). LS criterion represents a measure of how well the procedure or program generated line fits the data and describes the total of the differences between observed and calculated or predicted data points (Bourne, 2013, P.302). For this process, it is necessary to write the regression expression, declare parameter names and supply initial parameter values, such as starting parameters for the pharmacokinetic parameters. The estimation represents an iterative process in which procedure *nlin* first examines the starting values for in this case pharmacokinetic parameters and evaluates the residual sum of squares at each combination of values to optimise the order of parameters and minimise the sum of squares (SAS Instiute Inc., 1999). Several iterative methods exist that can be used such as Newton method, modified Gauss-Newton method etc. whereas the current evaluations were based upon the Marquardt method.

The non-linear regression of model parameters was mostly influenced by the high values, so a weight was included to each residual which considered not the absolute but the relative error. The weighting procedure was executed through the method $\frac{1}{(reference\ value)^2}$ (AGAH, 2001). Recent validations have shown that this weighting procedure was the most effective one and thus it was chosen in the present evaluation.

The following statements were included in the procedure *nlin* (SAS Instiute Inc., 1999):

BOUNDS	constrains the parameter estimates within specified bounds
BY	specifies variables to define subgroups for the analysis (e.g. subjects)
MODEL	defines the relationship between the dependent and independent variables
OUTPUT	creates an output data set containing statistics for each observation
PARMS	identifies parameters to be estimated and the starting values for each parameter

Given that many study data generally include fewer sampling points in the absorption phase, the pharmacokinetic parameter k_a could be imprecise. For this case, the model for the concentration of unchanged drug in plasma and the amount of unchanged drug in urine included a fixed value for k_a. In pharmacokinetic modeling with real study data, this would be a substance-specific value for k_a. This method was intended to improve the results of iteration processes.

3.2.4.3 Simulation and Correlation

With the predicted values of pharmacokinetic parameters, concentration-over-time profiles for unchanged drug and metabolite in plasma as well as cumulative amount excreted in urine for each subject were calculated during the validation process.

A correlation analysis was undertaken by correlating predicted and reference pharmacokinetic parameters as well as reference concentrations/amounts and predicted concentrations/amounts. The dimension of correlation was expressed by the parameters intercept and slope. Through calculation of both, the optimal grade of prediction could be achieved.

The slope generally represents the dependence between predicted and measured values and is proportional to the correlation (Bortz and Schuster, 2010, P.188-189). A value close to one for the slope means that the linear dependence of the model fits, whereas for the coefficient of determination a value around 1 means a precise result (Bortz and Schuster, 2010, P.188-189).

The correlation was defined by the coefficient of determination by using the procedure *reg* in SAS.

3.2.4.4 Statistics

The adequacy of the system was evaluated by typical validation characteristics namely accuracy and precision of the pharmacokinetic parameters and were calculated as follows:

29) $$\text{Error} = Val_{reference} - Val_{iterated}$$

30) $$Relative\ error\ (\%) = \frac{Error}{Val_{reference}} \cdot 100$$

31) $$Accuracy\ (\%) = mean(relative\ error)$$

32) $$Precision\ (\%) = SD\ of\ relative\ error$$

$Val_{reference}$ stands for reference value and $Val_{iterated}$ for iterated value of pharmacokinetic parameter.

Accuracy and precision were chosen as desired statistical measures for evaluation, as proposed in the ICH Harmonised Tripartite Guideline of Validation of Analytical Procedures and were evaluated in the light of the Food and Drug Administration (FDA) guideline for bioanalytical method validation (FDA, 2001, ICH, 1994). It has to be considered that the FDA generally stated a replicate of samples or measurements that were necessary for these determinations, whereas the present evaluation was based upon the precision and accuracy of iterated values compared with generated values (as representatives for measured values), of the individual subjects of a fictive study population. The mean value of accuracy should be within 15% of the actual value and the precision at each concentration level should not exceed 15% of the coefficient of variation (FDA, 2001).

The SAS programs for the pharmacokinetic modeling process including the correlation and simulation analysis as well as the statistical analysis can be seen in Appendix 5.

3.2.5 Pharmacokinetic Modeling with SP641 Study Data

3.2.5.1 Study Design and Population

Methods of study design and population were taken from the publication of Cawello et al. and of the clinical trial report of study SP641 (Cawello, Fuhr et al., 2013, UCB, 2006).

Study SP641 was an open-label, single dose, phase I trial and was conducted in accordance with the Declaration of Helsinki, the local laws of the country involved and the ICH Tripartite Guideline (Guideline for Good Clinical Practice, May 1996). The study was a sequential group comparison to investigate the pharmacokinetics, safety and tolerability of 100 mg lacosamide in male and female subjects with renal impairment including subjects requiring dialysis compared with male and female healthy subjects following single dose administration. Participants were allocated to one of four groups based upon their creatinine clearance (CL_{CR}), determined according to Cockroft-Gault:

Group 1 (healthy controls)	$CL_{CR} \geq 80$ mL/min
Group 2 (mild renal impairment)	80 mL/min > $CL_{CR} \geq 50$ mL/min
Group 3 (moderate renal impairment)	50 mL/min > $CL_{CR} \geq 30$ mL/min
Group 4 (severe renal impairment)	CL_{CR} of 20-30 mL/min or $CL_{CR} < 20$ mL/min
Group 5 (end-stage renal disease)	$CL_{CR} < 15$mL/min

None of Group 4 was on haemodialysis two weeks prior to or during the trial. Each group comprised eight subjects.

All further information and evaluations excluded patients with end-stage renal disease requiring dialysis, given that the present evaluation only considered healthy subjects and those subjects with mild-to-moderate renal impairment.

Demographic parameters of subjects included in study SP641 can be seen in Table 3-5.

The SAS programs written for the generation of study SP641 demographic data sets can be seen in Appendix 9.

Table 3-5 Demographic Parameters of Subjects in Study SP641

Parameter	Mean	SD	Range	N
Age (years)	53.19	10.91	25.0–68.0	32
BMI (kg/m^2)	25.59	3.16	20.1–33.1	32
CR_{CL} (mL/min)	55.42	33.34	10.4–142.1	32
Height (m)	171.19	8.23	155–189	32
Weight (kg)	75.30	12.74	56.7–103.7	32

BMI= Body Mass Index; CL_{CR}= Creatinine clearance; N= Number of subjects

3.2.5.2 Treatment and Sample Collection

Among participants in Groups 1-4, single blood and urine samples were collected pre-dose and subsequently a single oral dose of 100 mg lacosamide was administered on day 1. The pharmacokinetics of lacosamide and its *O*-desmethyl metabolite were assessed 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, 48, 60, 72, 84 and 96 hours after lacosamide administration. Urine samples for the determination of renal excretion of lacosamide and its metabolite were collected 0-4, 4-8, 8-12, 12-24, 26-36 and 36-48 hours post-dose. The validated LLQ for both lacosamide and the *O*-desmethyl metabolite was 0.01 µg/mL. The calibration range was 0.01-10 µg/mL for lacosamide and 0.01-5.0 µg/mL for the metabolite. Precision was 0.2-6.3% for lacosamide and 0.7-6.5% for the metabolite whereas accuracy was 96.4-107.4% and 97.6-102.6%, respectively. A similar method was performed on urine samples. The calibration range was 0.2-200 µg/mL and precision was 0.8-3.7% for lacosamide and 0.3-4.3% for the metabolite. Accuracy was 88.3-108.3% for lacosamide and 93.4-103.0% for the metabolite.

3.2.5.3 Pharmacokinetic Modeling Process

Given that study SP641 included healthy subjects as well as subjects with renal impairment, it was considered necessary to include individual starting parameters during the modeling process to achieve better iteration results. The individual starting parameters were taken from recent pharmacokinetic modeling using the Bateman function. The evaluation of Cawello et al. examined the relationship between pharmacokinetic parameters and renal clearance (Cawello, Fuhr et al., 2013). They mentioned a correlation between renal clearance of lacosamide and total body clearance whereas for the metabolite the same correlation could be observed. Metabolic clearance was mentioned being a prominent component of total body clearance (Cawello, Fuhr et al., 2013). Based upon this evaluation, estimations for k_{ren} and k_m could be formed using the known renal clearance derived from k_e. The following equations (Equations 33-35) derived from Figure 2 of the publication of Cawello, Fuhr et al. were used for the estimations for k_{ren} and k_m (Cawello, Fuhr et al., 2013):

33) $$k_e = \frac{1.2+0.8 \cdot CL_{ren}}{50}$$

34) $$k_m = \frac{1.1+0.6 \cdot CL_{ren}}{50}$$

35) $$k_{ren} = \frac{0.7 \cdot CL_{ren}}{50}$$

From the expected dependence between k_{me} and CL_{CR}, the starting parameters for the iteration of k_{me} could be determined. Recent validations have shown that, due to fewer sampling points in the absorption phase, the iteration process for k_a could be imprecise (see also section 3.2.4.2). For this reason, k_a was fixed for each individual subject, with that value taken from recent pharmacokinetic modeling with SP641 data (using the Bateman function).

3.2.5.4 Statistics of Pharmacokinetic Modeling Results of SP641 Study Data

The pharmacokinetic parameters developed in SAS through iteration processes were evaluated by descriptive statistics. For V_d, V_{dm}, k_{ren}, k_m and k_{me}, arithmetic means with corresponding SD were calculated whereas median was used as an appropriate point estimate for absorption lag time. Range was determined for all pharmacokinetic parameters. During the iteration process, the weighted least square was used to estimate the best fit when analysing data and finding the parameter values.

The regression analysis was conducted to correlate measured and predicted concentrations/amounts as well as CL_{CR} and k_{me}, k_{ren} and k_m. Regarding the assumed connection between volume of distribution and total body water and increasing muscle mass a further correlation analysis was conducted between the two parameters. The regression analysis was evaluated by calculating the coefficient of determination, along with the line parameters of intercept and slope.

The SAS programs for the pharmacokinetic modeling process including all simulation and correlation analysis as well as statistical analysis can be seen in Appendix 9.

3.3 Results

3.3.1 Mathematical Pharmacokinetic Models

The following equations resulted from the back-transformation of generated Laplace equations for description of drug and metabolite transports after oral administration in central circulation as well as renal elimination of unchanged drug. F represented the relative bioavailability (F was set as 1 for a 100% bioavailability) and D represented the dose. The way of back-transformation of generated Laplace equations was illustrated with an equation for the metabolite in plasma given that no equation has been developed thus far. Based upon the methods for Laplace back-transformation described in section 3.2.1.2, the following Laplace equations have been developed and back-transformed. The partial equations were represented by the expression X(1-4).

The creation of the Laplace equation exemplary for the pharmacokinetics of the metabolite in plasma was conducted through putting together the input, disposition and sample site function, which can be found in tables 3-1, 3-2, 3-3 (Equation 38). For the input function the oral route of administration was chosen (Equation 16 from Table 3-1). The Laplace disposition function was a one-compartment model whereby Equation 17 from Table 3-2 was taken whereas for the sample site the metabolite in central compartment was chosen (Equation 24 from Table 3-3). To find the roots of the polynomial in the denominator, each of the factors were set to zero and used to find the root for s. The roots for the denominator were s=0, s=-k_a, s=-k_e and s=-k_{me}. Subsequently, all instances of 's' in the remaining equation were replaced by the current root and multiplied by $e^{root \cdot t}$ (Equations 39-41). To calculate a value for the concentration at a chosen point of time, the volume of distribution of the chosen component had to be included. The sum of all the terms from each root can be seen in Equation 42. In Equation 43, the sum of k_{ren} and k_m as representative for k_e was included and allowed the calculation of the concentration of the metabolite in plasma due to having considered its fictive volume of distribution. Equation 44 was simplified to Equation 43.

Based upon the consideration that k_e represented the sum of k_{ren} and k_m, each k_e in the mathematical equation was replaced by the expression k_{ren}+k_m during

validation processes and evaluations of study data SP641. V_{dm} was included to calculate the concentration of metabolite in plasma.

Unchanged Drug in Plasma (Bateman Function)

36) $$C(t) = \frac{F \cdot D}{V_d} \cdot \frac{k_a}{k_a - (k_m + k_{ren})} \cdot (e^{-(k_m + k_{ren}) \cdot t} - e^{-k_a \cdot t})$$

Unchanged Drug Excreted in Urine

37) $$U(t) = \frac{F \cdot D \cdot k_a \cdot k_{ren}}{(k_{ren} + k_m)} \left(\frac{1}{k_a} - \frac{e^{-(k_m + k_{ren}) \cdot t}}{k_a - (k_m + k_{ren})} + \frac{(k_m + k_{ren}) \cdot e^{-k_a \cdot t}}{k_a \cdot (k_a - (k_m + k_{ren}))} \right)$$

Metabolite in Plasma

Laplace equation:

38) $$\frac{F \cdot D \cdot k_a}{(s + k_a)} \cdot \frac{1}{(s + k_e)} \cdot \frac{k_m}{(s + k_{me})} = F \cdot D \cdot k_a \cdot k_m \cdot \frac{1}{(s + k_a)} \cdot \frac{1}{(s + k_e)} \cdot \frac{1}{(s + k_{me})}$$

Partial equations X(1-4):

39) $$X1 = F \cdot D \cdot k_a \cdot k_m \cdot e^{-k_a \cdot t \cdot} \cdot \frac{1}{(-k_a + k_e)} \cdot \frac{1}{(-k_a + k_{me})}$$

40) $$X2 = F \cdot D \cdot k_a \cdot k_m \cdot e^{-k_e \cdot t \cdot} \cdot \frac{1}{(-k_e + k_a)} \cdot \frac{1}{(-k_e + k_{me})}$$

41) $$X3 = F \cdot D \cdot k_a \cdot k_m \cdot e^{-k_{me} \cdot t \cdot} \cdot \frac{1}{(-k_{me} + k_a)} \cdot \frac{1}{(-k_{me} + k_e)}$$

Summary of all partial equations including the fictive volume of distribution of the metabolite to calculate the concentration C(met) at point of time t

42) $$Cmet(t) = \frac{F \cdot D \cdot k_a \cdot k_m}{V_{dm}} \cdot \left(\frac{e^{-k_a \cdot t}}{(-k_a + k_e) \cdot (-k_a + k_{me})} + \frac{e^{-k_e \cdot t}}{(-k_e + k_a) \cdot (-k_e + k_{me})} + \frac{e^{-k_{me} \cdot t}}{(-k_{me} + k_a) \cdot (-k_{me} + k_e)} \right)$$

Replacement of k_e by the sum of k_{ren} and k_m

43) $$Cmet(t) = \frac{\mathrm{F \cdot D} \cdot k_a \cdot k_m}{V_{dm}} \cdot \left(\frac{e^{-(k_{ren} + k_m) \cdot t}}{(k_{me} - (k_{ren} + k_m)) \cdot (k_a - (k_{ren} + k_m))} + \frac{e^{-k_{me} \cdot t}}{((k_{ren} + k_m) - k_{me}) \cdot (k_a - k_{me})} + \frac{e^{-k_a \cdot t}}{(k_a - k_{me}) \cdot (k_a - (k_{ren} + k_m))} \right)$$

44) $$Cmet(t) = \frac{\mathrm{F \cdot D} \cdot k_a \cdot k_m}{V_{dm} \cdot (k_a - k_{me}) \cdot (k_a - (k_{ren} + k_m)) \cdot (k_{me} - (k_{ren} + k_m))} \cdot \left[(k_a - k_{me}) \cdot e^{-(k_{ren} + k_m) \cdot t} - \left(k_a - (k_{ren} + k_m) \right) \cdot e^{-k_{me} \cdot t} + (k_{me} - (k_{ren} + k_m)) \cdot e^{-k_a \cdot t} \right]$$

3.3.2 Validation Results

3.3.2.1 Results of Generated Pharmacokinetic Data

The concentration of unchanged drug in central circulation (dr=1 and ti=1) and the cumulative amount of unchanged drug excreted in the tissue urine (dr=1 and ti=0) as well as the concentration of the metabolite in plasma (dr=0 and ti=1) at time t were described by the following equation (Equation 45):

45)

$$y = F \cdot D \cdot k_a \cdot \frac{ti \cdot dr}{V_d \cdot (k_a - (k_m + k_{ren}))} \cdot \left(e^{-(k_m + k_{ren}) \cdot t} - e^{-k_a \cdot t}\right) - \frac{(ti-1) \cdot dr \cdot k_{ren}}{(k_{ren} + k_m)} \left(\frac{1}{k_a} - \frac{e^{-(k_m + k_{ren}) \cdot t}}{k_a - (k_m + k_{ren})} + \frac{(k_m + k_{ren}) \cdot e^{-k_a \cdot t}}{k_a \cdot (k_a - (k_m + k_{ren}))}\right) - \frac{(dr-1) \cdot ti \cdot k_m}{V_{dm} \cdot (k_a - k_{me}) \cdot (k_a - (k_{ren} + k_m)) \cdot (k_{me} - (k_{ren} + k_m))} \cdot \left[(k_a - k_{me}) \cdot e^{-(k_{ren} + k_m) \cdot t} - \left(k_a - (k_{ren} + k_m)\right) \cdot e^{-k_{me} \cdot t} + (k_{me} - (k_{ren} + k_m)) \cdot e^{-k_a \cdot t}\right]$$

The factors *dr* and *ti* were used to connect the three mathematical models in SAS.

By using developed equations, concentrations of unchanged drug and metabolite in plasma as well as the cumulative amount of unchanged drug excreted in urine for each subject were calculated by inserting the individual values of pharmacokinetic parameters at each sampling point or end of the collection interval (for urine data) at time t. Furthermore, calculated plasma concentrations and amounts excreted in urine were assigned a proportional error.

The source data of plasma and urine data from each subject of the fictive study population are listed in Appendix 6.

3.3.2.2 Results of Descriptive Statistics

The results of descriptive statistics of reference as well as iterated pharmacokinetic parameters are shown in Table 3-6. For all pharmacokinetic parameters, the arithmetic mean was calculated.

Reference values were generated in SAS as described in section 3.2.4.1. For V_d and V_{dm}, reference values were between 32 and 80 L and between 34 and 143 L, respectively. Iterated values were between 31 and 78 L for V_d and between 35 and 154 L for V_{dm}. The same could be observed for the rate constants. The differences between reference and iterated values were small, whereas this observation could be supported by the results of precision and accuracy presented in Table 3-7.

Pharmacokinetic parameters V_d and V_{dm} were determined with an accuracy of 0.57 and −0.26% and a precision of 3.2 and 5.21%, respectively. The rate constants k_a, k_{me}, k_m and k_{ren} were determined with an accuracy of 0.97, −0.03, −0.40 and −0.53% and a precision of 8.68, 4.89, 3.74 and 3.53%, respectively. Table 3-8 presents the results of regression analysis between reference and predicted concentrations of unchanged drug in plasma, metabolite in plasma and cumulative amount of unchanged drug in urine, as well as between reference and predicted pharmacokinetic parameters.

A summary of generated reference and iterated pharmacokinetic parameters from each subject from the fictive study population is listed in Appendices 7 and 8.

Table 3-6 Descriptive Statistics of Pharmacokinetic Parameters for Unchanged Drug in Plasma and its Amount Excreted in Urine as well as Metabolite in Plasma During Validation

Parameter	Mean	SD	Range	N
k_{a_ref} (1/h)	1.9978	0.2132	1.5485-2.3689	20
V_{d_ref}	49.85	10.99	31.77-80.26	20
k_{ren_ref} (1/h)	0.0507	0.0057	0.0394-0.0627	20
V_{dm_ref} (L)	99.79	30.30	34.14-143.36	20
k_{me_ref} (1/h)	0.0194	0.0032	0.0120-0.0228	20
k_{m_ref} (1/h)	0.0692	0.0101	0.0526-0.0871	20
k_{a_iter} (1/h)	1.9726	0.2277	1.7045-2.4148	20
V_{d_iter} (L)	49.50	10.58	30.63-78.39	20
k_{ren_iter} (1/h)	0.0510	0.0059	0.0409-0.0642	20
V_{dm_iter} (L)	99.90	30.88	34.59-154.42	20
k_{me_iter} (1/h)	0.0194	0.0032	0.0125-0.0229	20
k_{m_iter} (1/h)	0.0696	0.0109	0.0514-0.0879	20
_ref, reference values; _iter, iterated values				

Table 3-7 Accuracy and Precision for Evaluation of Pharmacokinetic Parameters During Validation

	Accuracy (%)	Precision (%)
k_a	0.97	8.68
V_d	0.57	3.20
k_{ren}	-0.53	3.53
V_{dm}	-0.26	5.21
k_{me}	-0.03	4.89
k_m	-0.40	3.74

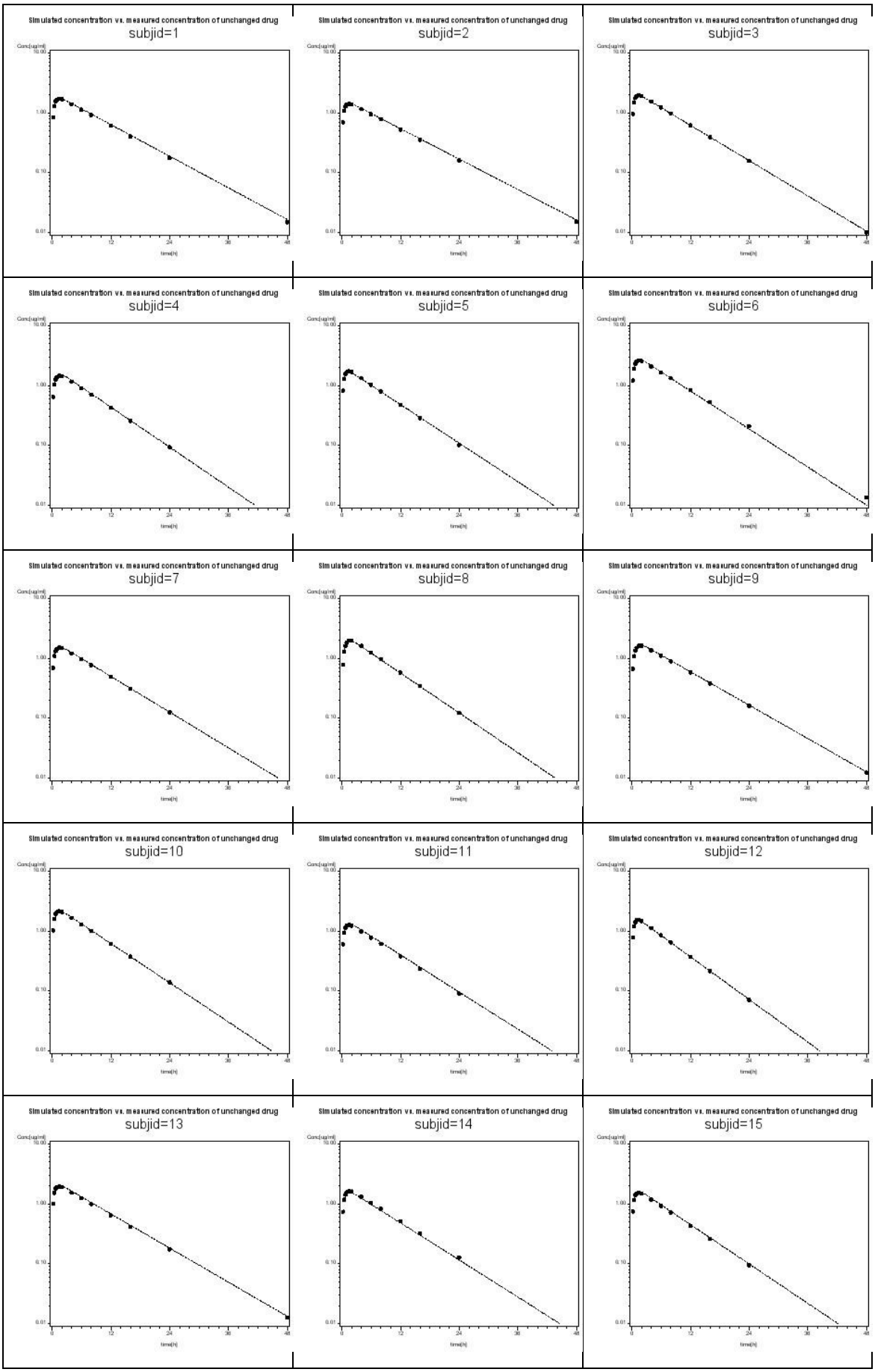
3.3.2.3 Results of Simulation and Regression

As described in section 3.2.4.3, a simulation and correlation analysis was conducted. For each subject, concentration-over-time profiles for unchanged drug and metabolite in plasma as well as the amount-over-time profiles for unchanged drug excreted in urine were calculated and can be seen in Figures 3-3, 3-4 and 3-5.

Table 3-8 and Figure 3-6 show the results of regression analysis between reference and predicted pharmacokinetic models and reference and predicted pharmacokinetic parameters with the calculated parameters coefficient of determination, intercept and slope.

Regarding the correlation between the reference and predicted concentration of unchanged drug in plasma a good correlation could be declared due to a coefficient of determination of 0.9961. Intercept was 0.0022 and slope 0.9988. Similar results could be observed when regarding the regression analysis between reference and predicted values for amounts of unchanged drug in urine and concentrations of metabolite in plasma. R^2 for unchanged drug in urine was 0.9930, whereas intercept was -0.0509 and slope 1.0034. R^2 for the correlation of reference and predicted concentrations of metabolite in plasma was 0.9972, whereas intercept and slope were 0.0007 and 0.9950, respectively.

The same regression analysis was conducted with reference and predicted pharmacokinetic parameters. R^2 values for reference and iterated pharmacokinetic parameters V_d and V_{dm} were 0.9792 and 0.9682 with intercepts of 1.9795 and -0.1700 and slopes of 0.9534 and 1.0028, respectively. When correlating reference and predicted rate constants of elimination k_{ren} and k_{me}, values for R^2 were 0.9113 and 0.9112. Intercept was 0.0008 and 0.0004 and slope was 0.9887 and 0.9808, respectively. R^2 values for the rate constants of absorption k_a and k_m were 0.4727 and 0.9432, respectively, with an intercept of 0.5057 and -0.0030 and a slope of 0.7342 and 1.0486.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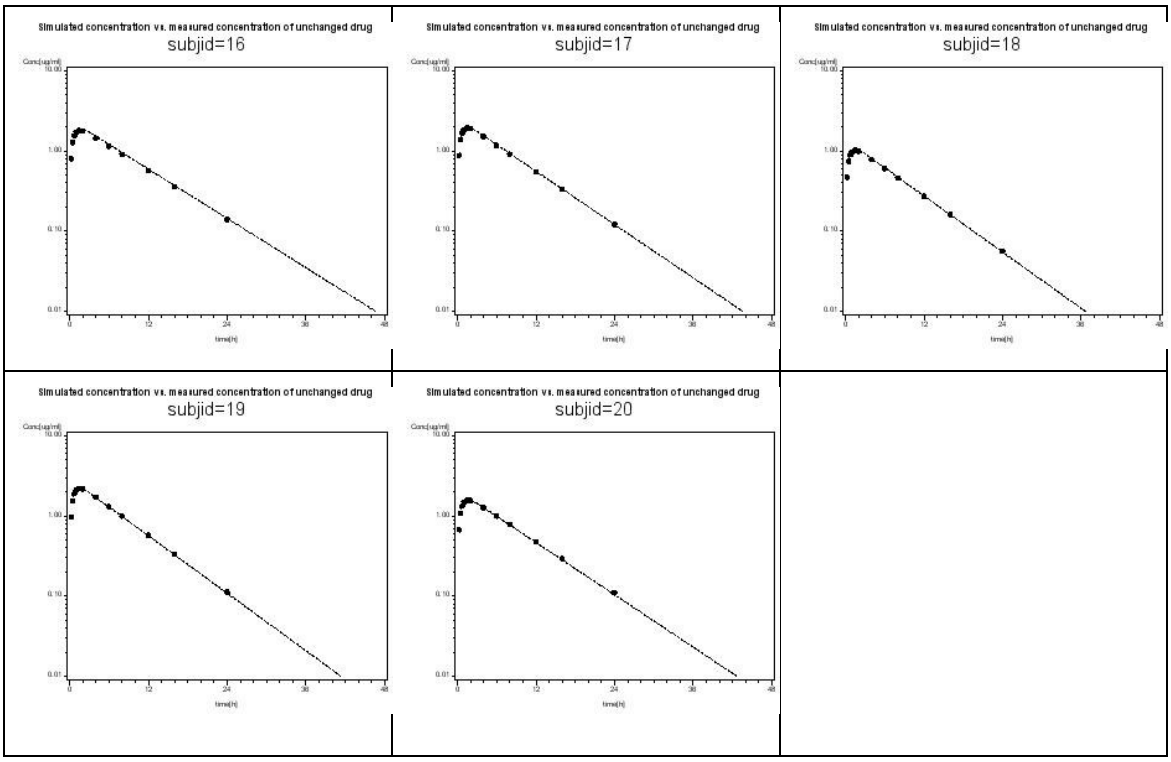
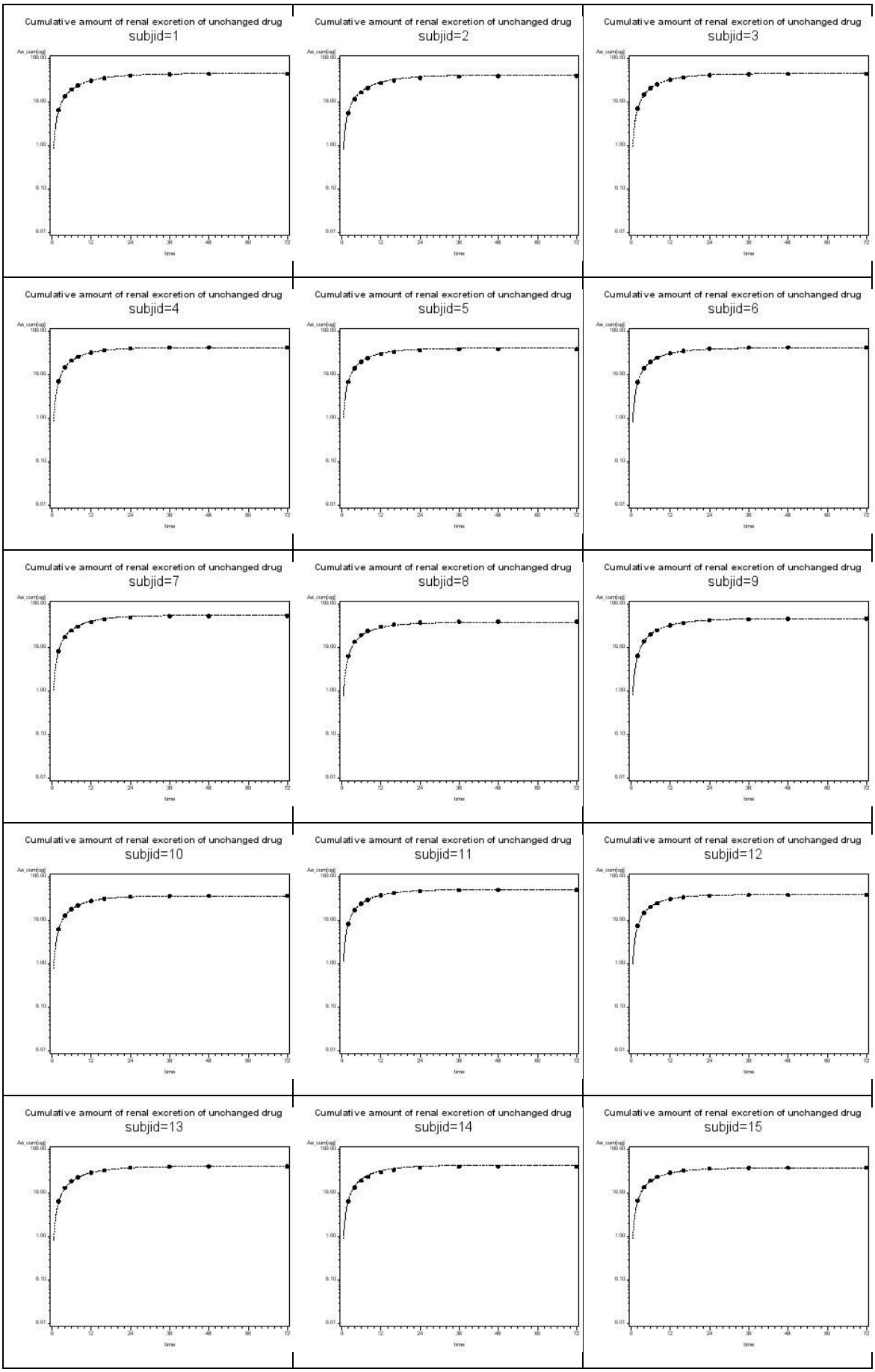


Figure 3-3 Simulation of Concentration of Unchanged Drug in Plasma

Simulation of concentration-over-time profiles for each subject of unchanged drug in plasma. The points represent reference and the lines the iterated values. The simulations are presented on a semi-logarithmic scale where on the x-axis the time in hours and on the y-axis the concentration of unchanged drug in plasma is presented. The unit of concentration is set as µg/mL but could be in each other unit depending on the way in which the drug concentration is specified.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Cumulative amount of renal excretion of unchanged drug
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Cumulative amount of renal excretion of unchanged drug
subjid=15

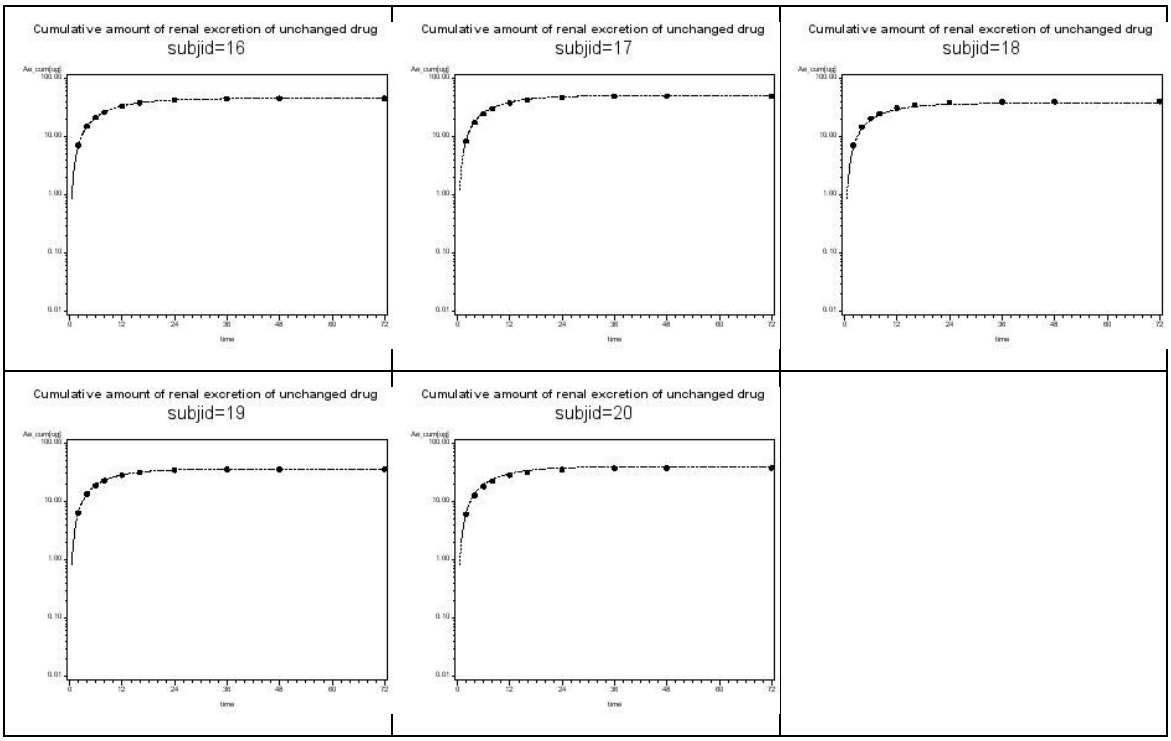
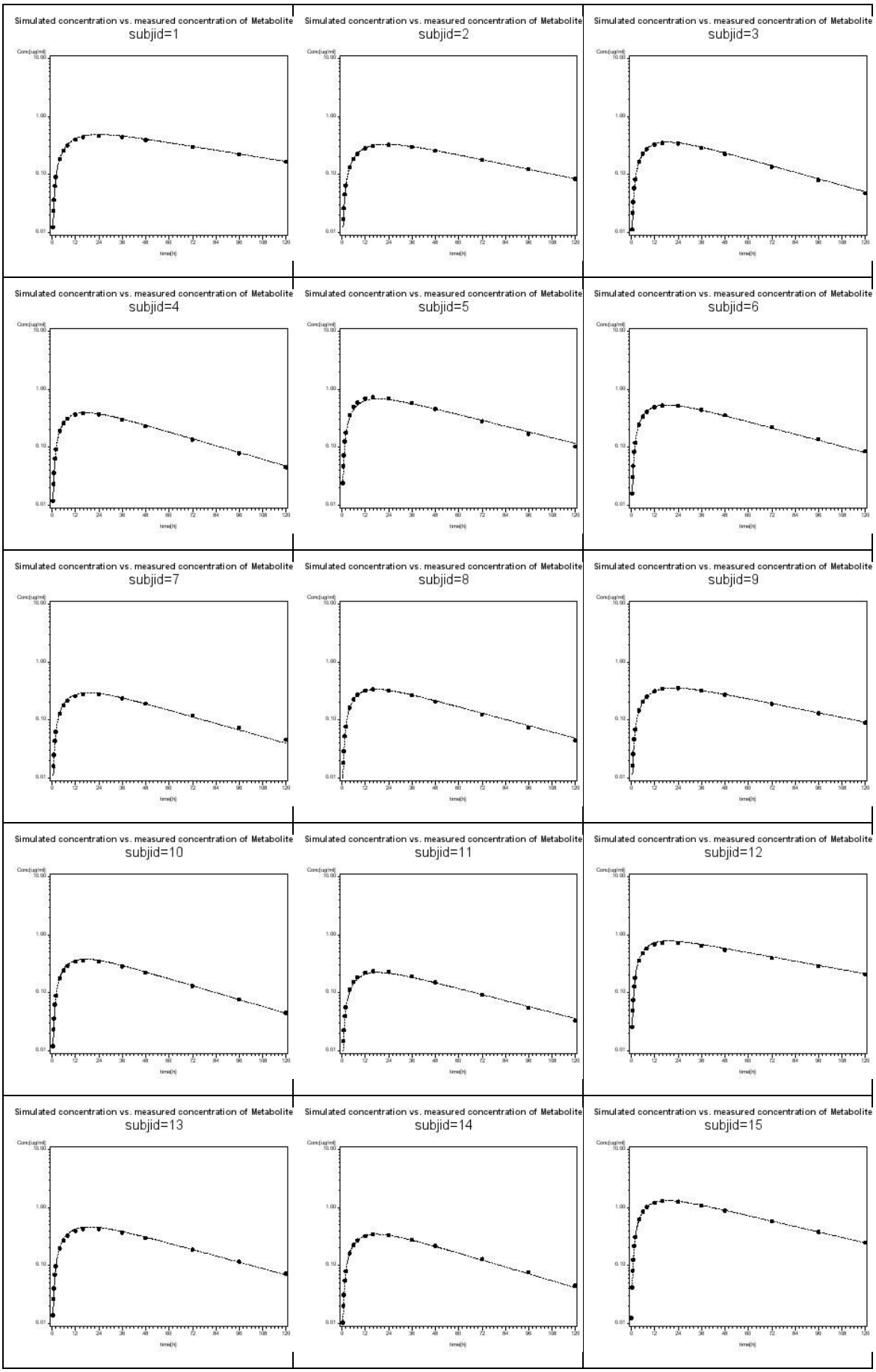


Figure 3-4 Simulation of the Amount of Unchanged Drug in Urine

Simulation of amount-over-time profiles for each subject of unchanged drug excreted in urine. The points represent reference and the lines the iterated values. The simulations are presented on a semi-logarithmic scale where on the x-axis the time in hours and on the y-axis the cumulative amount of unchanged drug in urine is presented. The unit of amount in urine is set as mg but could be in each other unit depending on the way in which the drug concentration is specified.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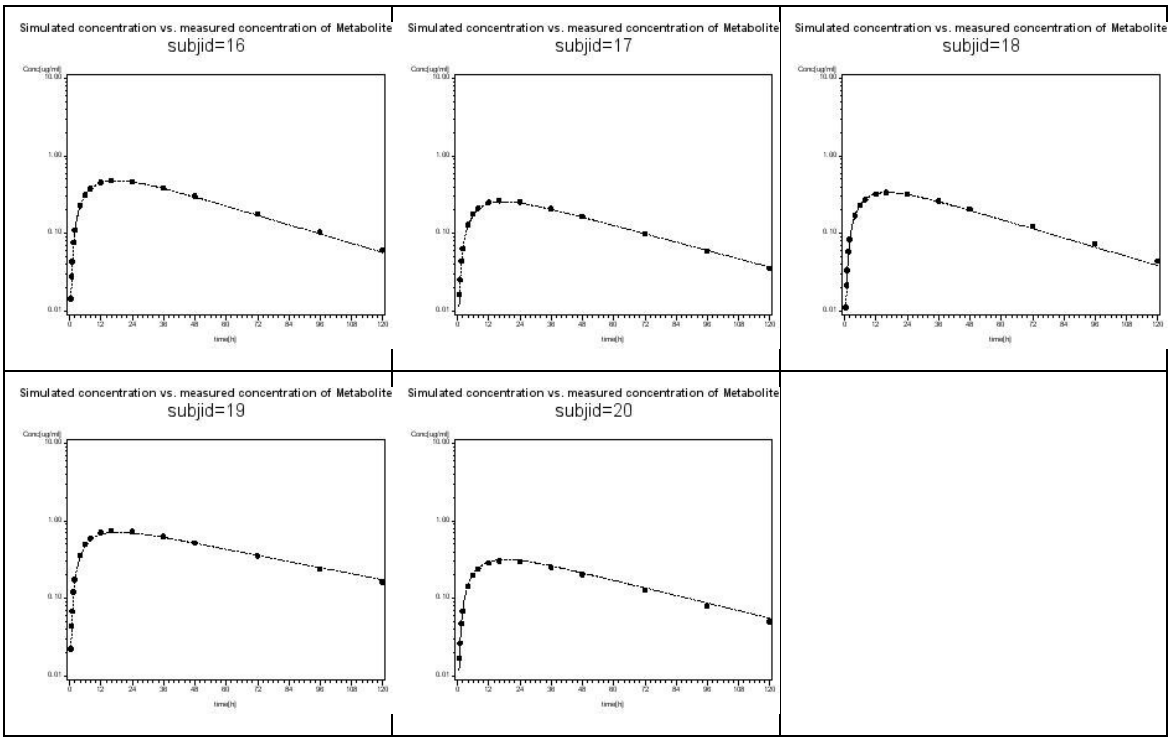


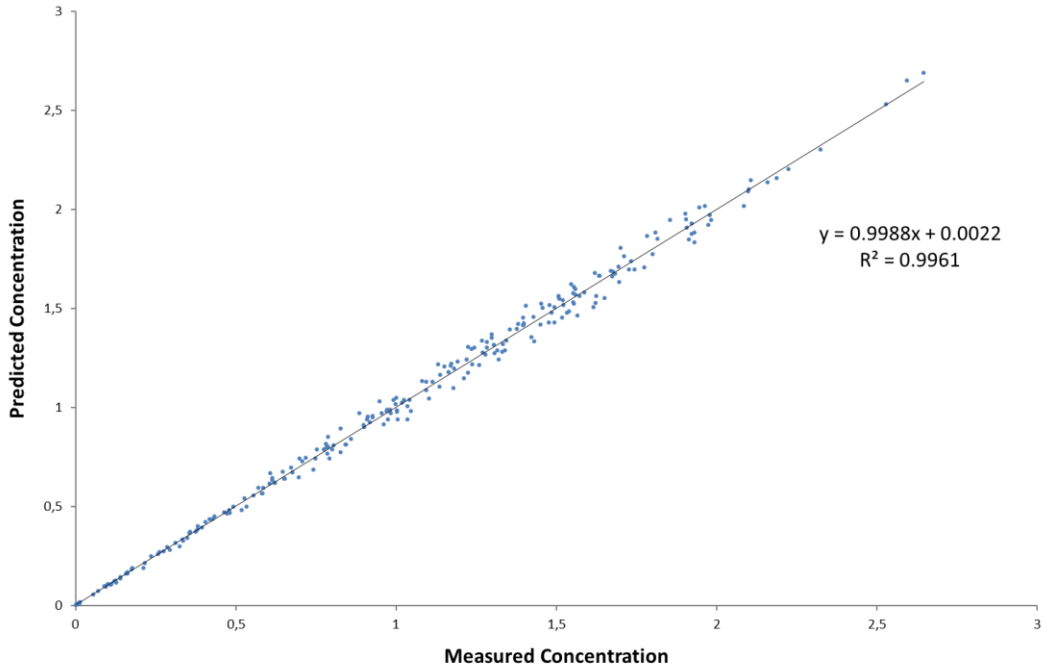
Figure 3-5 Simulation of Concentration of Metabolite in Plasma

Simulation of concentration-over-time profiles for each subject of metabolite in plasma. The points represent reference and the lines the iterated values. The simulations are presented on a semi-logarithmic scale where on the x-axis the time in hours and on the y-axis the concentration of unchanged drug in plasma is presented. The unit of concentration is set as µg/mL but could be in each other unit depending on the way in which the drug concentration is specified.

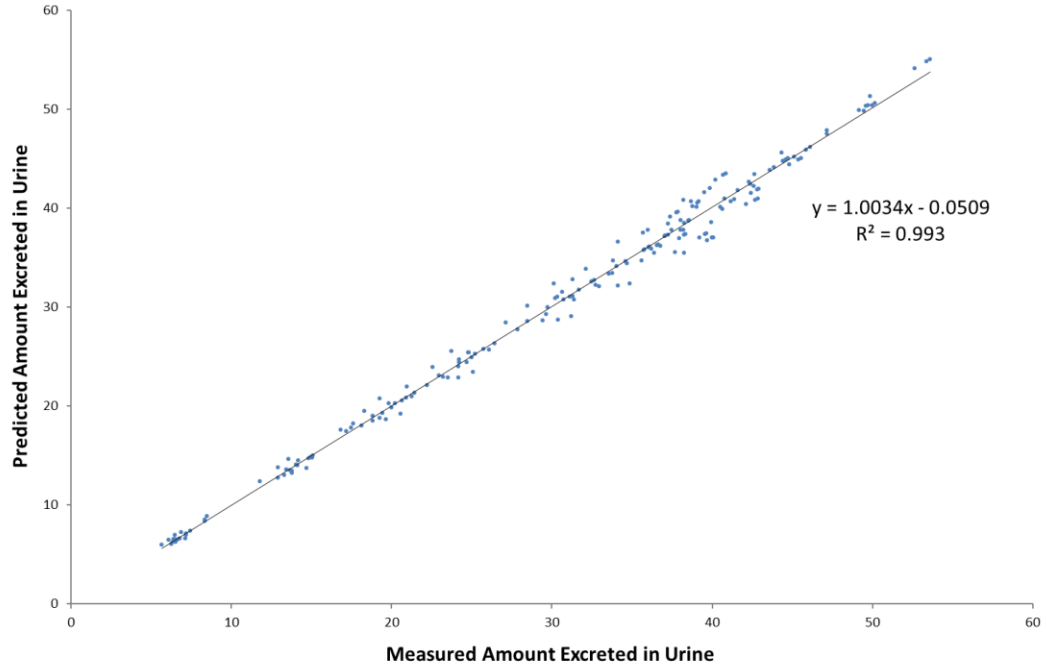
Table 3-8 Regression Analysis Between Reference and Predicted Pharmacokinetic Models and Pharmacokinetic Parameters

	Pharmacokinetic Models			Pharmacokinetic Parameters					
	Unchanged Drug in Plasma	Unchanged Drug in Urine	Metabolite in Plasma	V_d	V_{dm}	k_{ren}	k_a	k_m	k_{me}
R^2	0.9961	0.9930	0.9972	0.9792	0.9682	0.9113	0.4727	0.9432	0.9112
Intercept	0.0022	−0.0509	0.0007	1.9795	−0.1700	0.0008	0.5057	−0.0030	0.0004
Slope	0.9988	1.0034	0.9950	0.9534	1.0028	0.9887	0.7342	1.0486	0.9808

a


Predicted Concentration
Measured Concentration
y = 0.9988x + 0.0022
R² = 0.9961
0
0,5
1
1,5
2
2,5
3

b


Predicted Amount Excreted in Urine
Measured Amount Excreted in Urine
y = 1.0034x - 0.0509
R² = 0.993
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10
20
30
40
50
60

C

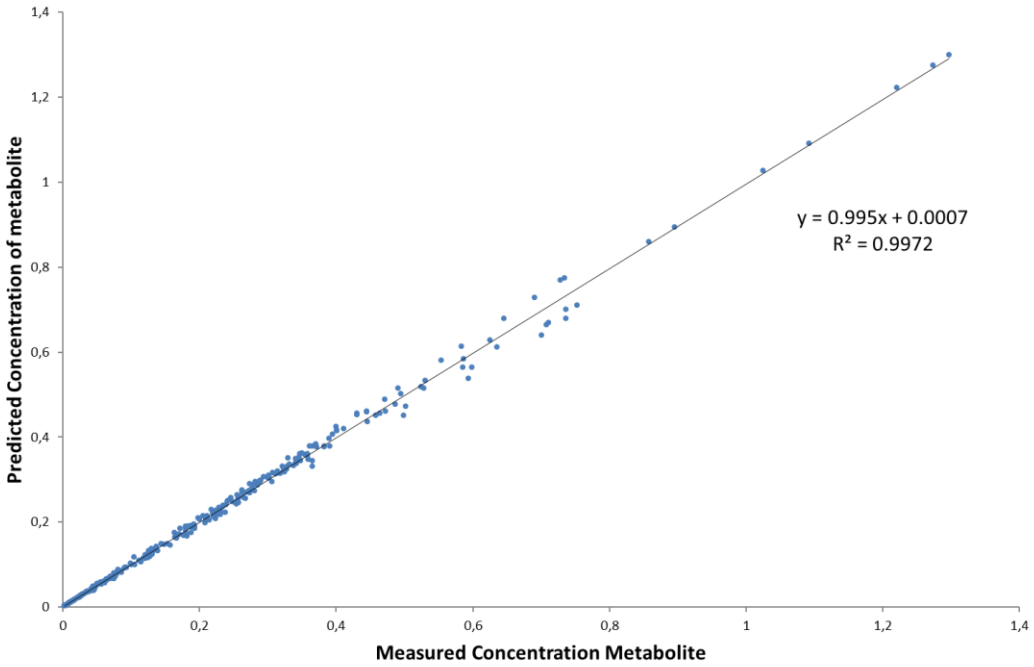


Figure 3-6 Predicted versus Measured Values

Predicted versus measured concentration/amount of a) unchanged drug in plasma, b) unchanged drug excreted in urine and c) metabolite in plasma

3.3.3 Results of Evaluation with SP641 Data

3.3.3.1 Results of Descriptive Statistics

The SP641 study population comprised male and female subjects aged between 25 and 68 years. Further information on demographic parameters can be seen in Table 3-5. The pharmacokinetic modeling could be conducted for all subjects except subject 80306, for whom no urine data were available.

Descriptive statistics of pharmacokinetic parameters can be seen in Table 3-9. The range of pharmacokinetic parameter k_a resulting from the pharmacokinetic modeling with the Bateman function was between 2.249 and 10.0/h with an arithmetic mean of 8.884/h and a SD of 2.490/h whereas it has to be considered that the iteration of k_a was restricted by an upper limit of 10/h. Thus, the value of 10/h should not be regarded as the actual iterated value. The metabolic rate constant of absorption ranged between 0.016 and 0.051/h with an arithmetic mean of 0.034/h and a SD of 0.009/h. V_d and V_{dm} ranged between 24.78 and 61.53 L and between 41.25 and 138.67 L, respectively. The renal rate constant of elimination was iterated with a mean value of 0.011/h with a SD of 0.006/h, whereas k_{me} was iterated with an arithmetic mean of 0.063/h with a SD of 0.042/h. The pharmacokinetic parameter tlag was determined with a median of 0.135 h in a range of 0-0.467 h.

Table 3-9 Descriptive Statistics of Pharmacokinetic Parameters Resulting from Evaluation of SP641 Study Data

Parameter	Mean	SD	Range	N
k_a	8.884*	2.490	2.249-10.0	32
V_d	39.92*	9.1	24.78-61.53	32
k_{ren}	0.011*	0.006	0.003-0.026	32
V_{dm}	78.33*	27.30	41.52-138.67	32
k_{me}	0.063*	0.042	0.013-0.161	32
k_m	0.034*	0.009	0.016-0.051	32
tlag	0.135**		0-0.467	32

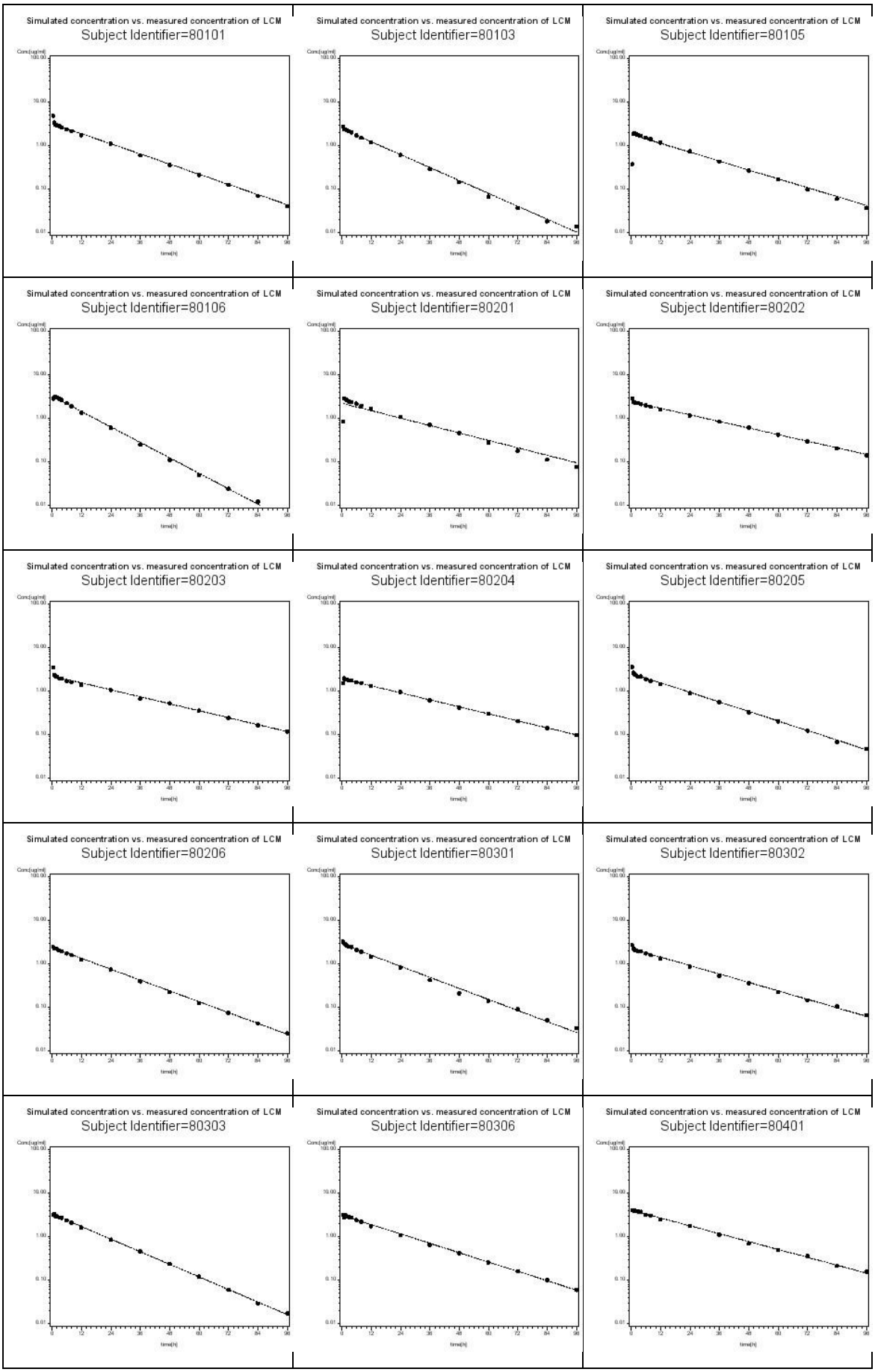
*Arithmetic mean; **Median

3.3.3.2 Results of Simulation and Correlations

As was the case during the validation process, a simulation and correlation analysis was conducted. For each subject, concentration-over-time profiles for lacosamide and *O*-desmethyl-metabolite in plasma as well as amount-over-time profiles for lacosamide excreted in urine were calculated. For subject number 80306, only the simulation curve can be seen, given that no urine data were available.

The results of regression analysis between measured and predicted concentrations of lacosamide and its metabolite in plasma as well as between measured and predicted amounts of lacosamide excreted in urine can be seen in Table 3-10 and Figures 3-10.

Regarding the regression between the measured and predicted concentration of lacosamide in plasma, the coefficient of correlation was 0.9697 with an intercept of 0.0585 and a slope of 0.9230. For the urine data, R^2 was 0.9758 with an intercept of -0.4676 and a slope of 1.0364. When correlating measured and predicted concentration of *O*-desmethyl-lacosamide with each other, the coefficient of determination was 0.9772 with an intercept of -0.0120 and a slope of 1.0784.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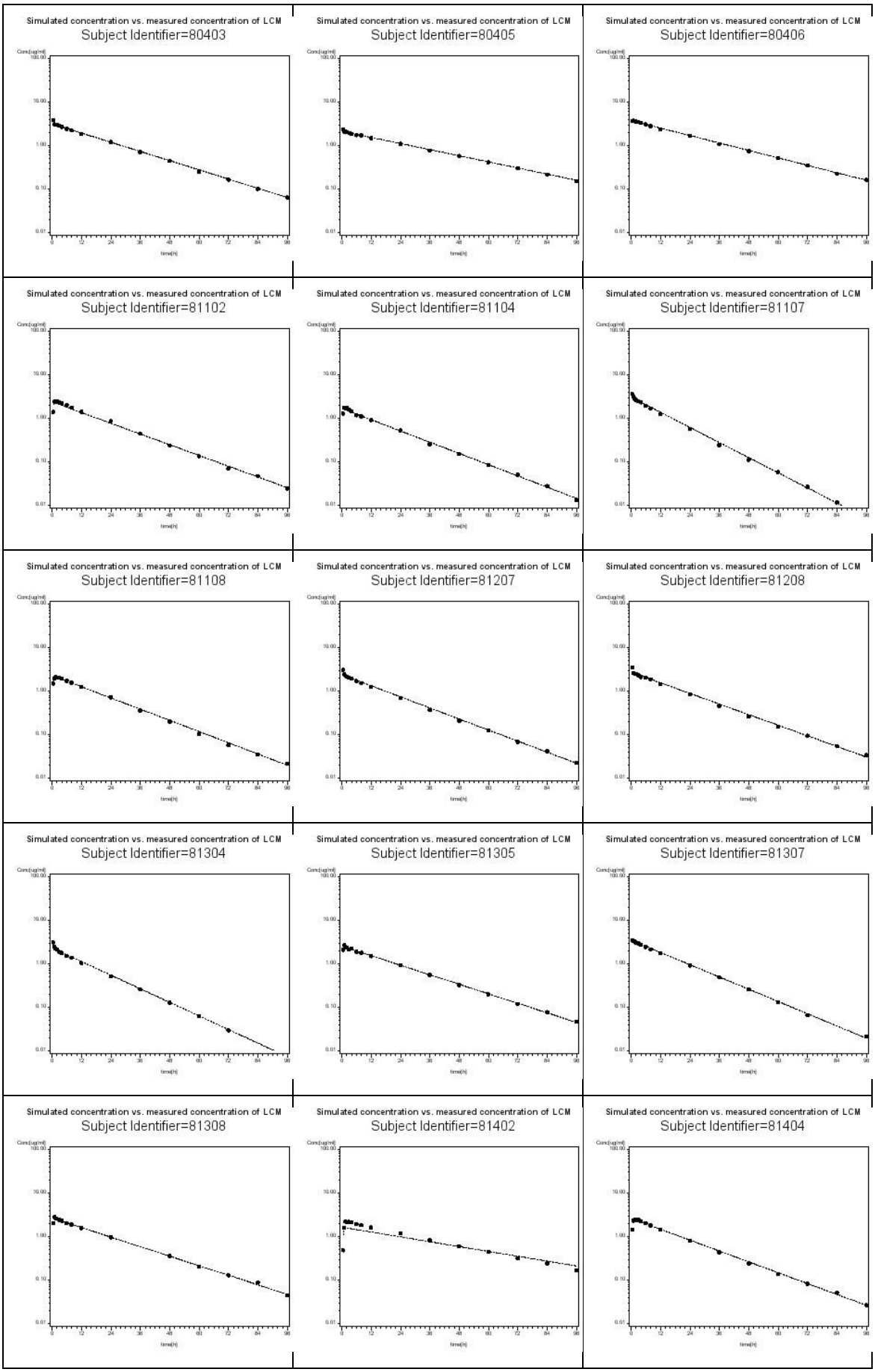
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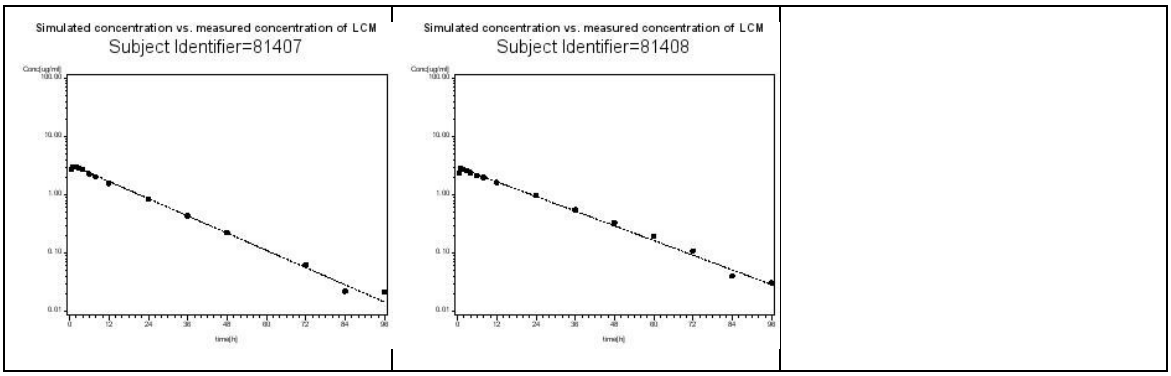
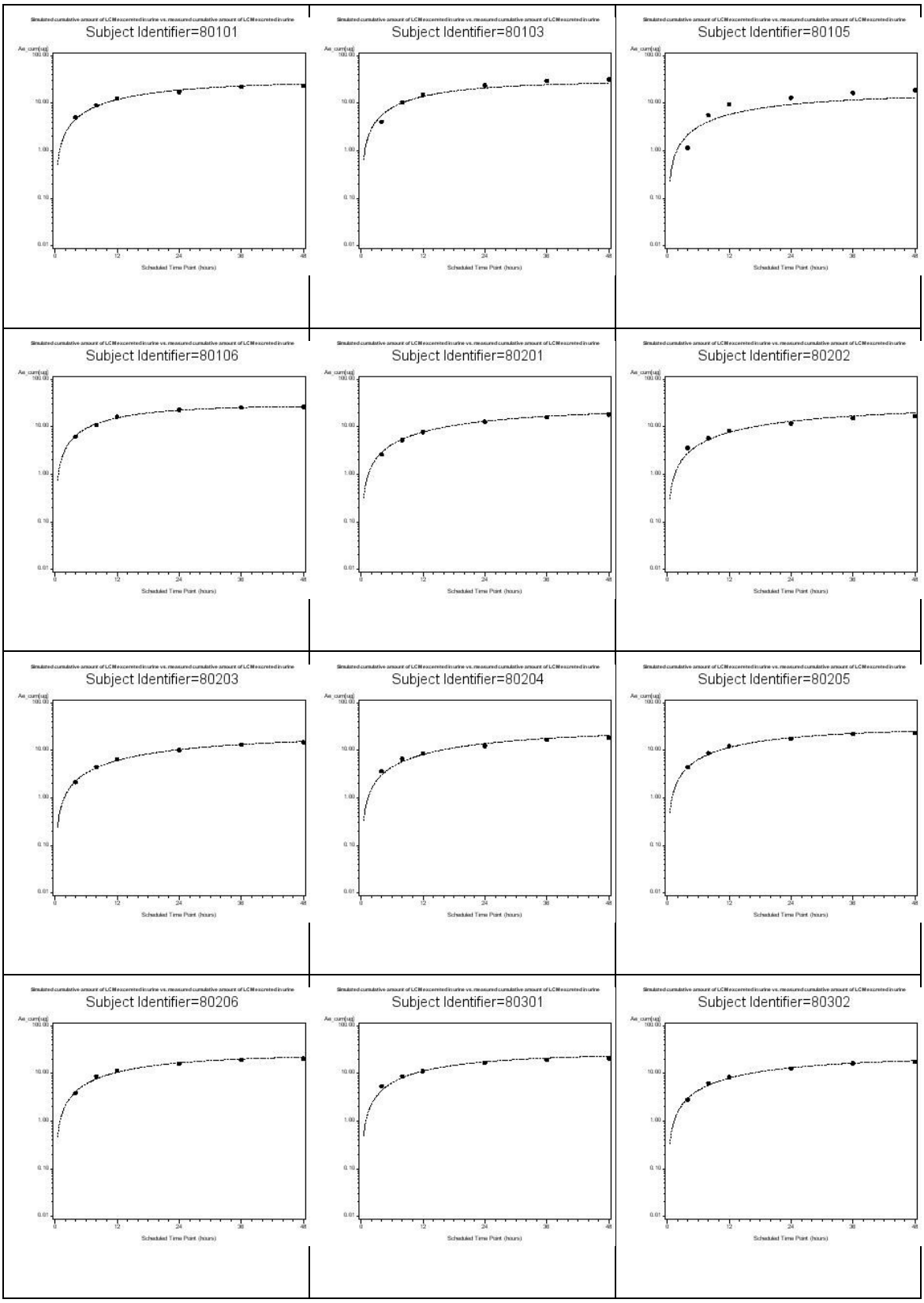


Figure 3-7 Simulation of Lacosamide Concentration in Plasma

Simulation of concentration-over-time profiles for each subject of lacosamide in plasma. The points represent the measured and the lines the simulated concentrations. The simulations are presented on a semi-logarithmic scale where on the x-axis the time in hours and on the y-axis the concentration of lacosamide in µg/mL in plasma is presented.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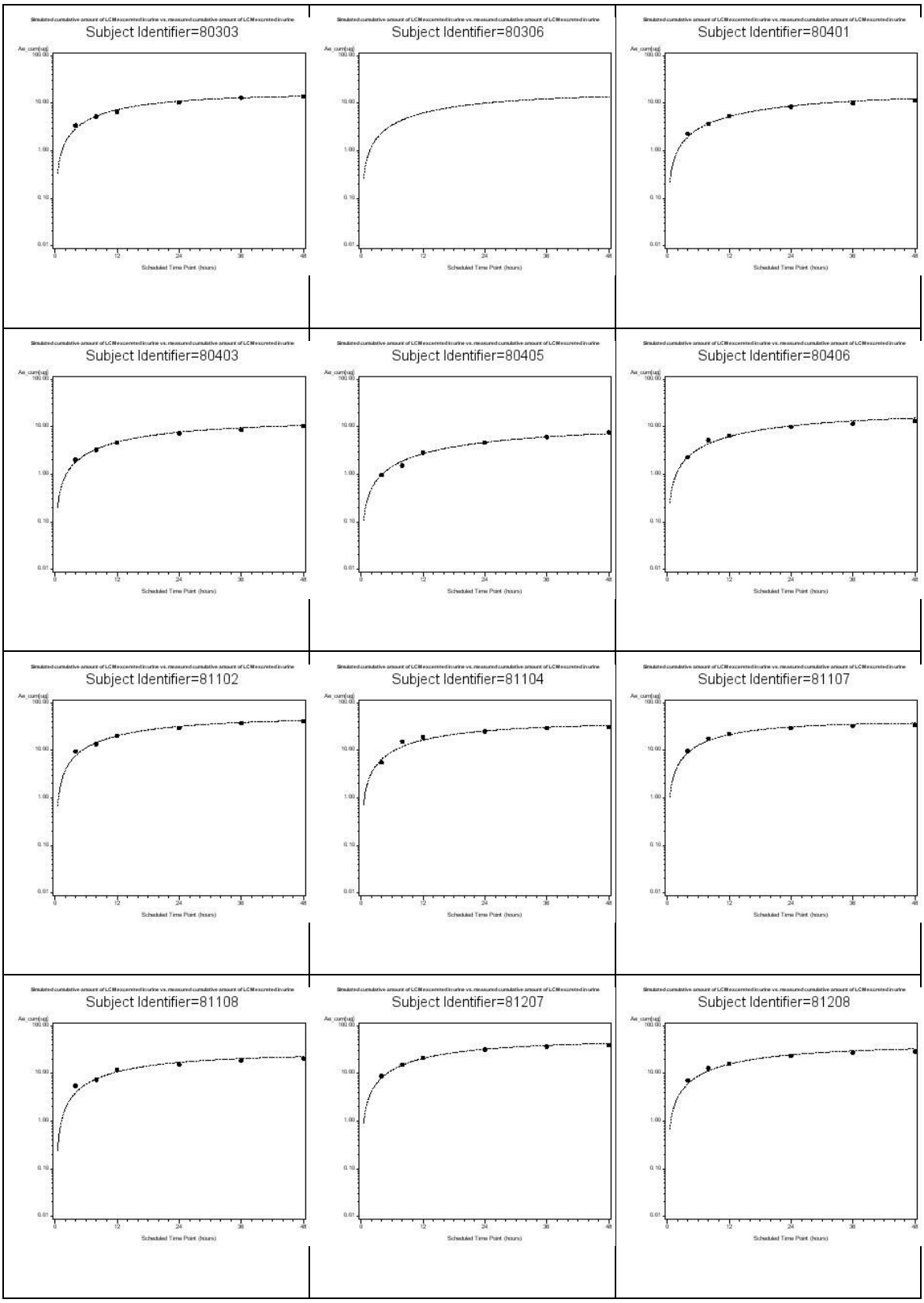
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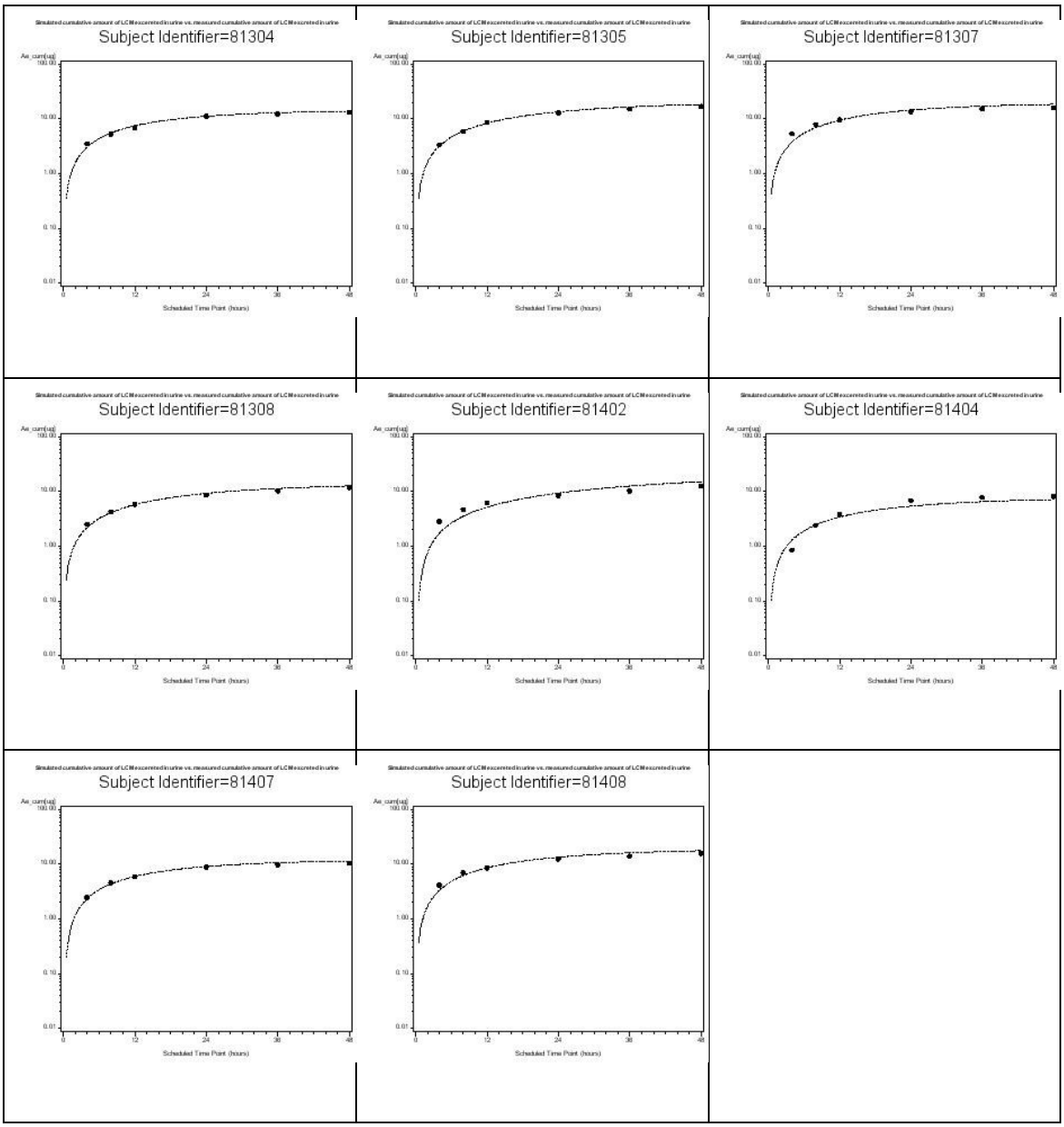
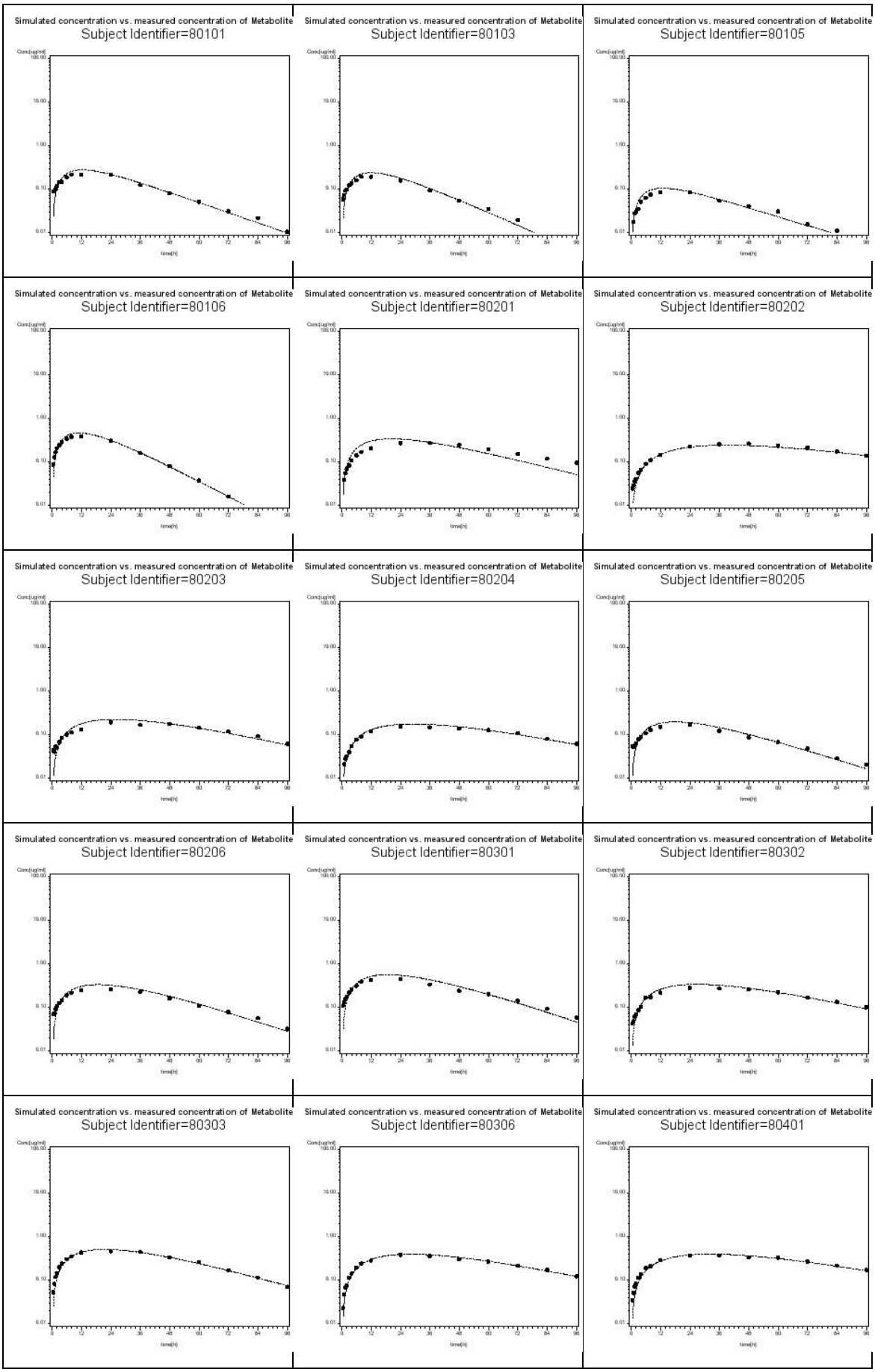
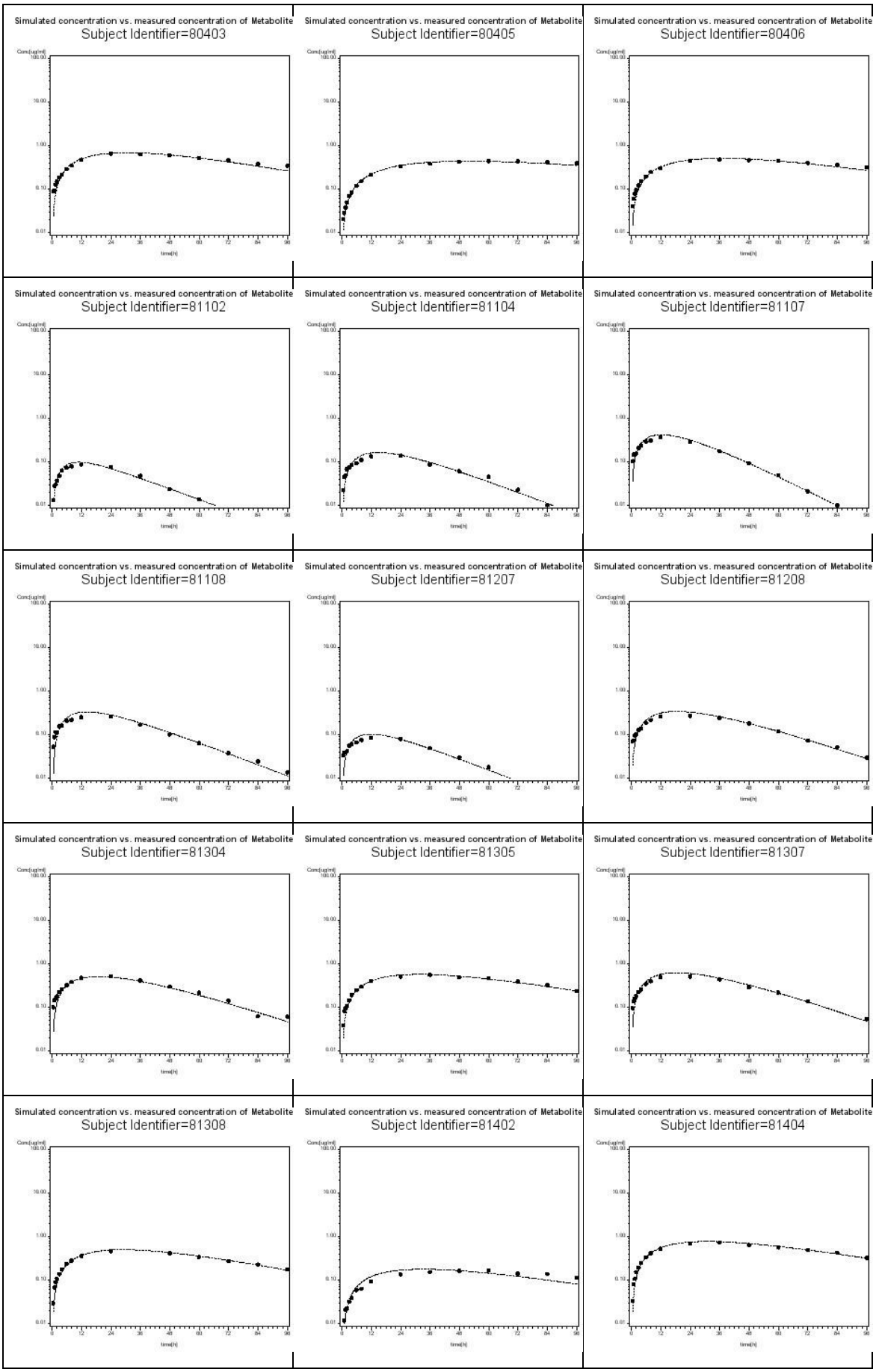


Figure 3-8 Simulation of the Amount of Lacosamide Excreted in Urine

Simulation of amount-over-time profiles for each subject of lacosamide excreted in urine. The points represent the measured and the lines the simulated values of amounts excreted in urine. The simulations are presented on a semi-logarithmic scale where on the x-axis the time in hours and on the y-axis the cumulative amount of unchanged drug in mg in urine is presented.


Simulated concentration vs. measured concentration of Metabolite
Subject Identifier=80101
Simulated concentration vs. measured concentration of Metabolite
Subject Identifier=80103
Simulated concentration vs. measured concentration of Metabolite
Subject Identifier=80105
Simulated concentration vs. measured concentration of Metabolite
Subject Identifier=80106
Simulated concentration vs. measured concentration of Metabolite
Subject Identifier=80201
Simulated concentration vs. measured concentration of Metabolite
Subject Identifier=80202
Simulated concentration vs. measured concentration of Metabolite
Subject Identifier=80203
Simulated concentration vs. measured concentration of Metabolite
Subject Identifier=80204
Simulated concentration vs. measured concentration of Metabolite
Subject Identifier=80205
Simulated concentration vs. measured concentration of Metabolite
Subject Identifier=80206
Simulated concentration vs. measured concentration of Metabolite
Subject Identifier=80301
Simulated concentration vs. measured concentration of Metabolite
Subject Identifier=80302
Simulated concentration vs. measured concentration of Metabolite
Subject Identifier=80303
Simulated concentration vs. measured concentration of Metabolite
Subject Identifier=80306
Simulated concentration vs. measured concentration of Metabolite
Subject Identifier=80401


Simulated concentration vs. measured concentration of Metabolite
Subject Identifier=80403
Simulated concentration vs. measured concentration of Metabolite
Subject Identifier=80405
Simulated concentration vs. measured concentration of Metabolite
Subject Identifier=80406
Simulated concentration vs. measured concentration of Metabolite
Subject Identifier=81102
Simulated concentration vs. measured concentration of Metabolite
Subject Identifier=81104
Simulated concentration vs. measured concentration of Metabolite
Subject Identifier=81107
Simulated concentration vs. measured concentration of Metabolite
Subject Identifier=81108
Simulated concentration vs. measured concentration of Metabolite
Subject Identifier=81207
Simulated concentration vs. measured concentration of Metabolite
Subject Identifier=81208
Simulated concentration vs. measured concentration of Metabolite
Subject Identifier=81304
Simulated concentration vs. measured concentration of Metabolite
Subject Identifier=81305
Simulated concentration vs. measured concentration of Metabolite
Subject Identifier=81307
Simulated concentration vs. measured concentration of Metabolite
Subject Identifier=81308
Simulated concentration vs. measured concentration of Metabolite
Subject Identifier=81402
Simulated concentration vs. measured concentration of Metabolite
Subject Identifier=81404

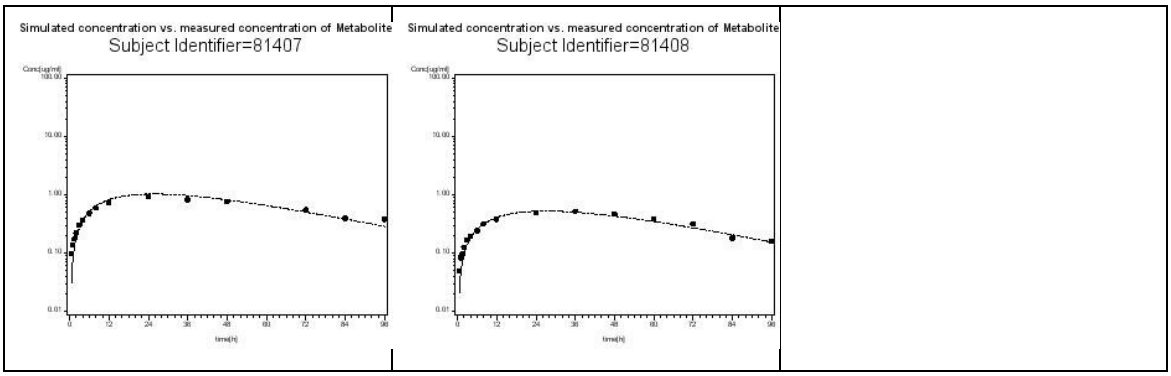


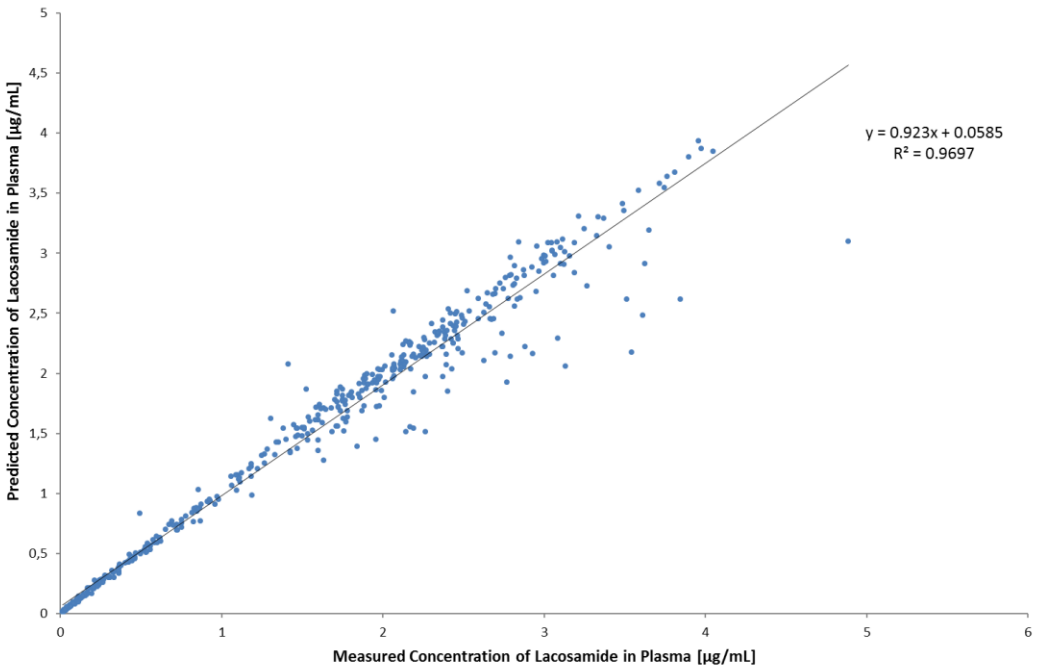
Figure 3-9 Simulation of Concentration of *O*-Desmethyl-Lacosamide in Plasma

Simulation of concentration-over-time profiles for each subject of *O*-desmethyl-lacosamide in plasma. The points represent the measured and the lines the simulated concentrations of the metabolite in plasma. The simulations are presented on a semi-logarithmic scale where on the x-axis the time in hours and on the y-axis the concentration in µg/mL of the metabolite is presented.

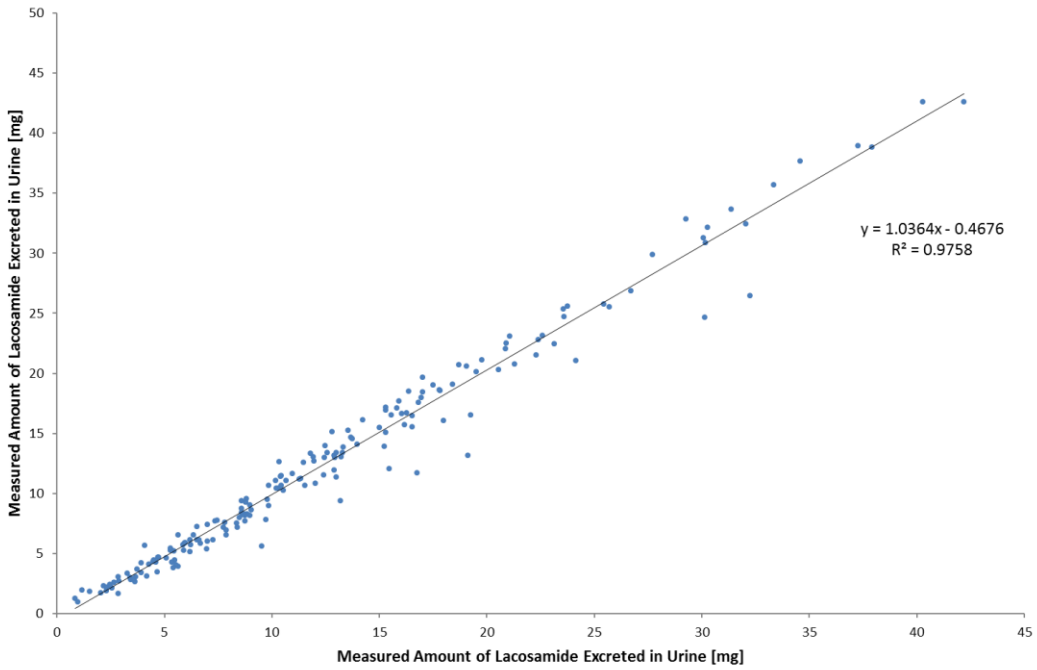
Table 3-10 Regression Analysis Between Measured and Predicted Values

	Pharmacokinetic Model		
	Lacosamide in Plasma	Lacosamide in Urine	O-Desmethyl-Lacosamide in Plasma
R^2	0.9697	0.9758	0.9772
Intercept	0.0585	-0.4676	-0.0120
Slope	0.9230	1.0364	1.0784

a


Predicted Concentration of Lacosamide in Plasma [µg/mL]
Measured Concentration of Lacosamide in Plasma [µg/mL]
y = 0.923x + 0.0585
R² = 0.9697
0
0,5
1
1,5
2
2,5
3
3,5
4
4,5
5
0
1
2
3
4
5
6

b


Measured Amount of Lacosamide Excreted in Urine [mg]
Measured Amount of Lacosamide Excreted in Urine [mg]
y = 1.0364x - 0.4676
R² = 0.9758
0
5
10
15
20
25
30
35
40
45
50
0
5
10
15
20
25
30
35
40
45

C

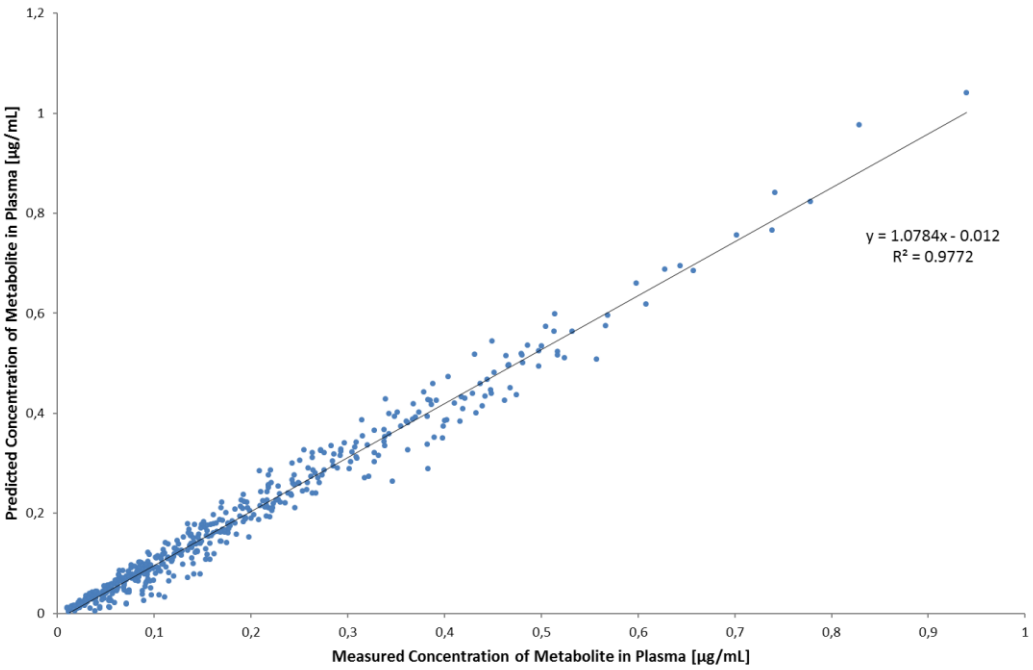


Figure 3-10 Predicted versus Measured Values of Study Data SP641

Predicted versus measured concentration/amount of a) lacosamide in plasma, b) lacosamide excreted in urine and c) *O*-desmethyl-lacosamide in plasma

3.3.3.3 Results of Correlation with Creatinine Clearance

As mentioned in section 3.2.5.4, it is assumed that the elimination rate constant comprises the sum of renal excretion and metabolism and could be replaced by the sum of pharmacokinetic parameters k_m and k_{ren}. The correlation between k_{me} and CR_{CL} showed a coefficient of determination of 0.6799 with an intercept of 0.00470 and a slope of 0.00105. Whereas the coefficient of determination for the correlation between k_{ren} and CL_{CR} was 0.3488 with an intercept of 0.00487 and a slope of 0.0001055, the coefficient of determination for the correlation of k_m and CR_{CL} was only 0.00008 with an intercept of 0.0338 and a slope of -0.000002.

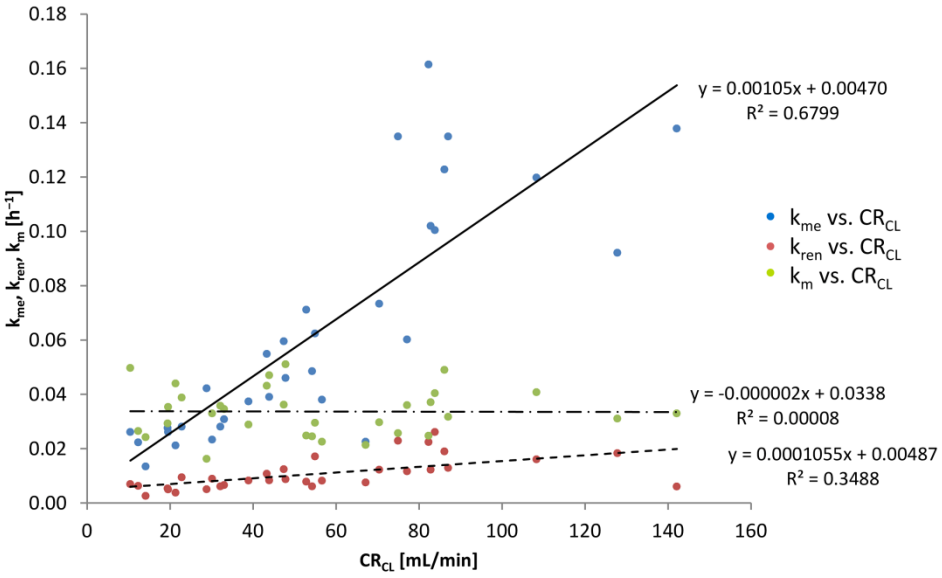


Figure 3-11 Correlation of CR_{CL} and Rate Constants k_{me}, k_{ren} and k_m

Correlation between the values for k_{me}, k_{ren} and k_m versus measured creatinine clearance of each subject with the indication of the coefficient of determination, the intercept and the slope.

3.3.3.4 Results of Correlation Between Body Height and Volume of Distribution

The correlation between body height and volume of distribution showed a coefficient of determination of 0.7051 with an intercept of 140.86 and a slope of 0.7597.

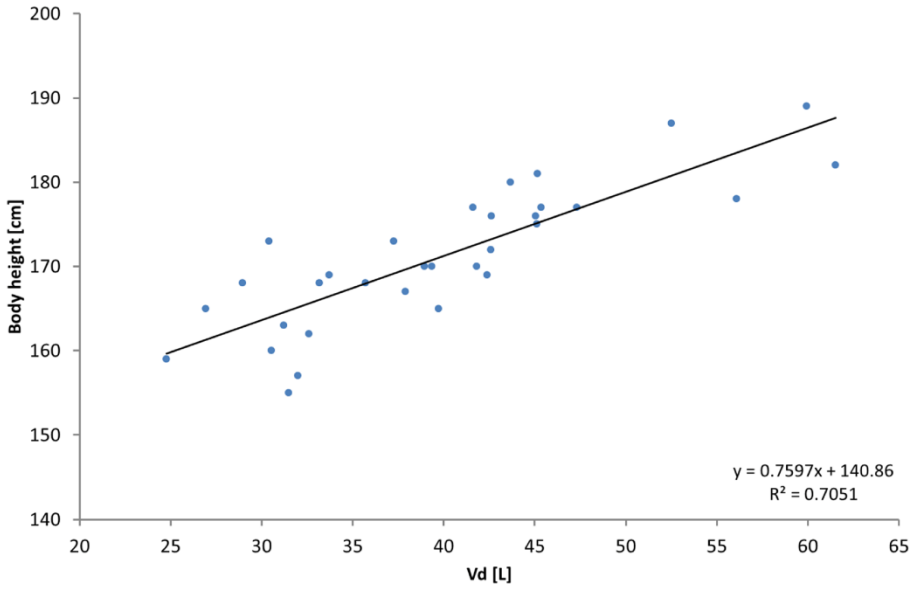


Figure 3-12 Correlation Between Body Height and Volume of Distribution

Correlation between body height and volume of distribution of each subject with the indication of the coefficient of determination, the intercept and the slope.

3.4 Discussion

In the present evaluation, an overview was provided of the concepts of pharmacokinetic model development, including methods of Laplace back-transformation as well as pharmacokinetic modeling and simulation including software validation. By considering these aspects, a new mathematical model was developed that described different pharmacokinetic processes at the same time. Exploiting this connection should yield progress in understanding different drug concentration time curves in plasma and drug exposures in subjects with physiological restrictions such as renal impairment.

All modeling and simulation processes were based upon a compartmental pharmacokinetic approach to relate pharmacokinetic parameters to physiological processes. During the pharmacokinetic model development, the use of Laplace equations and the method of Laplace back-transformation were usual methods to create a pharmacokinetic model and thus presented the basis of the present pharmacokinetic model development.

The consideration of combining mathematical equations that describe different pharmacokinetic circumstances such as the concentration/amount-time-profile of unchanged drug in plasma or in urine as well as of the main metabolite in plasma was successfully conducted and represented a new and further step in the area of pharmacokinetic modeling. The methods of pharmacokinetic modeling were described in detail to introduce the necessary background information on pharmacokinetic modeling and software programs. It was necessary to deal with software validation methods, which represent an inevitable tool to conduct pharmacokinetic modeling. These validation processes were described and executed with the aim of deriving a validated procedure that is adequate for pharmacokinetic modeling with the developed pharmacokinetic model.

A pharmacokinetic model is useful when it can be adapted to data from real subjects or patients. It was described that physiological restrictions such as renal impairment could lead to alterations in the pharmacokinetic profile of a drug and thus to possible necessary modifications in the dosing regimen. For this reason, the study SP641 was taken as an appropriate study to apply the developed model to real study data of lacosamide in subjects with and without renal impairment. Each metabolite

generally has its own pharmacokinetic profile, which consequently has to be considered when building up a pharmacokinetic model. For this reason, lacosamide was supposed to be a good prime example due to having only one main metabolite and a distinctive renal excretion (Cawello, Boekens et al., 2012), both of which could be incorporated in the pharmacokinetic model. It was shown that the developed model was able to describe the kinetic of lacosamide in healthy subjects as well as in subjects with renal impairment and thus it was in line with present understanding of the dependence of renal elimination on renal function and the independence of lacosamides' metabolism on renal function.

3.4.1 Validation

In the validation process, the equations for the model-dependent pharmacokinetics of unchanged drug in plasma and its amount excreted in urine as well as the pharmacokinetics of the metabolite in plasma were connected to a system of equations to involve the pharmacokinetic parameters into each of the mentioned pharmacokinetic systems of subunits. The equations representing the kinetics of a drug after oral administration - namely the Bateman function - as well as the kinetics of unchanged drug excreted in urine are well known and described in many pharmacokinetic text books (e.g. (Gibaldi and Perrier, 1975)). However, the intention of the present evaluation was to develop an equation describing the pharmacokinetics of the metabolite in plasma. The connection of metabolite kinetics with the kinetics of unchanged drug in plasma and its amount excreted in urine should be used for the evaluation of the dependency or independency of renal excretion and metabolism on renal function. Hitherto, no equations could be found in literature that describes the model-dependent pharmacokinetics of a metabolite in plasma. Through Laplace equations and the methods of Laplace back-transformation, it was possible to develop an integrated function that described the pharmacokinetics of a metabolite in plasma.

The validation analysis of the *nlin* procedure with a fictive study population demonstrated that the procedure worked well for pharmacokinetic modeling. Other software packages such as NONMEM, Kinetica, WinNonlin and Topfit have already been evaluated as adequate tools for pharmacokinetic modeling and the evaluation of correct values for pharmacokinetic parameters (AGAH, 2001). The AGAH working group used the Gauss-Newton as well as the Marquardt algorithm of iteration and

concentrated on the description of single pharmacokinetic systems. The current evaluation was based upon the Marquardt algorithm as a modified Gauss-Newton algorithm and considered a pharmacokinetic system that combined three different pharmacokinetic circumstances, as described above. Although SAS do not represent the typical pharmacokinetic modeling software, it represents a common and famous statistical tool in the pharmaceutical industry. Its use as an adequate modeling software tool could be demonstrated through validation in the present evaluation.

Regarding the results of accuracy and precision chosen as adequate statistical methods for evaluation as proposed in the ICH Harmonised Tripartite Guideline of Validation of Analytical Procedures, all values for pharmacokinetic parameters were located under the 10% border and thus met the guidance level of the FDA. A strong linear relationship could be observed for all pharmacokinetic parameters due to the values for the coefficient of determination around 0.9-1.0, apart from the rate constant of absorption, which was around 0.5. The reason for this lower correlation could be explained by fewer plasma samples being taken during the absorption phase. Due to the rapid absorption, it was difficult to obtain sufficient plasma samples in this short time to describe an adequate kinetic profile for the absorption phase. Most plasma samples were taken during the elimination phase, whereby the iteration for k_a was not as precise as for the other pharmacokinetic parameters. This was one reason to restrict the iteration for k_a to an upper limit of 10/h to achieve better iteration results. The correlation analysis between predicted and reference plasma concentrations of unchanged drug and metabolite as well as between predicted and reference amounts of unchanged drug in urine showed a strong linear relationship and led to the statement that the chosen procedure in SAS could be used for pharmacokinetic modeling.

3.4.2 Pharmacokinetic Modeling With SP641 Data

The validation of the *nlin* procedure to evaluate the mathematical pharmacokinetic models employed a fictive study population generated in SAS. The question of interest was whether the model could be used for pharmacokinetic modeling using lacosamide concentrations and amounts in plasma and urine, as well as metabolite plasma concentrations of healthy subjects and subjects with renal impairment. A further aspect of interest was whether the predicted pharmacokinetic parameters would reflect the current understanding of how pharmacokinetic parameters change within populations due to physiological restrictions. It is known that the effect of renal impairment as well as hepatic impairment on the dosing regimen depend on the fraction of AED eliminated by hepatic or renal excretion (Anderson and Hakimian, 2014). For lacosamide, it is known that the elimination is predominantly performed via the kidneys and the liver through hepatic biotransformation (Hoy, 2013). It is obvious that renal impairment could thus enforce dosing regimen changes or dose adaption due to a possible decrease in renal excretion or hepatic metabolism (Anderson and Hakimian, 2014). In the first part of the present thesis, it was shown that lacosamide did not require dose adjustment based upon age and gender. Moreover, Cawello et al. showed that this was also the case for subjects with mild-to-moderate renal impairment (Cawello, Fuhr et al., 2013). Nevertheless, one point of interest was whether the developed model could be used for pharmacokinetic modeling in populations – as previously mentioned – as well as differentiating the extent to which renal and metabolic elimination present part of total drug elimination.

The iteration and simulation results of lacosamide and its metabolite underlined that the developed model could be used for healthy subjects and subjects with renal impairment. The simulations of concentration time profiles and amounts of lacosamide in urine were almost consistent with measured values. In some cases, measured lacosamide concentrations were higher than the predicted concentrations (e.g. subject 80101 or 80203). This is possibly due to the fast absorption of lacosamide, whereby distribution processes have not been completed at time of sampling. The result would be a higher lacosamide concentration than after distribution processes whereby the pharmacokinetic model behind could be assumed to be a two-

compartment model as long as distribution processes have not been finished. Nevertheless, the more compartments that are considered in a pharmacokinetic model, the more parameters that have to be iterated by the chosen software procedure. This could lead to more difficulties during the modeling process and thus would not reflect a realistic pharmacokinetic evaluation. Thus, the decision was taken to concentrate on a pharmacokinetic one-compartment analysis. Regarding the simulation of lacosamide urine profiles, there was one subject where only the simulation curve could be seen (Figure 3-8). This could possibly be explained by the fact that urine data for that subject were missing. The regression analysis between measured and predicted values for lacosamide and its metabolic concentrations in plasma as well as for the measured and predicted amounts of lacosamide in urine showed good correlation with values for the coefficient of determination near one (Figure 3-10).

Rate constant of elimination includes all elimination processes proceeding in the human body. The fact that the developed system of equations included renal and metabolic elimination processes resulted in the consideration to describe each elimination process with its own rate constant of elimination. The basic thought was that the overall rate constant of elimination comprised the sum of renal and metabolic elimination (leaving out other elimination processes). Cawello et al. have already evaluated the relationship between pharmacokinetic parameters and creatinine clearance when correlating renal, metabolic and total body clearance versus renal function expressed as creatinine clearance (Cawello, Fuhr et al., 2013). Whereas the evaluation of Cawello et al. was based upon a model-independent approach, the present evaluation was based upon a model-dependent analysis of pharmacokinetic parameters. In the work of Cawello et al., a possible correlation between renal, metabolic and total clearance versus renal function following a model-independent analysis was demonstrated, whereas metabolic clearance was shown to be a prominent component of total body clearance (> 60% of total clearance in healthy subjects). The assumption that the renal rate constant of elimination and the metabolic rate constant of elimination were dependent on renal function was confirmed through the correlation between renal and metabolic elimination rate constant and creatinine clearance (Figure 3-11). Both correlations showed a

dependency of the rate of elimination on renal function. The elimination of lacosamide via hepatic biotransformation was around 34%, whereby it could be assumed that the rate constant of metabolic creation k_m would not be affected by renal impairment (Cawello, Fuhr et al., 2013). This assumption was confirmed when regarding the lack of correlation between k_m and renal clearance (Figure 3-11). Indeed, the regression line was almost parallel to the x-axis with values constantly fluctuating between 0.02 and 0.05/h.

The volume of distribution of lacosamide is approximately 0.6 L/kg and thus close to that of total body water (Hoy, 2013, Schiltmeyer, Cawello et al., 2005, UCB, 2014a). It is known that with advancing age total body water decreases but can increase with muscle mass - which mostly comprises water - whereby a correlation between volume of distribution and body height could be expected (Klotz, 2009). Schaefer, Cawello et al. showed that the volume of distribution approximated for each individual subject by using an empirical equation accounting for gender, body weight, height and age involved a scaling factor to describe the differences in lacosamide plasma concentrations in healthy subjects of different age and gender (Schaefer, Cawello et al., 2015). As it can be seen in Figure 3-12, a correlation between the iterated volume of distribution after a model-dependent pharmacokinetics and the measured body height of each individual subject could be observed. The values for the model-independent determined volume of distribution were ranged between 25 and 65 L and thus were in line with the results of calculated volume of distributions by Schaefer, Cawello et al. which were ranged between 31 and 78 L (Schaefer, Cawello et al., 2015). Moreover, in a population pharmacokinetic evaluation, Schiltmeyer, Cawello et al. showed that height and gender were identified as covariate on the volume of distribution after a model-dependent evaluation and thus confirmed the results of the present evaluation. The variability might be explained by the fact that an increase in body height could be related to an increase in muscle mass as well as body fat. Body fat generally comprises less water than muscle mass, whereby a higher body fat would not lead to an increase in the volume of distribution. Schaefer et al. also presented that women generally had a lower volume of distribution. This fact could be related to a higher fat mass in contrast to the male population, resulting in higher lacosamide plasma concentrations in women (Schaefer, Cawello et al., 2015). Nevertheless, the

results of correlation between volume of distribution and body height supported the fact that the developed pharmacokinetic model in the present analysis could be used in subjects with renal impairment, as well as subjects of different body compositions.

3.5 Conclusion

In the present work, a new pharmacokinetic model was developed that described the model-dependent pharmacokinetics of an unchanged drug in plasma and urine, as well as the pharmacokinetics of the metabolite in plasma. Moreover, it could be shown that the model could be applied to lacosamide and metabolite plasma concentrations as well as lacosamide amounts excreted in urine of healthy subjects and subjects with mild to severe renal impairment of a phase I trial. The pharmacokinetic parameters were consistent with the present understanding of the drug's behaviour in this population, which thus led to a better understanding of lacosamide's metabolism, its renal elimination and the dependency or independency of both on renal function. It could be shown that the developed pharmacokinetic model and the results of the pharmacokinetic modeling study revealed progress in the understanding of lacosamide's behaviour in healthy populations, as well as populations that were characterised by different body compositions or physiological restrictions such as renal impairment.

4 CHAPTER 4: FINAL SUMMARY OF THE THESIS AND PERSPECTIVES

4.1 Conclusion

The present thesis has provided an overview of the concepts, structures and applications in the area of pharmacokinetic model development, as well as in the area of pharmacokinetic modeling and simulation. A systematic approach was shown to develop a new pharmacokinetic model aiming to yield progress in understanding different drug concentration time curves in plasma and drug exposures in healthy and renal impaired subjects, as well as in subjects marked by different body compositions.

The first part of the thesis was aimed to provide an overview over the different areas in pharmacokinetic modeling to differentiate between compartmental, non-compartmental modeling and the physiological based pharmacokinetic modeling. Furthermore, it was worked out which steps generally have to be undertaken to develop a mathematical system of equations describing pharmacokinetic circumstances. Developing a new pharmacokinetic model that includes different pharmacokinetic processes of an unchanged drug and its main metabolite in different tissues would represent progress in developing new pharmacokinetic models. The steps that had to be executed during the pharmacokinetic model development resulted in two main evaluations, represented in chapters two and three.

In the second chapter of the thesis, the influence of age and gender on the pharmacokinetics of lacosamide in healthy subjects was evaluated and compared to results of a population pharmacokinetic analysis in adult patients with focal epilepsy taken from literature. Developing a pharmacokinetic model that could provide a framework for the prediction of time courses of exposure and response for different dosing regimens throughout a study population required an investigation about how pharmacokinetic parameters of a drug were influenced by age and gender. It could be shown that age and gender had no relevant effect on the rate of absorption of lacosamide as minor numerical differences could be explained by scaling factors such as body weight, or volume of distribution. The higher exposure and maximal plasma concentration in females compared to males in the age groups ‘older’ and ‘younger’ could largely be explained by the lower body weight and volume of distribution in females compared with males. The results of a population pharmacokinetic analysis in

adult patients with focal epilepsy were taken to evaluate the extent to which the results of the present evaluation in healthy subjects were in line with those taken from patient data. Although direct comparison was not possible due to different dosing regimens, it was worked out that lacosamide had a broadly similar pharmacokinetic profile in adults with focal epilepsy as that in healthy subjects. Given that most actual information on pharmacokinetic characteristics of anti-epileptic drugs is based upon healthy subjects of male gender and aged under 65 years, the present analysis broadens the spectrum through working out information of lacosamide pharmacokinetics in adults with focal epilepsy (information taken from literature, see section 3.2.4). Although some limitations should be considered when interpreting the results of the present post-hoc analysis – such as the missing urine data in female subjects and minor changes in age (females could have been up to 5 years younger than their male counterparts) – it could be shown that age and gender had no relevant effect on the rate of absorption and rate of elimination of lacosamide in healthy subjects. For this reason it could be stated that lacosamide represented an anti-epileptic drug with predictable exposure when administered twice daily in individuals, regardless of age and gender.

In the third chapter, a new pharmacokinetic model was developed through methods of Laplace back-transformation, including the model-dependent pharmacokinetics of a drug and its main metabolite in plasma as well as the pharmacokinetic of unchanged drug excreted in urine. It could be shown that the combination of different pharmacokinetic models into one mathematical equation represented a further step in the area of pharmacokinetic modeling; thus, it could be adapted to data from real subjects and patients. During the validation process, the chosen procedure in program SAS proved an adequate tool for pharmacokinetic modeling through statistical methods accuracy and precision as proposed in the ICH Harmonised Tripartite Guideline of Validation of Analytical Procedures.

It was worked out that physiological restrictions such as renal impairment represented important information that has to be considered when applying dosing regimens to patients. Study SP641 - which included healthy as well as mild to severe renal impaired subjects receiving oral lacosamide - was chosen as an adequate study for pharmacokinetic modeling with developed model, given that lacosamide generally

is excreted via the kidneys and metabolised to only one main metabolite. It could be shown that the developed model reflected the pharmacokinetics of lacosamide and its main metabolite in plasma, as well as the amount of lacosamide excreted in urine in an appropriate way and thus could be used for pharmacokinetic modeling. Furthermore, it was possible to underline the known information that lacosamide's elimination pathways split into renal and metabolic elimination. The renal rate constant of elimination as representative for renal elimination was dependent whereas the rate constant of metabolism was independent on renal function. The pharmacokinetic parameters were consistent with our present understanding of lacosamides' behaviour in healthy subjects but also in renal impaired populations leading to a better understanding of the independence between metabolism and the dependence of renal excretion on renal function.

The perspective of the present pharmacokinetic modeling approach was to enhance our understanding of other drugs and their behaviour in different patient populations through applying the developed model to existing and new developed drugs.

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APPENDICES

Appendix 1 SAS Programs for Data Extraction, Normalization and Statistical Evaluation of Study SP620

Appendix 2 SAS Programs for Data Extraction, Normalization and Statistical Evaluation of Study SP599

Appendix 3 SAS Program for Merging of SP599 and SP620

Appendix 4 SAS Program for Analysis of Variance

Appendix 5 SAS Programs for Validation

Appendix 6 Source Data of Plasma and Urine Data of the Fictive Study Population

Appendix 7 List of Generated Reference Pharmacokinetic Parameters for Each Subject of the Fictive Study Population

Appendix 8 List of Iterated Pharmacokinetic Parameters for Each Subject of the Fictive Study Population

Appendix 9 SAS Programs for Evaluation of Study Data SP641

Appendix 1 SAS Programs for Data Extraction, Normalization and Statistical Evaluation of Study SP620

Program 1

```
**********Program for Data Extraction of Non-compartimental Parameters of Study SP620 and Normalization Processes **********
********** Autor: Carina Schäfer *08.03.2013**********;

libname LCM
'M:\Sites\Monheim\NewMedicines\GED\SP\CSchaefer\LCM\SP620\SASdata';


data PP;
set LCM.Sp620_pp;
run;


proc sort data=Pp;
by SUBJID;
run;
data Pp;
set Pp;
rename SUBJID=subjid;
run;


data demo;
set LCM.Sp620_demo2;
run;


proc sort data=demo;
by subjid;
run;


data All;
merge Pp demo;
by subjid;
run;


data All2;
```

```
set all;
where ARMCD ne "" and (pptest in ('CL/f','AUC(0-inf)','Cmax','Cmax,ss','AUC(0-tau)ss'));

run;
*******Calculation of LBW and FFM*************;
data All2;
set All2;
BMI=weighkg/(heights*heights);
if SEX_C=1 then LBW=1.10*WEIGHKG-0.0128*BMI*WEIGHKG;
else LBW=1.07*WEIGHKG-0.0148*BMI*WEIGHKG;
if SEX_C=1 then FFM=9270*WEIGHKG/(6680+216*BMI);
else FFM=9270*WEIGHKG/(8780+244*BMI);
run;

********Normalization Processes*************;
data All3;
set All2;
if (pptest ne 'CL/f') then do;
weightNorm=ppstresn*weighkg;
heightNorm=ppstresn*heights;
LBWnorm=ppstresn*LBW;
FFMnorm=ppstresn*FFM;
end;
else do;
weightNorm=ppstresn/weighkg;
heightNorm=ppstresn/heights;
LBWnorm=ppstresn/LBW;
FFMnorm=ppstresn/FFM;
end;
run;

 data LCM.Sp620_pp_normall;
 set All3;
 run;
```

```
proc sort data=All3;
by ppparm pptest armcd;
run;

options orientation=landscape;
** ods pdf
file='M:\Sites\Monheim\NewMedicines\GED\SP\CSchaefer\LCM\SP620\PDF_Files\summary_norm.pdf';
****** Summary Statistics**********;
proc summary data=All3 print mean Std min max n median;
class ppparm pptest armcd;
var ppstresn weightNorm heightNorm LBWnorm FFMnorm;

run;
** ods pdf close;

**********preparation for geometric mean calculation**********;

data all4;
 set all3;
 ln_ppstresn=log(ppstresn);
 ln_weightNorm=log(weightNorm);
 ln_heightNorm=log(heightNorm);
 ln_LBWnorm=log(LBWnorm);
 ln_FFMnorm=log(FFMnorm);
run;

ods output summary=geomean;
proc summary data=All4 print mean Std n;
class ppparm pptest armcd;
var ln_ppstresn ln_weightNorm ln_heightNorm ln_LBWnorm ln_FFMnorm;
run;

proc transpose data=geomean out=geomean_m;
 by ppparm pptest armcd;
```

```
 var ln_ppstresn_mean ln_weightNorm_mean ln_heightNorm_mean
ln_LBWnorm_mean ln_FFMnorm_mean;
run;
proc transpose data=geomean out=geomean_std;
 by ppparm pptest armcd;
 var ln_ppstresn_StdDev ln_weightNorm_StdDev ln_heightNorm_StdDev
ln_LBWnorm_StdDev ln_FFMnorm_StdDev;
run;
data geomean_std;
 set geomean_std;
 rename COL1=std;
run;

data geomean2;
 merge geomean_m geomean_std;
 by ppparm pptest armcd;
 rename COL1=mean;
run;
data geomean2;
 set geomean2;
 geomean=exp(mean);
 CVgeo=100*sqrt(exp(std*std)-1);
 variab=substr(_NAME_,4,length(_NAME_)-10);
run;

ods pdf
file='M:\Sites\Monheim\NewMedicines\GED\SP\CSchaefer\LCM\SP620\PDF_Files\geo
mean_summary_norm.pdf';

proc print data=geomean2;
  var ppparm pptest armcd variab geomean CVgeo;
run;
ods pdf close;
```

Program 2

**********Program for Data Extraction of Concentration Data of Study SP620 and Normalization Processes **********

********** Autor: Carina Schäfer *01.03.2013**********;

```
libname LCM
'M:\Sites\Monheim\NewMedicines\GED\SP\CSchaefer\LCM\SP620\SASdata';

data LCMpc;
set LCM.Sp620_pc;
if timept_s=4.1 then timept_s=4;
if timept_s=1.1 then timept_s=1;
if timept_s=1.15 then timept_s=1;
if (timept_s>3.16 and timept_s<3.17) then timept_s=3;
run;

data LCMpc;
set LCMpc;
rename SUBJID=subjid;
run;

proc sort data=LCMpc;
by subjid;
run;
data demo;
set LCM.Sp620_demo2;
run;

proc sort data=demo;
by subjid;
run;

data All;
merge LCMpc demo;
```

```
by subjid;
run;

*******Calculation of LBW and FFM**********;
data All2;
set All;
where domain='PC';
BMI=weighkg/(heights*heights);
if SEX_C=1 then LBW=1.10*WEIGHKG-0.0128*BMI*WEIGHKG;
else LBW=1.07*WEIGHKG-0.0148*BMI*WEIGHKG;
if SEX_C=1 then FFM=9270*WEIGHKG/(6680+216*BMI);
else FFM=9270*WEIGHKG/(8780+244*BMI);
run;

**********Normalization Processes**********;
data All3;
set All2;
weightNorm=pcstresn*weighkg;
heightNorm=pcstresn*heights;
LBWnorm=pcstresn*LBW;
FFMnorm=pcstresn*FFM;
run;

data LCM.Sp620_PCnorm;
set All3;
run;

 ***** Summary Statistics*******;
proc sort data=All3;
by PCParm armcd timept_s;
run;
options orientation=landscape;
ods pdf file=
'M:\Sites\Monheim\NewMedicines\GED\SP\CSchaefer\LCM\SP620\PDF_Files\PC_Summary_norm.pdf';
```

```
proc summary data=All3 print mean Std min max n median;
class PCPARM armcd timept_s;
var pcstresn weightNorm heightNorm LBWnorm FFMnorm;
output out=Summary_PC mean=mean Std=Std min=min max=max n=n
median=median;
run;
ods pdf close;

data Summary_PC2;
set Summary_pc;
where _TYPE_=7;
run;

data _null_;
set Summary_PC2;
file
'M:\Sites\Monheim\NewMedicines\GED\SP\CSchaefer\LCM\SP620\Figures\Summary
_PC.xxx';
put PCPARM armcd timept_s mean Std min max n median;
run;
```

Program 3

**********Program for Data Extraction of Concentration Data of Study SP620 and Normalization Processes/Weight Normalization**********

********** Author: Carina Schäfer *03.04.2013**********;

```
libname LCM
'M:\Sites\Monheim\NewMedicines\GED\SP\CSchaefer\LCM\SP620\SASdata';

data LCMpc;
set LCM.Sp620_pc;
if timept_s=4.1 then timept_s=4;
if timept_s=1.1 then timept_s=1;
if timept_s=1.15 then timept_s=1;
if (timept_s>3.16 and timept_s<3.17) then timept_s=3;
run;

data LCMpc;
set LCMpc;
rename SUBJID=subjid;
run;

proc sort data=LCMpc;
by subjid;
run;
data demo;
set LCM.Sp620_demo2;
run;

proc sort data=demo;
by subjid;
run;

data All;
merge LCMpc demo;
```

```
by subjid;
run;

*******Calculation of LBW and FFM*************;
data All2;
set All;
where domain='PC';
BMI=weighkg/(heights*heights);
if SEX_C=1 then LBW=1.10*WEIGHKG-0.0128*BMI*WEIGHKG;
else LBW=1.07*WEIGHKG-0.0148*BMI*WEIGHKG;
if SEX_C=1 then FFM=9270*WEIGHKG/(6680+216*BMI);
else FFM=9270*WEIGHKG/(8780+244*BMI);
run;

*********Normalization Processes*************;
data All3;
set All2;
weightNorm=pcstresn*weighkg;
heightNorm=pcstresn*heights;
LBWnorm=pcstresn*LBW;
FFMnorm=pcstresn*FFM;
run;

data LCM.Sp620_PCnorm;
set All3;
run;

proc sort data=All3;
by PCParm armcd timept_s;
run;
options orientation=landscape;
ods pdf file=
'M:\Sites\Monheim\NewMedicines\GED\SP\CSchaefer\LCM\SP620\PDF_Files\PC_Summary_normWeight.pdf';
proc summary data=All3 print mean Std min max n median;
```

```
class PCPARM armcd timept_s;

var weightNorm;

output out=Summary_PC mean=mean Std=Std min=min max=max n=n
median=median;

run;

ods pdf close;


data Summary_PC2;

set Summary_pc;

where _TYPE_=7;

run;


data _null_;

set Summary_PC2;

file
'M:\Sites\Monheim\NewMedicines\GED\SP\CSchaefer\LCM\SP620\Figures\Summary
_PCweight.xxx';

put PCPARM armcd timept_s mean Std min max n median;

run;
```

Appendix 2 SAS Programs for Data Extraction, Normalization and Statistical Evaluation of Study SP599

Program 4

**********Program for Data Extraction of Non-compartimental Parameters of Study SP599 and Normalization Processes**********

********** Autor: Carina Schäfer *08.03.2013**********;

```
libname LCM
'M:\Sites\Monheim\NewMedicines\GED\SP\CSchaefer\LCM\SP599\SASdata';

data PP;
set LCM.Sp599_pp;
run;

proc sort data=PP;
by SUBJID;
run;
data PP;
set PP;
rename SUBJID=subjid;
run;

data demo;
set LCM.Sp599_demo2;
run;

proc sort data=demo;
by subjid;
run;

data All;
merge PP demo;
by subjid;
run;
```

```
data All2;
set All;
where ARMCD ne " " and (pptest in ('Cmax,ss','AUC(0-tau)ss'));

run;

*******Calculation of LBW and FFM*************;
data All2;
set All2;
BMI=weighkg/(heights*heights);
 LBW=1.07*WEIGHKG-0.0148*BMI*WEIGHKG;
 FFM=9270*WEIGHKG/(8780+244*BMI);
run;

******Normalization Processes*****************;
data All3;
set All2;
weightNorm=ppstresn*weighkg;
heightNorm=ppstresn*heights;
LBWnorm=ppstresn*LBW;
FFMnorm=ppstresn*FFM;
run;

********Summary Statistics************;
 data LCM.Sp599_pp_normall;
 set All3;
 run;
proc sort data=All3;
by pptest armcd ;
run;
options orientation=landscape;
ods pdf file='M:\Sites\Monheim\NewMedicines\GED\SP\CSchaefer\LCM\SP599\PDF
files\Sp_599_PPsummary.pdf';
proc summary data=All3 print mean Std min max n median;
```

```
class pptest;
var ppstresn weightNorm heightNorm LBWnorm FFMnorm;
output out=Summary_PP mean=mean Std=Std min=min max=max n=n
median=median;
run;
ods pdf close;

data Summary_PP2;
set Summary_pp;
where _TYPE_=1;
run;

data _null_;
set Summary_PP2;
file
'M:\Sites\Monheim\NewMedicines\GED\SP\CSchaefer\LCM\SP599\Figures\Summary
PP2.xxx';
put pptest  mean Std min max n median;
run;
```

Program 5

```
**********Program for Data Extraction of Non-compartimental Parameters of Study SP599 and Normalization Processes **********
********** Autor: Carina Schäfer *01.03.2013**********;


libname LCM
'M:\Sites\Monheim\NewMedicines\GED\SP\CSchaefer\LCM\SP599\SASdata';


data PP;
set LCM.Sp599_pp;
run;


proc sort data=PP;
by SUBJID;
run;
data PP;
set PP;
rename SUBJID=subjid;
run;


**********Normalization to a dose of 100 mg**********;
data PP;
set PP;
PPSTRESNnorm=PPSTRESN/2;
run;


data demo;
set LCM.Sp599_demo2;
run;


proc sort data=demo;
by subjid;
run;
```

```
data All;
merge PP demo;
by subjid;
run;

data All2;
set All;
where ARMCD ne " " and (pptest in ('Cmax,ss','AUC(0-tau)ss'));

run;

**********Calculation of FFM and LBW**********;
data All2;
set All2;
BMI=weighkg/(heights*heights);
 LBW=1.07*WEIGHKG-0.0148*BMI*WEIGHKG;
 FFM=9270*WEIGHKG/(8780+244*BMI);
run;

*****Normalization Processes***************;
data All3;
set All2;
weightNorm=ppstresnnorm*weighkg;
heightNorm=ppstresnnorm*heights;
LBWnorm=ppstresnnorm*LBW;
FFMnorm=ppstresnnorm*FFM;
run;

 data LCM.Sp599_pp_DoseNorm;
 set All3;
 run;
proc sort data=All3;
by pptest armcd ;
run;
```

```
*****Summary Statistics*****************;


options orientation=landscape;
***ods pdf
file='M:\Sites\Monheim\NewMedicines\GED\SP\CSchaefer\LCM\SP599\PDF
files\Sp_599_PPsummaryDoseNorm.pdf';
proc summary data=All3 print mean Std min max n median;
class pptest;
var ppstresnnorm weightNorm heightNorm LBWnorm FFMnorm;
output out=Summary_PPdoseNorm mean=mean Std=Std min=min max=max n=n
median=median;
run;
****ods pdf close;


******Preparation for Geometric Mean Calculation*****************;
data all4;
 set all3;
 ln_ppstresn=log(ppstresn);
 ln_weightNorm=log(weightNorm);
 ln_heightNorm=log(heightNorm);
 ln_LBWnorm=log(LBWnorm);
 ln_FFMnorm=log(FFMnorm);
run;


ods output summary=geomean; *** Einbringen in eine Exeltabelle***;
proc summary data=All4 print mean Std n;
class  pptest;
var ln_ppstresn ln_weightNorm ln_heightNorm ln_LBWnorm ln_FFMnorm;
run;


proc transpose data=geomean out=geomean_m; **** aus Zeilen werden Spalten***;
 by  pptest;
 var ln_ppstresn_mean ln_weightNorm_mean ln_heightNorm_mean
ln_LBWnorm_mean ln_FFMnorm_mean;
run;
proc transpose data=geomean out=geomean_std; ***s.o.***;
```

```
 by  pptest;
 var ln_ppstresn_StdDev ln_weightNorm_StdDev ln_heightNorm_StdDev
ln_LBWnorm_StdDev ln_FFMnorm_StdDev;
run;
data geomean_std;
 set geomean_std;
 rename COL1=std;
run;

data geomean2; *** Zusammenbringen der std und means***;
 merge geomean_m geomean_std;
 by pptest;
 rename COL1=mean;
run;
data geomean2; ***Berechnung des geometrischen Mittels und CV***;
 set geomean2;
 geomean=exp(mean);
 CVgeo=100*sqrt(exp(std*std)-1);
 variab=substr(_NAME_,4,length(_NAME_)-10);
run;

ods pdf file='M:\Sites\Monheim\NewMedicines\GED\SP\CSchaefer\LCM\SP599\PDF
files\Sp599_pp_geomean_summary_norm.pdf';

proc print data=geomean2;
  var pptest variab geomean CVgeo;
run;
ods pdf close;

options orientation=landscape;
ods pdf file='M:\Sites\Monheim\NewMedicines\GED\SP\CSchaefer\LCM\SP599\PDF
files\Sp_599_PPsummaryDoseNorm.pdf';
proc summary data=All3 print mean Std min max n median;
class pptest;
var ppstresnnorm weightNorm heightNorm LBWnorm FFMnorm;
```

```
output out=Summary_PPdoseNorm mean=mean Std=Std min=min max=max n=n
median=median;

run;

ods pdf close;


data Summary_PPdoseNorm;

set Summary_ppdoseNorm;

where _TYPE_=1;

run;


data _null_;

set Summary_PPdoseNorm;

file
'M:\Sites\Monheim\NewMedicines\GED\SP\CSchaefer\LCM\SP599\Figures\Summary
PPdoseNorm.xxx';

put pptest  mean Std min max n median;

run;
```

Program 6

```
**********Program for Data Extraction of Concentration Data of Study SP599 and
Normalization Processes **********
********** Author: Carina Schäfer *06.03.2013*****************;


libname LCM
'M:\Sites\Monheim\NewMedicines\GED\SP\CSchaefer\LCM\SP599\SASdata';


data PC;
set LCM.Sp599_pc;
run;
 data PC1;
 set PC;
 where EXTRT='SPM927';
 run;

proc sort data=PC1;
by SUBJID;
run;
data PC1;
set PC1;
rename SUBJID=subjid;
run;

data demo;
set LCM.Sp599_demo2;
run;

proc sort data=demo;
by subjid;
run;

data All;
merge PC1 demo;
```

```
by subjid;
run;

data All2;
set all;
where ARMCD ne " " ;
run;

******Calculation of LBW and FFM****************;
data All2;
set All2;
BMI=weighkg/(heights*heights);
 LBW=1.07*WEIGHKG-0.0148*BMI*WEIGHKG;
 FFM=9270*WEIGHKG/(8780+244*BMI);
run;

****Normalization Processes***********;
data All3;
set All2;
weightNorm=pcstresn*weighkg;
heightNorm=pcstresn*heights;
LBWnorm=pcstresn*LBW;
FFMnorm=pcstresn*FFM;
run;

*****Summary Statistics*************;
 data LCM.Sp599_pc_normall;
 set All3;
 run;
proc sort data=All3;
by timept_s subjid ;
run;
options orientation=landscape;
ods pdf file='M:\Sites\Monheim\NewMedicines\GED\SP\CSchaefer\LCM\SP599\PDF
files\Sp_599_PCsummary.pdf';
```

```
proc summary data=All3 print mean Std min max n median;
class timept_s;
var pcstresn weightNorm heightNorm LBWnorm FFMnorm;
output out=Summary_PC mean=mean Std=Std min=min max=max n=n
median=median;
run;
ods pdf close;

data Summary_PC2;
set Summary_pc;
where _TYPE_=1;
run;



data _null_;
set Summary_PC2;
file
'M:\Sites\Monheim\NewMedicines\GED\SP\CSchaefer\LCM\SP599\Figures\Summary
PC.xxx';
put timept_s mean Std min max n median;
run;
```

Program 7

```
**********Program for Data Extraction of Concentration Data of Study SP599 and
Normalization Processes with Dose Normalization **********
********** Author: Carina Schäfer *06.03.2013*****************;


libname LCM
'M:\Sites\Monheim\NewMedicines\GED\SP\CSchaefer\LCM\SP599\SASdata';


data PC;
set LCM.Sp599_pc;
run;
 data PC1;
 set PC;
 where EXTRT='SPM927' and visitdy>=12;
 run;

proc sort data=PC1;
by SUBJID;
run;
data PC1;
set PC1;
rename SUBJID=subjid;
run;

******Dose Normalization**************;
data PC1;
set PC1;
PCSTRESNnorm=PCSTRESN/2;
run;

data demo;
set LCM.Sp599_demo2;
run;
```

```
proc sort data=demo;
by subjid;
run;

data All;
merge PC1 demo;
by subjid;
run;

data All2;
set all;
where ARMCD ne " " ;
run;

data All2;
set All2;
BMI=weighkg/(heights*heights);
 LBW=1.07*WEIGHKG-0.0148*BMI*WEIGHKG;
 FFM=9270*WEIGHKG/(8780+244*BMI);
run;

data All3;
set All2;
weightNorm=pcstresnnorm*weighkg;
heightNorm=pcstresnnorm*heights;
LBWnorm=pcstresnnorm*LBW;
FFMnorm=pcstresnnorm*FFM;
run;

*****Summary Statistics*****;
 data LCM.Sp599_pc_DoseNorm;
 set All3;
 run;
proc sort data=All3;
```

```
by timept_s subjid ;
run;
options orientation=landscape;
ods pdf file='M:\Sites\Monheim\NewMedicines\GED\SP\CSchaefer\LCM\SP599\PDF files\Sp_599_PCsummaryDoseNorm.pdf';
proc summary data=All3 print mean Std min max n median;
class timept_s;
var pcstresnnorm weightNorm heightNorm LBWnorm FFMnorm;
output out=Summary_PCdoseNorm mean=mean Std=Std min=min max=max n=n median=median;
run;
ods pdf close;

data Summary_PCdoseNorm;
set Summary_PCdoseNorm;
where _TYPE_=1;
run;



data _null_;
set Summary_PCdoseNorm;
file 'M:\Sites\Monheim\NewMedicines\GED\SP\CSchaefer\LCM\SP599\Figures\Summary PCdoseNorm.xxx';
put timept_s mean Std min max n median;
run;
```

Program 8

```
**********Program for Data Extraction of Concentration Data of Study SP599 and Normalization Processes/ Separation Weight Normalization**********
********** Author: Carina Schäfer *20.03.2013*****************;


libname LCM
'M:\Sites\Monheim\NewMedicines\GED\SP\CSchaefer\LCM\SP599\SASdata';


data PC;
set LCM.Sp599_pc;
run;
 data PC1;
 set PC;
 where EXTRT='SPM927' and visitdy>=12;
 run;

proc sort data=PC1;
by SUBJID;
run;
data PC1;
set PC1;
rename SUBJID=subjid;
run;

data PC1;
set PC1;
PCSTRESNnorm=PCSTRESN/2;
run;

data demo;
set LCM.Sp599_demo2;
run;

proc sort data=demo;
```

```
by subjid;
run;

data All;
merge PC1 demo;
by subjid;
run;

data All2;
set all;
where ARMCD ne " " ;
run;

*****Calculation of LBW and FFM**********;
data All2;
set All2;
BMI=weighkg/(heights*heights);
 LBW=1.07*WEIGHKG-0.0148*BMI*WEIGHKG;
 FFM=9270*WEIGHKG/(8780+244*BMI);
run;

******Normalization Processes**********;
data All3;
set All2;
weightNorm=pcstresnnorm*weighkg;
heightNorm=pcstresnnorm*heights;
LBWnorm=pcstresnnorm*LBW;
FFMnorm=pcstresnnorm*FFM;
run;

*****Summary Statistics******;
 data LCM.Sp599_pc_DoseNorm;
 set All3;
 run;
```

```
proc sort data=All3;
by timept_s subjid ;
run;
options orientation=landscape;
ods pdf file='M:\Sites\Monheim\NewMedicines\GED\SP\CSchaefer\LCM\SP599\PDF files\Sp_599_PCsummaryDoseNormWeight.pdf';
proc summary data=All3 print mean Std min max n median;
class timept_s;
var  weightNorm;
output out=Summary_PCdoseNorm mean=mean Std=Std min=min max=max n=n median=median;
run;
ods pdf close;


data Summary_PCdoseNorm;
set Summary_PCdoseNorm;
where _TYPE_=1;
run;



data _null_;
set Summary_PCdoseNorm;
file 'M:\Sites\Monheim\NewMedicines\GED\SP\CSchaefer\LCM\SP599\Figures\Summary PCdoseNormWeight.xxx';
put timept_s mean Std min max n median;
run;
```

Appendix 3 SAS Program for Merging of SP620 and SP599 Data for Statistical Evaluation

Program 9

```
*********Descriptive Statistics of Dose - Normalized by Body Weight (SP599 and
SP620)
********** Author: Carina Schäfer *03.12.2014**********;
libname LCM
'N:\Sites\Monheim\NewMedicines\GED\SP\CSchaefer\LCM\Vergleich599_620\SASdat
a';


*****Merging Process**************;
data dose1;
 set LCM.Femalefemale_pp LCM.MaleFemale_pp LCM.Ymalefemale_pp;
 keep studyid subjid arm armcd weighkg;
run;
proc sort nodupkey;by studyid subjid;run;
data dose2;
  set dose1;
  if studyid='SP599' then group='YF'; else group='YM';
  if armcd='1 bid' then group='EM';
  if armcd='2 bid' then group='EF';
  if studyid='SP599' then dose=200; else dose=100;
  dosenorm=dose/weighkg;
run;
proc sort;by group;run;

******Macro Program*******;
%macro means(var);
proc means data=dose2 noprint;
by group;
var &var;
output out=desi_&var n=n mean=mean std=SD min=min max=max median=median;
run;
quit;
%mend means;
```

```
%means(dosenorm);

%macro report(var,label,format,formatm);
ODS PDF close;
ODS LISTING;

ods pdf
file="N:\Sites\Monheim\NewMedicines\GED\SP\CSchaefer\LCM\Vergleich599_620\Desi_&var..pdf"  ;
ods rtf
file="N:\Sites\Monheim\NewMedicines\GED\SP\CSchaefer\LCM\Vergleich599_620\Desi_&var..rtf"  ;
ods listing close;
options nodate pageno=1;
title1 J=L " ";
title2 J=C "Descriptive Statistics for &label";

proc report data=desi_&var NOWINDOWS HEADLINE SPLIT="\";
column group n mean sd min median max;
define group / "Group" order;
define n / format=3.0 "n" display;
define mean / format=&formatm "Mean" display;
define sd / format=&formatm "SD" display;
define min / format=&format "Min" display;
define median / format=&formatm "Median" display;
define max / format=&format "Max" display;

COMPUTE AFTER ;
  LINE @5 "Abbreviations:";
  LINE @5 "YM=young male, YF=young female, EM=elderly male, EF=elderly female";
ENDCOMP;
RUN;
quit;
ODS PDF close;
ODS RTF close;
```

```
ODS LISTING;


%mend report;


%report(dosenorm,Dose normalized by body weight [mg/kg],8.3 ,8.4 );
```

Appendix 4 SAS Program for Analysis of Variance

Program 10

```
*********Analysis of Variance for Cmax,ss/ Statistical Evaluation of Age and Gender**********************

************ Author: Carina Schäfer *05.08.2014***********;

libname abc 'N:\Sites\Monheim\NewMedicines\GED\SP\CSchaefer\LCM\Vergleich599_620\SASdata\';


data pp;
 set abc.Ymalefemale_pp  abc.Femalefemale_pp abc.Malefemale_pp;
 if studyid='SP599' then ppstresn=ppstresn/2; else ppstresn=ppstresn;  *** parameter normalized to 100mg bid;
 keep studyid subjid pptest ppstresn arm armcd armdose sex_c heights weighkg age BMI LBW FFM;
run;


proc sort data=pp nodupkey; by studyid subjid pptest;run;


**************** Normalization and Log-transformation for PK Parameters***;


data pp_select;
 set pp;
 where pptest='Cmax,ss';
 para_dnorm=log(ppstresn);
 para_bwnorm=log(ppstresn*weighkg);
 para_HTnorm=log(ppstresn*heights);
 para_LBWnorm=log(ppstresn*LBW);
 para_FFMnorm=log(ppstresn*FFM);
 if age<60 then age_g='Y';else age_g='E';
 if sex_c=1 then vd=0.3625*weighkg+0.2239*heights*100-0.1387*age-14.47;
  else vd=0.2363*weighkg+0.1962*heights*100-0.0272*age-10.26;
 para_vdnorm=log(ppstresn*vd);
run;
```

```
*************** ANOVA for PK parameters without as well with normalization****;
option nodate nonumber orientation=portrait;
ods rtf
file='N:\Sites\Monheim\NewMedicines\GED\SP\CSchaefer\LCM\Paper_AgeAndGende
r\Paper\Tabellen\ANOVA_001A.rtf' STARTPAGE=BYGROUP;
Title ' GLM with model Cmax,ss=group';
Title2 '*** without normalization  ***';

proc Annotate=LSMeanDiffCL; run;
proc glm data=pp_select;
  ods output LSMeanDiffCL=diffcl1;
  class sex_c age_g;
  model para_dnorm=sex_c age_g sex_c*age_g;
   lsmeans sex_c age_g sex_c*age_g /alpha=0.10 CL pdiff stderr;
   output out=result1 r=res p=fitted LCLM=LCLM UCLM=UCLM;
run;
quit;

Title2 '*** normalization by body weight **';
proc glm data=pp_select;
  ods output LSMeanDiffCL=diffcl2;
  class sex_c age_g;
  model para_bwnorm=sex_c age_g sex_c*age_g;
   lsmeans sex_c age_g sex_c*age_g/out=_lsout alpha=0.10 CL pdiff stderr;
   output out=result2 r=res p=fitted LCLM=LCLM UCLM=UCLM;
run;
quit;

Title2 '*** normalization by height **';
proc glm data=pp_select;
  ods output LSMeanDiffCL=diffcl3;
  class sex_c age_g;
  model para_HTnorm=sex_c age_g sex_c*age_g;
   lsmeans sex_c age_g sex_c*age_g/out=_lsout alpha=0.10 CL pdiff stderr;
```

```
  output out=result3 r=res p=fitted LCLM=LCLM UCLM=UCLM;
run;
quit;

Title2 '*** normalization by LBW **';
proc glm data=pp_select;
 ods output LSMeanDiffCL=diffcl4;
 class sex_c age_g;
 model para_LBWnorm=sex_c age_g sex_c*age_g;
  lsmeans sex_c age_g sex_c*age_g/out=_lsout alpha=0.10 CL pdiff stderr;
  output out=result4 r=res p=fitted LCLM=LCLM UCLM=UCLM;
run;
quit;

Title2 '*** normalization by FFM **';
proc glm data=pp_select;
 ods output LSMeanDiffCL=diffcl5;
 class sex_c age_g;
 model para_FFMnorm=sex_c age_g sex_c*age_g;
  lsmeans sex_c age_g sex_c*age_g/out=_lsout alpha=0.10 CL pdiff stderr;
  output out=result5 r=res p=fitted LCLM=LCLM UCLM=UCLM;
run;
quit;

Title2 '*** normalization by vd **';
proc glm data=pp_select;
 ods output LSMeanDiffCL=diffcl6;
 class sex_c age_g;
 model para_vdnorm=sex_c age_g sex_c*age_g;
  lsmeans sex_c age_g sex_c*age_g/out=_lsout alpha=0.10 CL pdiff stderr;
  output out=result6 r=res p=fitted LCLM=LCLM UCLM=UCLM;
run;
quit;
```

```
********** Summary of LSmeans and corresponding confidence intervals**;
Title2 '*** SUMMARY OF ANOVA FOR ALL NORMAIZATIONS **';

data result1;
 set result1;
 norm='0_without';
run;
data result2;
 set result2;
 norm='1_BW';
run;
data result3;
 set result3;
 norm='2_HT';
run;
data result4;
 set result4;
 norm='3_LBW';
run;
data result5;
 set result5;
 norm='4_FFM';
run;
data result6;
 set result6;
 norm='5_vd';
run;
data allres;
 set result1 result2 result3 result4 result5 result6;
 keep age_g sex_c norm fitted LCLM UCLM;
run;

proc sort data=allres nodupkey;by norm sex_c descending age_g;run;
```

```
data allres;
 set allres;
 ggg=age_g||sex_c;
 mean=exp(fitted);
 llim=exp(LCLM);
 ulim=exp(UCLM);
run;

proc print data=allres; run;

data _null_;
 set allres;
 file
'N:\Sites\Monheim\NewMedicines\GED\SP\CSchaefer\LCM\Vergleich599_620\Statistics\cmaxallstatH.xxx';
 put age_g sex_c ggg norm mean llim ulim;
run;
********** Summary of Mean Differences and Corresponding Confidence Intervals**;
data diffcl1;
 set diffcl11;
 norm='0_without';
run;
data diffcl2;
 set diffcl12;
 norm='1_BW  ';
run;
data diffcl3;
 set diffcl13;
 norm='2_HT';
run;
data diffcl4;
 set diffcl14;
 norm='3_LBW';
run;
```

```
data diffcl5;
 set diffcl15;
 norm='4_FFM';
run;
data diffcl6;
 set diffcl6;
 norm='5_vd';
run;
data allrescl;
 set diffcl1 diffcl2 diffcl3 diffcl4 diffcl5 diffcl6;
run;

proc sort data=allres nodupkey;by norm sex_c descending age_g;run;

data allrescl;
 set allrescl;
 mean=exp(Difference)*100;
 llim=exp(LowerCL)*100;
 ulim=exp(UpperCL)*100;
run;
ods rtf close;
option nodate nonumber orientation=landscape;
ods rtf
file='N:\Sites\Monheim\NewMedicines\GED\SP\CSchaefer\LCM\Paper_AgeAndGende
r\Paper\Tabellen\ANOVA_001B.rtf' STARTPAGE=BYGROUP;

proc print data=allrescl; run;

data _null_;
 set allrescl;
 file
'N:\Sites\Monheim\NewMedicines\GED\SP\CSchaefer\LCM\Vergleich599_620\Statisti
cs\cmaxallstatclH.xxx';
 put effect dependent i j mean llim ulim;
run;
```

```
ods rtf close;
```

Program 11

```
*********Analysis of Variance for AUC(tau,ss)/ Statistical Evaluation of Age and Gender************************

************ Author: Carina Schäfer *05.08.2014************;


libname abc
'N:\Sites\Monheim\NewMedicines\GED\SP\CSchaefer\LCM\Vergleich599_620\SASdata\';


data pp;
 set abc.Ymalefemale_pp  abc.Femalefemale_pp abc.Malefemale_pp;
 if studyid='SP599' then ppstresn=ppstresn/2; else ppstresn=ppstresn;  *** parameter normalized to 100mg bid;
 keep studyid subjid pptest ppstresn arm armcd armdose sex_c heights weighkg age BMI LBW FFM;
run;


proc sort data=pp nodupkey; by studyid subjid pptest;run;


**************** Normalization and Log-transformation for PK Parameters**;


data pp_select;
 set pp;
 where pptest='AUC(0-tau)ss';
 para_dnorm=log(ppstresn);
 para_bwnorm=log(ppstresn*weighkg);
 para_HTnorm=log(ppstresn*heights);
 para_LBWnorm=log(ppstresn*LBW);
 para_FFMnorm=log(ppstresn*FFM);
 if age<60 then age_g='Y';else age_g='E';
 if sex_c=1 then vd=0.3625*weighkg+0.2239*heights*100-0.1387*age-14.47;
  else vd=0.2363*weighkg+0.1962*heights*100-0.0272*age-10.26;
 para_vdnorm=log(ppstresn*vd);
run;
```

```
********** ANOVA for PK parameters without as well with normalization****;
option nodate nonumber orientation=portrait;
ods rtf
file='N:\Sites\Monheim\NewMedicines\GED\SP\CSchaefer\LCM\Paper_AgeAndGende
r\Paper\Tabellen\ANOVA_002A.rtf' STARTPAGE=BYGROUP;

Title ' GLM with model AUC,ss=group';
Title2 '*** without normalization  ***';

proc Annotate=LSMeanDiffCL; run;
proc glm data=pp_select;
  ods output LSMeanDiffCL=diffcl1;
  class sex_c age_g;
  model para_dnorm=sex_c age_g sex_c*age_g;
   lsmeans sex_c age_g sex_c*age_g /alpha=0.10 CL pdiff stderr;
   output out=result1 r=res p=fitted LCLM=LCLM UCLM=UCLM;
run;
quit;

Title2 '*** normalization by body weight **';
proc glm data=pp_select;
  ods output LSMeanDiffCL=diffcl2;
  class sex_c age_g;
  model para_bwnorm=sex_c age_g sex_c*age_g;
   lsmeans sex_c age_g sex_c*age_g/out=_lsout alpha=0.10 CL pdiff stderr;
   output out=result2 r=res p=fitted LCLM=LCLM UCLM=UCLM;
run;
quit;

Title2 '*** normalization by height **';
proc glm data=pp_select;
  ods output LSMeanDiffCL=diffcl3;
  class sex_c age_g;
  model para_HTnorm=sex_c age_g sex_c*age_g;
   lsmeans sex_c age_g sex_c*age_g/out=_lsout alpha=0.10 CL pdiff stderr;
```

```
  output out=result3 r=res p=fitted LCLM=LCLM UCLM=UCLM;
run;
quit;

Title2 '*** normalization by LBW **';
proc glm data=pp_select;
 ods output LSMeanDiffCL=diffcl4;
 class sex_c age_g;
 model para_LBWnorm=sex_c age_g sex_c*age_g;
  lsmeans sex_c age_g sex_c*age_g/out=_lsout alpha=0.10 CL pdiff stderr;
  output out=result4 r=res p=fitted LCLM=LCLM UCLM=UCLM;
run;
quit;

Title2 '*** normalization by FFM **';
proc glm data=pp_select;
 ods output LSMeanDiffCL=diffcl5;
 class sex_c age_g;
 model para_FFMnorm=sex_c age_g sex_c*age_g;
  lsmeans sex_c age_g sex_c*age_g/out=_lsout alpha=0.10 CL pdiff stderr;
  output out=result5 r=res p=fitted LCLM=LCLM UCLM=UCLM;
run;
quit;

Title2 '*** normalization by vd **';
proc glm data=pp_select;
 ods output LSMeanDiffCL=diffcl6;
 class sex_c age_g;
 model para_vdnorm=sex_c age_g sex_c*age_g;
  lsmeans sex_c age_g sex_c*age_g/out=_lsout alpha=0.10 CL pdiff stderr;
  output out=result6 r=res p=fitted LCLM=LCLM UCLM=UCLM;
run;
quit;
```

```
********** Summary of LSmeans and corresponding confidence intervals*****;

Title2 '*** SUMMARY OF ANOVA FOR ALL NORMAIZATIONS **';

data result1;
 set result1;
 norm='0_without';
run;
data result2;
 set result2;
 norm='1_BW  ';
run;
data result3;
 set result3;
 norm='2_HT';
run;
data result4;
 set result4;
 norm='3_LBW';
run;
data result5;
 set result5;
 norm='4_FFM';
run;
data result6;
 set result6;
 norm='5_vd';
run;
data allres;
 set result1 result2 result3 result4 result5 result6;
 keep age_g sex_c norm fitted LCLM UCLM;
run;

proc sort data=allres nodupkey;by norm sex_c descending age_g;run;
```

```
data allres;
 set allres;
 ggg=age_g||sex_c;
 mean=exp(fitted);
 llim=exp(LCLM);
 ulim=exp(UCLM);
run;

proc print data=allres; run;

data _null_;
 set allres;
 file
'N:\Sites\Monheim\NewMedicines\GED\SP\CSchaefer\LCM\Vergleich599_620\Statistics\allstatHAUC.xxx';
 put age_g sex_c ggg norm mean llim ulim;
run;

********** Summary of mean differences and corresponding confidence intervals**;
data diffcl1;
 set diffcl11;
 norm='0_without';
run;
data diffcl2;
 set diffcl12;
 norm='1_BW';
run;
data diffcl3;
 set diffcl13;
 norm='2_HT';
run;
data diffcl4;
 set diffcl14;
 norm='3_LBW';
run;
```

```
data diffcl5;
 set diffcl5;
 norm='4_FFM';
run;
data diffcl6;
 set diffcl16;
 norm='5_vd';
run;
data allrescl;
 set diffcl1 diffcl2 diffcl3 diffcl4 diffcl5 diffcl6;
run;

proc sort data=allres nodupkey;by norm sex_c descending age_g;run;

data allrescl;
 set allrescl;
 mean=exp(Difference)*100;
 llim=exp(LowerCL)*100;
 ulim=exp(UpperCL)*100;
run;
ods rtf close;
option nodate nonumber orientation=landscape;
ods rtf
file='N:\Sites\Monheim\NewMedicines\GED\SP\CSchaefer\LCM\Paper_AgeAndGende
r\Paper\Tabellen\ANOVA_002B.rtf' STARTPAGE=BYGROUP;

proc print data=allrescl; run;

data _null_;
 set allrescl;
 file
'N:\Sites\Monheim\NewMedicines\GED\SP\CSchaefer\LCM\Vergleich599_620\Statisti
cs\allstatclHAUC.xxx';
 put effect dependent i j mean llim ulim;
run;
ods rtf close;
```

Appendix 5 SAS Program for Validation

Program 12

```
*****Generation of Sorce Data of 20 Subjects for PK Modeling  - Pharmacokinetic of
Unchanged Drug and Its Metabolite in Plasma as Well as Amount of Unchanged Drug
in Urine*******
************ Author: Carina Schäfer *14.04.2014***********;


%let
inpath=N:\Sites\Monheim\NewMedicines\GED\SP\CSchaefer\Validierung_PKmodeling
\Modeling_oral_urine_met\;
%let runnr=PK01_A;
libname lcm "&inpath.SASdata";


data params;
seed=4711007;
mvd=50; svd=10;
mka=2; ska=0.2;
mkm=0.07;skm=0.01;
mkren=0.05;skren=0.005;
mkme=0.04;skme=0.005;
mvdm=100;svdm=30;
do i=1 to 20;
vd=mvd+rannor(seed)*svd;
ka=mka+rannor(seed)*ska;
km=mkm+rannor(seed)*skm;
kren=mkren+rannor(seed)*skren;
kme=mkme+rannor(seed)*skme;
vdm=mvdm+rannor(seed)*svdm;
output;
end;
run;
data params;
 set params;
 if km<=0 then km=-km;
 if kme<=0 then kme=-kme;
```

```
 if kren<=0 then kren=-kren;
 if kme>kren or kme>0.02 then kme=kme/2;
 ke=km+kren;
 patid=i;
run;

*** nur zu Überprüfungszwecken  *****/;
proc summary data=params print mean std;
 var vd ka km kren kme vdm;
run;

**************** PK of Unchanged Drug in Central Compartment*************;
data p_bateman;
 set params;
 ti=1; ** plasma;
 dr=1; ** unchanged drug;
 keep patid vd ka km kren ti dr;
run;

data time_bateman;
 input time;
 cards;
 0.25
 0.5
 0.75
 1
 1.5
 2
 4
 6
 8
 12
 16
 24
```

```
 48
;
run;
data time;
set time_bateman;
do i=1 to 20;
 patid=i;
 output;
end;
run;
proc sort;by patid time;run;
data bateman;
 merge time p_bateman;
 by patid;
run;

data bateman;
 set bateman;
 seed=6542387;
 tlag=0;
 dose=100;
 y=ti*dr*(dose/Vd)*ka/(ka-(km+kren))*(exp(-(km+kren)*(time-tlag))-exp(-ka*(time-
tlag)));
 y_prop=y*(1+rannor(seed)*0.1);
run;

data lcm.batman;
 set bateman;
run;

****************** Renal Elimination *********************/;
data renal;
 set params;
 ti=0; ** urine;
 dr=1; ** unchanged drug;
```

```
  keep patid vd ka km kren ti dr;
run;

data time_renal;
  input time;
  cards;
  2
  4
  6
  8
  12
  16
  24
  36
  48
  72
;
run;
data time;
set time_renal;
do i=1 to 20;
  patid=i;
  output;
end;
run;
proc sort;by patid time;run;
data renal;
  merge time renal;
  by patid;
run;

data renal;
  set renal;
  seed=6542387;
```

```
 tlag=0;
 dose=100;
 y=(1-ti)*dr*dose*ka*kren/(km+kren)*(1/ka+exp(-(km+kren)*(time-tlag))/((km+kren)-
ka)-(km+kren)*exp(-ka*(time-tlag))/(ka*((km+kren)-ka)));
 y_prop=y*(1+rannor(seed)*0.1);
run;

data lcm.renal;
 set renal;
run;

****************** PK of Metabolite in Central Compartment****************/;
data p_meta;
 set params;
 ti=1; ** plasma;
 dr=0; ** metabolite;
 keep patid vdm ka km kren kme ti dr;
run;

data time_meta;
 input time;
 cards;
 0.25
 0.5
 0.75
 1
 1.5
 2
 4
 6
 8
 12
 16
 24
 36
```

```
 48
 72
 96
 120
;
run;
data time;
set time_meta;
do i=1 to 20;
 patid=i;
 output;
end;
run;
proc sort;by patid time;run;
data metabol;
 merge time p_meta;
 by patid;
run;

data metabol;
 set metabol;
 seed=6542387;
 tlag=0;
 dose=100;
 y=dose*ka*km/Vdm*(exp(-(kren+km)*(time))/(-(kren+km)+kme)/(-
(kren+km)+ka)+exp(-kme*(time-tlag))/(-kme+(kren+km))/(-kme+ka)+exp(-ka*(time-
tlag))/(-ka+kme)/(-ka+(kren+km)));
 y_prop=y*(1+rannor(seed)*0.1);
run;

data lcm.metabol;
 set metabol;
run;

data all1;
```

```
  set bateman renal metabol;
  keep patid ti dr time y y_prop;
run;
proc sort;by patid ti dr time;run;
data all2;
   merge all1 params;
   by patid;
run;
data lcm.all;
  set all2;
  keep patid ti dr time y y_prop vd ka ke km kren kme vdm;
run;

proc sort data=all2;by ti dr;run;

symbol v=dot h=0.5 i=rl c=black;
proc gplot data=all2;
  plot y_prop*y;
  by ti dr;
run;
```

Program 13

```
****PK Modeling With Simulated Data - Pharmacokinetic of Unchanged Drug and its Metabolite in Plasma as Well as Amount of Unchanged Drug in Urine*******

************ Author: Carina Schäfer *14.04.2014***********;

%let inpath=N:\Sites\Monheim\NewMedicines\GED\SP\CSchaefer\Validierung_PKmodeling\Modeling_oral_urine_met\;

%let runnr=PK02_C;

libname lcm "&inpath.SASdata";


data pkmod;

 set lcm.all;

 rename patid=subjid ka=ka_ref vd=vd_ref km=km_ref kren=kren_ref kme=kme_ref vdm=vdm_ref;

run;


*  ods output ParameterEstimates=Parms;


proc nlin data=pkmod method=marquardt convergeobj=0.0001;

 parms Vd=50 Vdm=100 kren=0.05 km=0.07 kme=0.04 ka=2;

 ods output  ANOVA=ANOVA ParameterEstimates=Param  EstSummary=Iter;

 bounds 0<Vd<100;

 bounds km>0;

 bounds kren>0;

 bounds 0.1>kme>0;

 bounds Vd>0;

 bounds 500>Vdm>0;

 _weight_=1/(y*y);

 tlag=0;

 *  ka=2;

 dose=100;

 model y_prop=ti*dr*(dose/Vd)*ka/(ka-(km+kren))*(exp(-(km+kren)*(time-tlag))-exp(-ka*(time-tlag)))

      -(ti-1)*dr*dose*ka*kren/(km+kren)*(1/ka+exp(-(km+kren)*(time-tlag))/((km+kren)-ka)-(km+kren)*exp(-ka*(time-tlag))/(ka*((km+kren)-ka)))
```

```
      -(dr-1)*ti*100*ka*km/Vdm*(exp(-(kren+km)*(time))/(-(kren+km)+kme)/(-
(kren+km)+ka)+exp(-kme*(time-tlag))/(-kme+(kren+km))/(-kme+ka)+exp(-ka*(time-
tlag))/(-ka+kme)/(-ka+(kren+km)));
  output out=parameter parms= Vd Vdm kren km kme ka PRED=PRED;
  by subjid;
run;

data parameter;
  set iter_res;
run;

proc sort data=parameter;by dr ti;run;

symbol v=dot h=0.3 i=rl c=black;

proc gplot data=parameter;
   plot pred*y;
   by dr ti;
run;

data parameter;
  set parameter;
  label time='Time[h]';
run;

proc sort data=parameter nodupkey out=paras;
by SUBJID;
run;

proc sort data=parameter nodupkey out=paras;
by SUBJID;
run;

data all;
 set paras;
```

```
diff_Vd=Vd_ref-Vd;
diff_ka=ka_ref-ka;
diff_km=km_ref-km;
diff_kren=kren_ref-kren;
diff_kme=kme_ref-kme;
diff_Vdm=Vdm_ref-Vdm;
diff_Vd_p=(diff_Vd/Vd_ref)*100;
diff_ka_p=(diff_ka/ka_ref)*100;
diff_km_p=(diff_km/km_ref)*100;
diff_kren_p=(diff_kren/kren_ref)*100;
diff_kme_p=(diff_kme/kme_ref)*100;
diff_Vdm_p=(diff_Vdm/Vdm_ref)*100;

run;

proc sort data=pkmod;
 by SUBJID ;
run;

data urine_parameter;
 set parameter;
 where ti=0;
 rename y=Ae_cum;
run;

data urine_parameter;
 set urine_parameter;
 label Ae_cum='Ae_cum[ug]';
run;

data Met_parameter;
 set parameter;
 where ti=1 and dr=0;
run;
```

```
data Met_parameter;
 set Met_parameter;
 rename y=Conc;
run;

data Met_parameter;
 set Met_parameter;
 label conc='Conc[ug/ml]';
run;

data Met_parameter;
 set Met_parameter;
 label time='Time[h]';
run;

data oral_parameter;
 set parameter;
 where ti=1 and dr=1;
run;

data oral_parameter;
 set oral_parameter;
 rename y=Conc;
run;

data oral_parameter;
 set oral_parameter;
 label conc='Conc[ug/ml]';
run;

data oral_parameter;
 set oral_parameter;
 label time='Time[h]';
run;
```

```
***************** Summary Statistics ******************************;
libname abc
'N:\Sites\Monheim\NewMedicines\GED\SP\CSchaefer\Validierung_PKmodeling\Mode
ling_oral_urine_met\SASdata\';

data abc.pkmod_02C;
 set parameter;
run;

data abc.Anova_02C;
set ANOVA;
run;

data abc.Param_02C;
set Param;
run;

data abc.Iter_02C;
set Iter;
run;

***************** Saving of Tables as JPEGS *****************;
options nodate nonumber orientation=portrait ;
title "Summary statistics of PK parameter of PK02_C";

ods rtf file="&inpath.TablesRTF\&runnr._DesStat.rtf";

goptions reset=goptions device=JPEG target=JPEG
        ftext='Arial' ftitle='Arial/bold'
        xmax=9 in ymax=7 in ;

proc summary data=all print mean Std min max n median;
 var Vd_ref ka_ref kren_ref kme_ref km_ref Vdm_ref Vd Vdm kren km kme ka
diff_Vd_p diff_ka_p diff_km_p diff_kren_p diff_kme_p diff_Vdm_p ;
run;
```

```
ods rtf close;

proc sort data= paras;
by SUBJID;
run;

options nodate nonumber orientation=portrait ;
title "Summary statistics of PK parameter of PK02_C";

ods rtf file="&inpath.TablesRTF\&runnr._DesStat_group.rtf";

goptions reset=goptions device=JPEG target=JPEG
         ftext='Arial' ftitle='Arial/bold'
         xmax=9 in ymax=7 in ;

proc summary data=para_summary print mean Std min max n median;
  var Vd_ref ka_ref kren_ref kme_ref km_ref Vdm_ref Vd Vdm kren km kme ka;
* by ARM;
run;

ods rtf close;

****************** Simulations ***************************;
proc sort data= parameter nodupkey out=para;
by SUBJID ti dr;
run;
data para;
  set para;
  tlag=0;
  keep SUBJID ka Vd Vdm ke tlag kren km kme ti dr;
run;

data sim;
set para;
```

```
dose=100;
do dr=0 to 1;
do ti=0 to 1;
do time=0.5 to 120 by 0.1;
 simy=0;
 if time>tlag then simy=ti*dr*(dose/Vd)*ka/(ka-(km+kren))*(exp(-(km+kren)*(time-
tlag))-exp(-ka*(time-tlag)))
     -(ti-1)*dr*dose*ka*kren/(km+kren)*(1/ka+exp(-(km+kren)*(time-
tlag))/((km+kren)-ka)-(km+kren)*exp(-ka*(time-tlag))/(ka*((km+kren)-ka)))
     -(dr-1)*ti*100*ka*km/Vdm*(exp(-(kren+km)*(time))/(-(kren+km)+kme)/(-
(kren+km)+ka)+exp(-kme*(time-tlag))/(-kme+(kren+km))/(-kme+ka)+exp(-ka*(time-
tlag))/(-ka+kme)/(-ka+(kren+km)));
 output;
end;
end;
end;
run;

********** Preparation Datasets for Following Presentations******************;
data sim_oral;
 set sim;
 where ti=1 and dr=1;
 keep SUBJID time simy;
run;

data Pkmod_oral;
 set Pkmod;
 where ti=1 and dr=1;
run;
proc sort data=Pkmod_oral;by SUBJID time;run;
proc sort data=sim_oral;by SUBJID time;run;
data sim_oral;
 set sim_oral pkmod_oral;
 by SUBJID time;
run;
```

```
proc sort data=sim_oral;
 by SUBJID ;
run;

data sim_oral;
 set sim_oral;
 rename y=Conc;
run;

data sim_oral;
 set sim_oral;
 label simy='Conc[ug/ml]';
run;

data sim_oral;
 set sim_oral;
 label time=time[h];
run;

data sim_urine;
 set sim;
 where ti=0 and dr=1;
run;

data Pkmod_urine;
 set Pkmod;
 where ti=0;
run;
proc sort data=Pkmod_urine nodupkey;by SUBJID time;run;
proc sort data=sim_urine nodupkey;by SUBJID time;run;
data sim_urine;
 set sim_urine Pkmod_urine;
 by SUBJID time;
run;
```

```
data sim_urine;
 set sim_urine;
 rename y=Ae_cum;
run;

data sim_urine;
 set sim_urine;
 label simy='Ae_cum[ug]';

proc sort data=sim_urine;
 by SUBJID time;
run;

data sim_met;
 set sim;
 where ti=1 and dr=0;
 keep SUBJID time simy;
run;

data Pkmod_met;
 set Pkmod;
 where ti=1 and dr=0;
run;
proc sort data=Pkmod_met nodupkey;by SUBJID time;run;
proc sort data=sim_met nodupkey;by SUBJID time;run;
data sim_Met;
 set sim_met pkmod_Met;
 by SUBJID time;
run;

proc sort data=sim_Met;
 by SUBJID;
run;
```

```
data sim_Met;
 set sim_Met;
 rename y=Conc;
run;

data sim_Met;
 set sim_Met;
 label simy='Conc[ug/ml]';
run;

data sim_Met;
 set sim_Met;
 label time=time[h];
run;

****************** Illustrations RTF Files **************************;
******** Simulated Concentration vs. Measured Concentration of Unchanged Drug**;
options nonumber nodate orientation=portrait;
ods rtf file="&inpath.FiguresJPEG\&runnr.oral.rtf";
goptions reset=goptions device=JPEG target=JPEG
         ftext='Arial' ftitle='Arial/bold'
         xmax=8 cm in ymax=7 cm in ;
title "Simulated concentration vs. measured concentration of unchanged drug";
symbol1 v=none i=join c=black l=2;
symbol2 v=dot i=none c=black;
axis2 logbase=10 order= 0.01 0.1 1 10;
axis1 order=0 to 48 by 12;
proc gplot data=sim_oral;
plot simy*time=1 conc*time=2 /overlay vaxis=axis2 haxis=axis1;
by SUBJID;
run;
ods rtf close;

options nonumber nodate orientation=portrait;
```

```
ods rtf file="&inpath.FiguresJPEG\&runnr.corr_oral.rtf";

goptions reset=goptions device=JPEG target=JPEG
        ftext='Arial' ftitle='Arial/bold'
        xmax=12 cm in ymax=10 cm in ;

title "PRED vs. conc PKmodeling with data of &runnr." font="Albany AMT";
symbol1 v=dot i=rl c=black;
proc reg data=oral_parameter;
  model pred=conc;
  plot pred*conc;
run;
ods rtf close;

****************** Illustrations RTF Files *************************;
******** Simulated Cumulative Amount of LCM Excereted in Urine vs. Measured
Cumulative Amount of Unchanged Drug Excreted in Urine **************;
options nonumber nodate orientation=portrait;
ods rtf file="&inpath.FiguresJPEG\&runnr.urine.rtf";
goptions reset=goptions device=JPEG target=JPEG
        ftext='Arial' ftitle='Arial/bold'
        xmax=8 cm in ymax=7 cm in ;
title "SCumulative amount of renal excretion of unchanged drug";
symbol1 v=none i=join c=black l=2;
symbol2 v=dot i=none c=black;
axis2 logbase=10 order= 0.01 0.1 1 10 100 ;
axis1 order=0 to 72 by 12;
proc gplot data=sim_urine;
plot simy*time=1 Ae_cum*time=2 /overlay vaxis=axis2 haxis=axis1;
by SUBJID;
run;
ods rtf close;

ods rtf file="&inpath.FiguresJPEG\&runnr.corr_urine.rtf";
```

```
goptions reset=goptions device=JPEG target=JPEG
        ftext='Arial' ftitle='Arial/bold'
        xmax=12 cm in ymax=10 cm in ;

title "PRED vs. amount excreted - PKmodeling with data of &runnr." font="Albany AMT";
symbol1 v=dot i=rl c=black;
proc reg data=urine_parameter;
 model pred=Ae_cum;
 plot pred*Ae_cum;
run;
ods rtf close;
****************** Illustrations as RTF Files ************************;
******** Simulated Concentration vs. Measured Concentration of the Metabolite***;
options nonumber nodate orientation=portrait;
ods rtf file="&inpath.FiguresJPEG\&runnr.met.rtf";
goptions reset=goptions device=JPEG target=JPEG
        ftext='Arial' ftitle='Arial/bold'
        xmax=8 cm in ymax=7 cm in ;
title "Simulated concentration vs. measured concentration of Metabolite";
symbol1 v=none i=join c=black l=2;
symbol2 v=dot i=none c=black;
axis2 logbase=10 order= 0.01 0.1 1 10;
axis1 order=0 to 120 by 12;
proc gplot data=sim_Met;
plot simy*time=1 Conc*time=2 /overlay vaxis=axis2 haxis=axis1;
by SUBJID;
run;
ods rtf close;



options nonumber nodate orientation=portrait;
```

```
ods rtf file="&inpath.FiguresJPEG\&runnr.corr_met.rtf";


goptions reset=goptions device=JPEG target=JPEG
        ftext='Arial' ftitle='Arial/bold'
        xmax=12 cm in ymax=10 cm in ;


title "PRED vs. conc (metabolite) PKmodeling with data of &runnr." font="Albany
AMT";
symbol1 v=dot i=rl c=black;
proc reg data=Met_parameter;
  model pred=conc;
  plot pred*conc;
run;
ods rtf close;
```

Appendix 6 Source Data of Plasma and Urine Data of the Fictive Study Population

Subject No	Time[h]	Tissue 1=Plasma 0=Urine	Drug 1=unchanged 0=metabol.	Conc. or Amount	'error' added	Predicted Conc. or Amount
1	2	0	1	6.5805016	6.7548671	6.4186194
	4	0	1	13.666312	12.754309	13.482238
	6	0	1	19.454179	17.924917	19.279149
	8	0	1	24.16524	25.361382	24.013516
	12	0	1	31.120493	32.464467	31.037003
	16	0	1	35.728023	33.205267	35.720834
	24	0	1	40.802282	38.43603	40.927433
	36	0	1	43.61691	48.603989	43.860759
	48	0	1	44.435161	42.79056	44.730731
	72	0	1	44.742192	46.698137	45.065273
	0.25	1	0	0.0037054	0.0038035	0.003614
	0.5	1	0	0.0124884	0.011655	0.0122877
	0.75	1	0	0.0240013	0.0221146	0.0237875
	1	1	0	0.036899	0.0387255	0.0367893
	1.5	1	0	0.0641069	0.0668754	0.0644881
	2	1	0	0.0911544	0.084718	0.0922501
	4	1	0	0.186938	0.1760969	0.1912656
	6	1	0	0.2625027	0.2925168	0.2696702
	8	1	0	0.3213879	0.3094929	0.3309036
	12	1	0	0.4011836	0.4187217	0.4141177
	16	1	0	0.445206	0.4686492	0.4601815
	24	1	0	0.4716557	0.5434739	0.487901
	36	1	0	0.4444546	0.4571001	0.4589531
	48	1	0	0.3952641	0.4328018	0.4066142
	72	1	0	0.2997426	0.2866505	0.3052383
	96	1	0	0.2249245	0.2022274	0.226507
	120	1	0	0.1685827	0.1813475	0.1678549
	0.25	1	1	0.8443269	0.8666993	0.8112923
	0.5	1	1	1.3082657	1.2209603	1.2736032
	0.75	1	1	1.5540694	1.4319065	1.5288303

	1	1	1	1.6750013	1.7579113	1.6613683
	1.5	1	1	1.7338591	1.8087377	1.7387897
	2	1	1	1.6940928	1.5744729	1.70935
	4	1	1	1.3990543	1.3179188	1.4226996
	6	1	1	1.1389486	1.2691739	1.1622362
	8	1	1	0.9270071	0.8926974	0.949121
	12	1	1	0.6140987	0.6409446	0.6329509
	16	1	1	0.4068116	0.4282331	0.422103
	24	1	1	0.1785269	0.2057109	0.187722
	48	1	1	0.0150881	0.0155174	0.0165122
2	2	0	1	5.6616258	5.9597493	5.9841212
	4	0	1	11.776574	13.569772	12.347364
	6	0	1	16.812388	17.290731	17.596729
	8	0	1	20.946171	22.935397	21.917461
	12	0	1	27.124602	25.939854	28.400865
	16	0	1	31.287495	28.130282	32.793087
	24	0	1	35.982223	38.706741	37.784421
	36	0	1	38.691593	44.591162	40.701921
	48	0	1	39.520336	38.045307	41.60902
	72	0	1	39.851371	44.90694	41.97874
	0.25	1	0	0.0026773	0.0030856	0.002821
	0.5	1	0	0.0090058	0.0086697	0.0094024
	0.75	1	0	0.0172805	0.0194727	0.0179083
	1	1	0	0.0265324	0.0254734	0.0273337
	1.5	1	0	0.0460098	0.0484471	0.0469986
	2	1	0	0.0653418	0.0598742	0.0663871
	4	1	0	0.1337091	0.1388534	0.1347143
	6	1	0	0.1875442	0.2065876	0.1885745
	8	1	0	0.2293359	0.2489187	0.2304722
	12	1	0	0.2853077	0.2881399	0.2867571
	16	1	0	0.3150733	0.3281333	0.3168297
	24	1	0	0.3291375	0.2783958	0.3312268
	36	1	0	0.3010786	0.2871876	0.3029608
	48	1	0	0.2581392	0.2563523	0.2593994

	72	1	0	0.1802269	0.1716148	0.1802549
	96	1	0	0.1240098	0.1210195	0.1233294
	120	1	0	0.0851607	0.090346	0.0841982
	0.25	1	1	0.7003161	0.7668241	0.7427142
	0.5	1	1	1.0826284	1.0353414	1.13286
	0.75	1	1	1.2840024	1.154434	1.3297548
	1	1	1	1.382595	1.4872829	1.4209076
	1.5	1	1	1.4305265	1.6486485	1.4565679
	2	1	1	1.3988784	1.3466677	1.4184993
	4	1	1	1.1639066	1.3115606	1.1792989
	6	1	1	0.9555563	0.9174158	0.9707516
	8	1	1	0.7843609	0.8259109	0.7990044
	12	1	1	0.5284854	0.4842632	0.5412904
	16	1	1	0.3560821	0.3697819	0.3667005
	24	1	1	0.1616532	0.1780675	0.168296
	48	1	1	0.0151247	0.0164162	0.016269
3	2	0	1	7.1843542	6.8975949	7.0926307
	4	0	1	14.807724	15.592134	14.719584
	6	0	1	20.89807	19.149375	20.832832
	8	0	1	25.74457	26.735061	25.709639
	12	0	1	32.669652	35.986942	32.702762
	16	0	1	37.054372	40.218412	37.152351
	24	0	1	41.588448	42.001286	41.784969
	36	0	1	43.852347	45.670063	44.124547
	48	0	1	44.427002	37.577878	44.727223
	72	0	1	44.609896	42.551702	44.922465
	0.25	1	0	0.0034077	0.0031023	0.0033174
	0.5	1	0	0.0114867	0.0109381	0.0112406
	0.75	1	0	0.0220679	0.0227449	0.0216887
	1	1	0	0.0339002	0.0321036	0.033437
	1.5	1	0	0.0587475	0.063169	0.0582584
	2	1	0	0.0832461	0.0757581	0.0828603
	4	1	0	0.1677006	0.1826637	0.1681262
	6	1	0	0.2308649	0.212442	0.2321794

	8	1	0	0.276986	0.2918466	0.279145
	12	1	0	0.3319654	0.3721346	0.3355826
	16	1	0	0.3537408	0.3609077	0.3584589
	24	1	0	0.3457854	0.3199409	0.3517372
	36	1	0	0.2892663	0.2799863	0.2955009
	48	1	0	0.2286709	0.2377301	0.2343672
	72	1	0	0.1372524	0.1499312	0.1414296
	96	1	0	0.0815734	0.0870801	0.0844709
	120	1	0	0.0484301	0.0481527	0.0503941
	0.25	1	1	0.9634605	0.9730245	0.9127088
	0.5	1	1	1.4948136	1.5567748	1.4269888
	0.75	1	1	1.7764533	1.5025849	1.7063756
	1	1	1	1.9140854	1.8257741	1.8475523
	1.5	1	1	1.976572	1.9628902	1.9205093
	2	1	1	1.9235277	1.8316122	1.875991
	4	1	1	1.5551027	1.5176038	1.5244705
	6	1	1	1.2377336	1.3130975	1.2164468
	8	1	1	0.9848893	0.8966267	0.9703319
	12	1	1	0.6235975	0.5938187	0.6174035
	16	1	1	0.3948401	0.4069529	0.3928419
	24	1	1	0.1582906	0.1499018	0.1590433
	48	1	1	0.010199	0.0109666	0.0105538
4	2	0	1	7.1625567	7.1129775	6.962605
	4	0	1	15.056419	14.33695	14.795044
	6	0	1	21.253999	20.741492	20.936989
	8	0	1	26.074046	27.661659	25.69258
	12	0	1	32.735191	29.801571	32.220661
	16	0	1	36.761673	35.006184	36.129922
	24	0	1	40.666798	41.914363	39.872817
	36	0	1	42.418626	40.170615	41.516093
	48	0	1	42.805547	46.027163	41.868981
	72	0	1	42.90988	39.050117	41.961037
	0.25	1	0	0.0034925	0.0030508	0.0033772
	0.5	1	0	0.0120326	0.012755	0.0117284

	0.75	1	0	0.0235374	0.0267304	0.0230988
	1	1	0	0.0366952	0.0345335	0.0362194
	1.5	1	0	0.0649832	0.0629142	0.0647172
	2	1	0	0.093374	0.0841882	0.0935795
	4	1	0	0.1916696	0.1909034	0.1942628
	6	1	0	0.2635657	0.2379819	0.2678925
	8	1	0	0.3143844	0.3019873	0.3197397
	12	1	0	0.3712965	0.3882887	0.3774236
	16	1	0	0.3899199	0.4023742	0.3959828
	24	1	0	0.3717187	0.3733727	0.3770387
	36	1	0	0.3025495	0.3328439	0.3070011
	48	1	0	0.2345687	0.2792659	0.2385392
	72	1	0	0.1367609	0.1399442	0.1399697
	96	1	0	0.0792318	0.0811971	0.0816659
	120	1	0	0.0458782	0.0443864	0.047627
	0.25	1	1	0.6507816	0.5922435	0.6391315
	0.5	1	1	1.0428593	1.1359083	1.0363461
	0.75	1	1	1.2716866	1.1702067	1.2762172
	1	1	1	1.3977089	1.4726974	1.413954
	1.5	1	1	1.480372	1.6595031	1.514638
	2	1	1	1.4574618	1.4869902	1.5009745
	4	1	1	1.1709256	1.0834089	1.2111642
	6	1	1	0.9113489	0.8821116	0.9388961
	8	1	1	0.7085795	0.7366514	0.7266151
	12	1	1	0.4283176	0.4678838	0.4351257
	16	1	1	0.2589064	0.276384	0.2605695
	24	1	1	0.0946011	0.0940592	0.0934418
	48	1	1	0.0046148	0.0040312	0.0043092
5	2	0	1	6.8935433	7.5086193	7.1984204
	4	0	1	14.171959	13.041044	14.50221
	6	0	1	19.834251	20.898378	20.222109
	8	0	1	24.214862	27.14496	24.691194
	12	0	1	30.224803	30.837161	30.910996
	16	0	1	33.821058	31.293223	34.707784

24	0	1	37.260691	36.065321	38.44028
36	0	1	38.7682	40.304083	40.152947
48	0	1	39.091199	42.702276	40.542523
72	0	1	39.175233	41.819779	40.651296
0.25	1	0	0.0072535	0.0060478	0.0072987
0.5	1	0	0.0246209	0.0249802	0.0242326
0.75	1	0	0.0475616	0.0501281	0.0459794
1	1	0	0.0733733	0.0721475	0.0699163
1.5	1	0	0.1278586	0.1386966	0.1193361
2	1	0	0.1816713	0.1674739	0.1673592
4	1	0	0.3653453	0.4192215	0.330383
6	1	0	0.4990943	0.3782311	0.4506117
8	1	0	0.5937445	0.5487694	0.5373658
12	1	0	0.7004626	0.5313809	0.6391289
16	1	0	0.7367504	0.6198104	0.6784612
24	1	0	0.7075674	0.7194388	0.6635462
36	1	0	0.5858894	0.5859722	0.5633736
48	1	0	0.4638447	0.3802746	0.4564333
72	1	0	0.2830672	0.3092724	0.2910388
96	1	0	0.1718527	0.1606096	0.1844845
120	1	0	0.1042926	0.1288954	0.1168854
0.25	1	1	0.8287831	0.8785396	0.8915748
0.5	1	1	1.2986591	1.4748275	1.3519953
0.75	1	1	1.5545445	1.4629678	1.5774875
1	1	1	1.6831502	1.6295606	1.6753208
1.5	1	1	1.7451686	1.5734859	1.6959348
2	1	1	1.696372	1.6895909	1.6305946
4	1	1	1.3401737	1.2100859	1.2870187
6	1	1	1.0371558	0.9962578	1.0056565
8	1	1	0.8023027	0.8390197	0.785724
12	1	1	0.4800856	0.4954198	0.4796338
16	1	1	0.2872758	0.2885541	0.2927855
24	1	1	0.1028631	0.1131628	0.1091011
48	1	1	0.0047222	0.005622	0.005645

6	2	0	1	6.7715632	6.7327702	6.628217
	4	0	1	14.131195	12.343979	13.989971
	6	0	1	20.018327	21.220139	19.816544
	8	0	1	24.700055	28.050718	24.384506
	12	0	1	31.38273	29.534005	30.77043
	16	0	1	35.607848	34.474135	34.692885
	24	0	1	39.968123	36.03622	38.582072
	36	0	1	42.137952	41.96951	40.392347
	48	0	1	42.686344	38.542872	40.811862
	72	0	1	42.859971	41.169881	40.93161
	0.25	1	0	0.0047142	0.0044899	0.0045403
	0.5	1	0	0.0160635	0.017642	0.0156585
	0.75	1	0	0.0311401	0.026341	0.0306608
	1	1	0	0.0481944	0.0419958	0.0478469
	1.5	1	0	0.0844617	0.0990388	0.0848892
	2	1	0	0.1206061	0.1146392	0.1221936
	4	1	0	0.2464664	0.2591928	0.2524989
	6	1	0	0.3412482	0.3642732	0.3494067
	8	1	0	0.4109191	0.4485996	0.4193394
	12	1	0	0.495261	0.5148044	0.5011789
	16	1	0	0.5304559	0.5354447	0.5323999
	24	1	0	0.5240166	0.4581848	0.5188274
	36	1	0	0.4460628	0.4446864	0.4356526
	48	1	0	0.3593489	0.3235452	0.3480238
	72	1	0	0.2244485	0.2026326	0.2151238
	96	1	0	0.1389242	0.1449942	0.1320741
	120	1	0	0.0859082	0.0800484	0.0810383
	0.25	1	1	1.225904	1.2544389	1.1739983
	0.5	1	1	1.9322409	1.9801689	1.8836952
	0.75	1	1	2.3255171	2.2499034	2.2998427
	1	1	1	2.5305224	2.1098711	2.530754
	1.5	1	1	2.6474646	2.6860942	2.6865124
	2	1	1	2.5942202	2.7342132	2.6506602
	4	1	1	2.1089819	2.0737491	2.1470493

	6	1	1	1.6777615	1.8199776	1.6845392
	8	1	1	1.3340719	1.2298156	1.3202738
	12	1	1	0.8434665	0.9678495	0.8109586
	16	1	1	0.5332813	0.4041392	0.4981187
	24	1	1	0.2131735	0.197026	0.1879322
	48	1	1	0.0136165	0.0103296	0.0100927
7	2	0	1	8.3467789	8.7287652	8.340032
	4	0	1	17.481021	18.039374	17.80829
	6	0	1	24.80941	24.919805	25.422465
	8	0	1	30.652217	33.721432	31.481612
	12	0	1	39.022836	46.45866	40.135447
	16	0	1	44.342101	45.374235	45.611162
	24	0	1	49.870354	51.107355	51.268245
	36	0	1	52.653992	50.941958	54.088579
	48	0	1	53.368315	44.496846	54.80307
	72	0	1	53.59866	54.380728	55.029932
	0.25	1	0	0.0024539	0.0027315	0.0023785
	0.5	1	0	0.0083789	0.0079022	0.0082359
	0.75	1	0	0.0162715	0.017858	0.0161834
	1	1	0	0.0252204	0.0236634	0.0253326
	1.5	1	0	0.0443026	0.035518	0.0451753
	2	1	0	0.0633692	0.0669733	0.0652899
	4	1	0	0.1299843	0.1288342	0.1363039
	6	1	0	0.1803333	0.21308	0.1898232
	8	1	0	0.2174784	0.1992924	0.2289345
	12	1	0	0.262748	0.2399934	0.2756234
	16	1	0	0.2819715	0.3005164	0.2942252
	24	1	0	0.2794003	0.3354029	0.2878877
	36	1	0	0.2385822	0.2562074	0.2407128
	48	1	0	0.1926081	0.1991441	0.190074
	72	1	0	0.1206601	0.1177523	0.1137845
	96	1	0	0.0748706	0.0641234	0.0674413
	120	1	0	0.0464111	0.0381713	0.0399304
	0.25	1	1	0.6977737	0.5870202	0.6462152

	0.5	1	1	1.103198	1.1217072	1.0433704
	0.75	1	1	1.3311364	1.3313247	1.2809471
	1	1	1	1.4515217	1.1900033	1.4164483
	1.5	1	1	1.5232584	1.6642756	1.5158046
	2	1	1	1.4955907	1.3977443	1.5051127
	4	1	1	1.2205139	1.508434	1.241114
	6	1	1	0.9734832	0.9271776	0.9885296
	8	1	1	0.7760348	0.8522936	0.7863688
	12	1	1	0.4931459	0.4171458	0.4975757
	16	1	1	0.3133787	0.2730727	0.3148409
	24	1	1	0.1265487	0.1483894	0.1260536
	48	1	1	0.0083334	0.0079211	0.00809
8	2	0	1	6.3723801	6.7162554	6.2551499
	4	0	1	13.795628	13.565157	13.206
	6	0	1	19.649935	21.31557	18.653798
	8	0	1	24.183111	22.293227	22.877532
	12	0	1	30.401711	34.884944	28.687749
	16	0	1	34.121956	25.858811	32.177385
	24	0	1	37.679025	34.82491	35.532068
	36	0	1	39.23716	29.76587	37.014904
	48	0	1	39.570769	33.289933	37.336164
	72	0	1	39.657491	40.322856	37.420845
	0.25	1	0	0.0026678	0.0030296	0.0029053
	0.5	1	0	0.0093644	0.0096659	0.0100475
	0.75	1	0	0.0186153	0.0164825	0.0197184
	1	1	0	0.0294253	0.0319785	0.0308267
	1.5	1	0	0.0532624	0.0636386	0.0548304
	2	1	0	0.0777592	0.0765813	0.0790371
	4	1	0	0.1646336	0.1355366	0.163314
	6	1	0	0.2287518	0.2159295	0.2252643
	8	1	0	0.2741039	0.2549635	0.2693096
	12	1	0	0.3250607	0.3067433	0.319433
	16	1	0	0.3421686	0.3454845	0.3370537
	24	1	0	0.3276753	0.3659152	0.3250083

	36	1	0	0.2691317	0.2954555	0.2701448
	48	1	0	0.2110291	0.2362565	0.2144779
	72	1	0	0.1261847	0.1334604	0.1315236
	96	1	0	0.0750406	0.084387	0.0802196
	120	1	0	0.0446071	0.0437228	0.0489077
	0.25	1	1	0.7885372	0.8292538	0.8501266
	0.5	1	1	1.2990393	1.3866892	1.3695718
	0.75	1	1	1.621542	1.7702343	1.6774292
	1	1	1	1.8171547	1.8888611	1.8501762
	1.5	1	1	1.9802542	1.9988779	1.969164
	2	1	1	1.9843882	1.7350909	1.9438269
	4	1	1	1.6245098	1.6194971	1.5628032
	6	1	1	1.2605477	1.1349531	1.212785
	8	1	1	0.9751687	0.8803844	0.9399387
	12	1	1	0.583395	0.6088851	0.5645313
	16	1	1	0.3490122	0.325206	0.3390593
	24	1	1	0.1249098	0.1390418	0.1223069
	48	1	1	0.0057262	0.0054004	0.0057409
9	2	0	1	6.4716337	6.4725491	6.5325181
	4	0	1	14.028537	11.501038	14.090372
	6	0	1	20.210778	22.081811	20.265234
	8	0	1	25.205982	23.556924	25.254206
	12	0	1	32.497613	40.163823	32.537176
	16	0	1	37.252781	35.480778	37.287129
	24	0	1	42.376129	46.540318	42.405507
	36	0	1	45.115902	38.162969	45.143203
	48	0	1	45.875769	39.975343	45.902702
	72	0	1	46.144966	54.109007	46.171857
	0.25	1	0	0.0024375	0.0023956	0.0024647
	0.5	1	0	0.0084961	0.0092895	0.0085649
	0.75	1	0	0.0167939	0.0190311	0.0168858
	1	1	0	0.0264284	0.0263551	0.026514
	1.5	1	0	0.0475604	0.0467503	0.047548
	2	1	0	0.0692563	0.0692387	0.0690648

	4	1	0	0.1477489	0.1591005	0.1466454
	6	1	0	0.2091604	0.2095526	0.2073031
	8	1	0	0.2560236	0.2824558	0.2536319
	12	1	0	0.3170039	0.2921012	0.3140403
	16	1	0	0.3476996	0.2774625	0.3446076
	24	1	0	0.3586818	0.3189626	0.3560094
	36	1	0	0.3240367	0.3240461	0.3225126
	48	1	0	0.2761084	0.2655099	0.2756578
	72	1	0	0.1921766	0.1721616	0.1931167
	96	1	0	0.1323586	0.1520793	0.1338923
	120	1	0	0.0910535	0.0921578	0.0927242
	0.25	1	1	0.672541	0.7381163	0.6967325
	0.5	1	1	1.0958012	1.0281525	1.1300653
	0.75	1	1	1.3560731	1.0871802	1.3931177
	1	1	1	1.5099271	1.5958038	1.5462471
	1.5	1	1	1.6339303	1.6194731	1.6654389
	2	1	1	1.6360064	1.9330892	1.6627035
	4	1	1	1.3770686	1.2619153	1.3946693
	6	1	1	1.1139651	1.0174934	1.1279386
	8	1	1	0.89965	0.958819	0.9109611
	12	1	1	0.5866998	0.7042972	0.5941298
	16	1	1	0.3826106	0.4108758	0.3874912
	24	1	1	0.1627192	0.168241	0.164825
	48	1	1	0.0125166	0.0122149	0.0126855
10	2	0	1	6.2831639	5.9723082	6.0582049
	4	0	1	12.923336	13.59064	12.732161
	6	0	1	18.133016	19.356502	17.973011
	8	0	1	22.200228	24.235947	22.050114
	12	0	1	27.853684	28.952812	27.686829
	16	0	1	31.298778	31.593134	31.096003
	24	0	1	34.67745	30.320947	34.405006
	36	0	1	36.221585	36.109816	35.891354
	48	0	1	36.571003	32.927253	36.220197
	72	0	1	36.667965	33.103917	36.309048

	0.25	1	0	0.0036188	0.0031826	0.0034004
	0.5	1	0	0.0122523	0.0124345	0.0117126
	0.75	1	0	0.0236202	0.0252568	0.0229071
	1	1	0	0.0363798	0.0345677	0.0357068
	1.5	1	0	0.0632512	0.0587833	0.0632174
	2	1	0	0.0897537	0.0845833	0.0908214
	4	1	0	0.1803216	0.1912453	0.1863748
	6	1	0	0.2465942	0.2386847	0.2562646
	8	1	0	0.2937312	0.2645111	0.3056663
	12	1	0	0.3472007	0.4538656	0.3609852
	16	1	0	0.3654706	0.3880865	0.3790668
	24	1	0	0.3503318	0.3285935	0.3612871
	36	1	0	0.2874004	0.2883691	0.2938908
	48	1	0	0.2244226	0.2369259	0.2276893
	72	1	0	0.1325925	0.1423259	0.1325325
	96	1	0	0.077814	0.0811043	0.0766519
	120	1	0	0.0456397	0.0399391	0.0443087
	0.25	1	1	1.0358823	0.8871873	0.9393462
	0.5	1	1	1.6175612	1.3303814	1.504795
	0.75	1	1	1.9313084	2.1932049	1.834504
	1	1	1	2.0873698	2.1545791	2.0158849
	1.5	1	1	2.1607452	1.9131809	2.1345206
	2	1	1	2.1006593	2.2829349	2.101199
	4	1	1	1.6715271	1.9971601	1.6881928
	6	1	1	1.3053172	1.2855447	1.3142982
	8	1	1	1.0189729	0.8388821	1.0221641
	12	1	1	0.6209401	0.5861343	0.6182215
	16	1	1	0.3783874	0.3519649	0.37391
	24	1	1	0.1405111	0.1325932	0.136777
	48	1	1	0.007195	0.0072648	0.006695
11	2	0	1	8.3299572	8.6939161	8.521913
	4	0	1	17.189094	16.01662	17.40785
	6	0	1	24.200198	26.938159	24.435072
	8	0	1	29.722212	28.031262	29.973641

12	0	1	37.495849	41.151838	37.778912
16	0	1	42.317172	39.704743	42.62704
24	0	1	47.162028	37.810369	47.508835
36	0	1	49.468142	52.281629	49.840509
48	0	1	50.018325	49.575757	50.39927
72	0	1	50.1809	59.293261	50.565259
0.25	1	0	0.0022785	0.0021208	0.0022147
0.5	1	0	0.007715	0.0066498	0.0074304
0.75	1	0	0.0148755	0.0152246	0.0142195
1	1	0	0.022917	0.0239894	0.0217731
1.5	1	0	0.0398723	0.0381463	0.0375471
2	1	0	0.05663	0.0459375	0.0530224
4	1	0	0.1142791	0.1140922	0.1060662
6	1	0	0.1570194	0.1203391	0.1455287
8	1	0	0.1879032	0.1592289	0.1742325
12	1	0	0.2240642	0.2334132	0.2083426
16	1	0	0.2377589	0.2506598	0.2219234
24	1	0	0.2311488	0.1818737	0.21771
36	1	0	0.192965	0.2033043	0.1845464
48	1	0	0.1529289	0.1454341	0.1486632
72	1	0	0.0927855	0.1129403	0.0932924
96	1	0	0.0558607	0.0603911	0.0581144
120	1	0	0.033606	0.0312742	0.0361766
0.25	1	1	0.6075961	0.678503	0.6688457
0.5	1	1	0.9487989	1.041601	1.0301763
0.75	1	1	1.1330953	1.2685504	1.2169356
1	1	1	1.2251739	1.2958165	1.3048251
1.5	1	1	1.2698908	1.4280565	1.3378268
2	1	1	1.2367033	1.2121874	1.2956815
4	1	1	0.99235	0.9752819	1.0355019
6	1	1	0.7817862	0.8547915	0.81626
8	1	1	0.6156897	0.6977063	0.6433137
12	1	1	0.3818598	0.3808005	0.3995848
16	1	1	0.236835	0.2328013	0.2481962

	24	1	1	0.0911024	0.0910792	0.0957563
	48	1	1	0.0051854	0.0055838	0.005499
12	2	0	1	7.457601	6.8339808	7.3440999
	4	0	1	14.952229	13.657335	14.83449
	6	0	1	20.654948	22.013401	20.558422
	8	0	1	24.979875	29.986807	24.914796
	12	0	1	30.747069	33.0185	30.753197
	16	0	1	34.063863	35.219791	34.134602
	24	0	1	37.068449	36.175136	37.227235
	36	0	1	38.270918	32.777345	38.484958
	48	0	1	38.499654	31.664474	38.729298
	72	0	1	38.551442	43.779238	38.785988
	0.25	1	0	0.0077627	0.0075269	0.0078865
	0.5	1	0	0.0259595	0.0253336	0.0265232
	0.75	1	0	0.0495336	0.0509019	0.0508456
	1	1	0	0.075646	0.0742496	0.0779466
	1.5	1	0	0.1298462	0.1411153	0.1345557
	2	1	0	0.1826354	0.2050713	0.1899837
	4	1	0	0.3608522	0.3746376	0.3780289
	6	1	0	0.490514	0.508047	0.5153013
	8	1	0	0.5830428	0.6092874	0.6135
	12	1	0	0.6909906	0.7921984	0.7284198
	16	1	0	0.7342803	0.6459183	0.7746819
	24	1	0	0.7285995	0.8491048	0.7685391
	36	1	0	0.6460116	0.6828752	0.6794735
	48	1	0	0.5540461	0.5538447	0.5802731
	72	1	0	0.4010454	0.4687002	0.4160088
	96	1	0	0.2895997	0.2777713	0.2974542
	120	1	0	0.2090984	0.2099421	0.2126557
	0.25	1	1	0.7921059	0.7935913	0.7424202
	0.5	1	1	1.2126527	1.3378487	1.1462552
	0.75	1	1	1.4242183	1.3123367	1.3553922
	1	1	1	1.518613	1.2118456	1.4528987
	1.5	1	1	1.5406442	1.3700385	1.4845152

	2	1	1	1.4771197	1.4771622	1.4290699
	4	1	1	1.1355113	1.0919245	1.105245
	6	1	1	0.8612883	0.771586	0.8413431
	8	1	1	0.6531706	0.7504894	0.6402898
	12	1	1	0.3756476	0.3802035	0.3708343
	16	1	1	0.2160402	0.1900003	0.2147747
	24	1	1	0.0714566	0.0725189	0.0720426
	48	1	1	0.0025856	0.0027648	0.002719
13	2	0	1	6.5124124	6.7220996	6.2234765
	4	0	1	13.322791	11.796351	13.008054
	6	0	1	18.814262	20.446788	18.489564
	8	0	1	23.231265	27.756986	22.894935
	12	0	1	29.641407	29.192408	29.279851
	16	0	1	33.788291	27.81663	33.402947
	24	0	1	38.206538	36.064934	37.784791
	36	0	1	40.525516	37.695656	40.075004
	48	0	1	41.153371	38.834361	40.691713
	72	0	1	41.369384	41.77028	40.902499
	0.25	1	0	0.004225	0.0037588	0.0040186
	0.5	1	0	0.0140854	0.0147231	0.0136662
	0.75	1	0	0.0268228	0.0259894	0.026451
	1	1	0	0.0409185	0.0426595	0.0408875
	1.5	1	0	0.070229	0.074105	0.0715422
	2	1	0	0.0989524	0.0926414	0.1020792
	4	1	0	0.1982524	0.2138565	0.2087504
	6	1	0	0.2737255	0.3038133	0.2897481
	8	1	0	0.3300003	0.3310127	0.3498398
	12	1	0	0.3998889	0.4334198	0.4236731
	16	1	0	0.4309245	0.4786363	0.4554574
	24	1	0	0.4303158	0.4054908	0.4517392
	36	1	0	0.3705201	0.4015714	0.384254
	48	1	0	0.3006693	0.2973678	0.307717
	72	1	0	0.1894687	0.1913083	0.188636
	96	1	0	0.1180495	0.1140078	0.1142866

	120	1	0	0.073454	0.0695168	0.0691448
	0.25	1	1	1.0053664	0.9552888	0.9395022
	0.5	1	1	1.5344284	1.4260423	1.4767881
	0.75	1	1	1.8007756	1.6970383	1.7738911
	1	1	1	1.922523	2.0389874	1.927826
	1.5	1	1	1.9642508	1.9012475	2.0151914
	2	1	1	1.9041011	1.7146828	1.9760947
	4	1	1	1.5481753	2.0237964	1.6206523
	6	1	1	1.2453693	1.322435	1.3028793
	8	1	1	1.0016727	0.9395181	1.0469887
	12	1	1	0.6480075	0.6501917	0.6760989
	16	1	1	0.4192125	0.4425682	0.4365947
	24	1	1	0.1754457	0.1883249	0.1820601
	48	1	1	0.0128608	0.0134046	0.0132015
14	2	0	1	6.4681226	7.2229573	6.937674
	4	0	1	13.58453	14.913235	14.616291
	6	0	1	19.26284	21.565604	20.723381
	8	0	1	23.760312	25.130313	25.539686
	12	0	1	30.142068	33.896281	32.331018
	16	0	1	34.143914	33.467059	36.552693
	24	0	1	38.226996	37.569502	40.808338
	36	0	1	40.220589	43.976496	42.844665
	48	0	1	40.712171	46.135476	43.333809
	72	0	1	40.863276	40.749917	43.479529
	0.25	1	0	0.0030579	0.0026842	0.0030209
	0.5	1	0	0.0104805	0.0112714	0.0103877
	0.75	1	0	0.0204146	0.0203057	0.0202894
	1	1	0	0.031719	0.0367758	0.0315953
	1.5	1	0	0.0559073	0.0534172	0.0558734
	2	1	0	0.0801225	0.0694905	0.0802424
	4	1	0	0.1644447	0.1616074	0.1650932
	6	1	0	0.2273767	0.2188417	0.2280611
	8	1	0	0.2730234	0.2928626	0.2733614
	12	1	0	0.3267496	0.3138917	0.3258056

	16	1	0	0.3472846	0.3928645	0.3448306
	24	1	0	0.337618	0.3249491	0.3325973
	36	1	0	0.2804378	0.2515029	0.273396
	48	1	0	0.2204181	0.2012198	0.2128927
	72	1	0	0.1310333	0.1389883	0.1244069
	96	1	0	0.0771908	0.073534	0.0720799
	120	1	0	0.0454302	0.0395412	0.0417268
	0.25	1	1	0.7424747	0.6497355	0.6860839
	0.5	1	1	1.1806872	1.0989469	1.0964336
	0.75	1	1	1.4311896	1.233578	1.334381
	1	1	1	1.5661043	1.6028535	1.4647375
	1.5	1	1	1.6506436	1.7278886	1.5500947
	2	1	1	1.6234926	1.5532142	1.5275214
	4	1	1	1.3205841	1.0712422	1.2410121
	6	1	1	1.046499	1.0447881	0.979335
	8	1	1	0.8287191	0.6351272	0.7721613
	12	1	1	0.51967	0.4403676	0.4799965
	16	1	1	0.3258725	0.3394695	0.2983787
	24	1	1	0.1281409	0.1350939	0.1152994
	48	1	1	0.0077913	0.0061304	0.0066528
15	2	0	1	6.7201548	6.6056983	6.5578514
	4	0	1	13.763072	13.759567	13.399119
	6	0	1	19.258977	20.738644	18.738763
	8	0	1	23.527739	23.571859	22.888589
	12	0	1	29.417928	32.455077	28.619599
	16	0	1	32.970795	30.380724	32.0806
	24	0	1	36.406471	29.052182	35.432968
	36	0	1	37.940156	33.738792	36.933597
	48	0	1	38.276734	38.277835	37.264107
	72	0	1	38.366808	36.894092	37.352934
	0.25	1	0	0.0126276	0.0116268	0.012817
	0.5	1	0	0.0426858	0.0430371	0.0432285
	0.75	1	0	0.0821853	0.0721144	0.0830758
	1	1	0	0.1264546	0.1472955	0.1276306

	1.5	1	0	0.2195616	0.2475629	0.2211053
	2	1	0	0.3113379	0.3229905	0.3130589
	4	1	0	0.6256762	0.6434749	0.6275458
	6	1	0	0.8575535	0.7270899	0.8594643
	8	1	0	1.0246427	0.9638904	1.0265795
	12	1	0	1.2207638	1.2449231	1.2226481
	16	1	0	1.297583	1.4229108	1.2992487
	24	1	0	1.2739825	1.2638007	1.2748341
	36	1	0	1.0919185	1.2860199	1.0912518
	48	1	0	0.8955298	0.8110519	0.8935867
	72	1	0	0.5869595	0.4692757	0.5836793
	96	1	0	0.3828122	0.4199849	0.3793333
	120	1	0	0.2495773	0.2495663	0.2464362
	0.25	1	1	0.7539079	0.7943032	0.7873772
	0.5	1	1	1.1736916	1.116171	1.2217483
	0.75	1	1	1.397769	1.701391	1.4511818
	1	1	1	1.5074948	1.6297548	1.561933
	1.5	1	1	1.5553993	1.4474759	1.607355
	2	1	1	1.5086173	1.4627939	1.556976
	4	1	1	1.1931982	1.1644297	1.2306426
	6	1	1	0.9270038	0.9526107	0.9566258
	8	1	1	0.7199611	0.7066716	0.7434118
	12	1	1	0.4342691	0.4719584	0.4489521
	16	1	1	0.2619442	0.2941228	0.2711256
	24	1	1	0.0953034	0.0989442	0.0988807
	48	1	1	0.0045899	0.004754	0.0047966
16	2	0	1	7.1677657	6.4212503	7.0366915
	4	0	1	15.101484	17.351521	14.960348
	6	0	1	21.43662	21.696607	21.285695
	8	0	1	26.454908	23.266218	26.283351
	12	0	1	33.576538	34.07566	33.348026
	16	0	1	38.043075	40.67892	37.754831
	24	0	1	42.601321	40.479337	42.218405
	36	0	1	44.827763	41.661303	44.37137

	48	0	1	45.377033	42.762999	44.893925
	72	0	1	45.545969	48.305095	45.05154
	0.25	1	0	0.0042297	0.0041118	0.0040051
	0.5	1	0	0.0145289	0.013831	0.0138505
	0.75	1	0	0.0283534	0.0206798	0.0271845
	1	1	0	0.0441229	0.0439427	0.0425076
	1.5	1	0	0.0779566	0.0683421	0.0756577
	2	1	0	0.1119066	0.1222036	0.1091596
	4	1	0	0.2303442	0.2352538	0.2266309
	6	1	0	0.3187361	0.3290367	0.3141394
	8	1	0	0.3827881	0.3307062	0.3772212
	12	1	0	0.4580009	0.5187857	0.4504982
	16	1	0	0.4865082	0.4636523	0.4773366
	24	1	0	0.4722269	0.5100129	0.4608604
	36	1	0	0.3911134	0.353853	0.3787741
	48	1	0	0.3064135	0.3049495	0.2946106
	72	1	0	0.1809106	0.1821961	0.1715672
	96	1	0	0.1058303	0.0883754	0.0990217
	120	1	0	0.0618502	0.0589273	0.0570994
	0.25	1	1	0.8051007	0.8413409	0.8077629
	0.5	1	1	1.2845533	1.4726989	1.3018922
	0.75	1	1	1.5613832	1.3734891	1.5957299
	1	1	1	1.7123705	1.9955848	1.7618849
	1.5	1	1	1.8104439	1.9137541	1.8804543
	2	1	1	1.7839064	1.7832579	1.8628583
	4	1	1	1.4538988	1.6991662	1.5242372
	6	1	1	1.1523586	1.1052915	1.2053443
	8	1	1	0.9126281	0.9163108	0.9520208
	12	1	1	0.5723816	0.5092134	0.5938527
	16	1	1	0.3589857	0.3752396	0.3704336
	24	1	1	0.1412083	0.1368209	0.1441364
	48	1	1	0.0085943	0.0089599	0.0084911
17	2	0	1	8.4617392	8.1903289	8.8742532
	4	0	1	17.609514	15.857735	18.177629

6	0	1	24.772103	32.382438	25.424524
8	0	1	30.339118	32.216555	31.042354
12	0	1	38.026786	35.667195	38.772306
16	0	1	42.668967	42.812785	43.416705
24	0	1	47.164825	49.792525	47.883829
36	0	1	49.176859	52.786866	49.860113
48	0	1	49.619873	51.717992	50.288768
72	0	1	49.738895	43.526235	50.401909
0.25	1	0	0.0024862	0.002569	0.0025485
0.5	1	0	0.0085074	0.011678	0.008619
0.75	1	0	0.0165461	0.0154022	0.0166002
1	1	0	0.0256712	0.0228949	0.0255468
1.5	1	0	0.0451258	0.0408032	0.0443581
2	1	0	0.0645105	0.0608488	0.0628811
4	1	0	0.1312532	0.1210019	0.1260213
6	1	0	0.1801104	0.1372484	0.1720948
8	1	0	0.2147982	0.2204248	0.2048104
12	1	0	0.2540879	0.2279703	0.2419494
16	1	0	0.2675667	0.2419478	0.254867
24	1	0	0.2569135	0.2718369	0.2454678
36	1	0	0.2118495	0.2111436	0.2039934
48	1	0	0.1666269	0.1797152	0.1620043
72	1	0	0.1001213	0.0946225	0.099427
96	1	0	0.0598054	0.059542	0.0606955
120	1	0	0.0357064	0.0368572	0.0370365
0.25	1	1	0.8870905	0.93605	0.9711231
0.5	1	1	1.4069465	1.3172134	1.5135259
0.75	1	1	1.7010515	1.8349383	1.8039933
1	1	1	1.8566829	2.0607691	1.9467754
1.5	1	1	1.9472042	1.953178	2.0099073
2	1	1	1.9058828	2.0656923	1.9496633
4	1	1	1.5210763	1.6894894	1.5398255
6	1	1	1.1828345	1.1145966	1.1939889
8	1	1	0.9191705	0.9962013	0.9255067

	12	1	1	0.5550393	0.5489447	0.5560738
	16	1	1	0.3351592	0.3384132	0.3341067
	24	1	1	0.1222098	0.1180257	0.1206121
	48	1	1	0.0059248	0.0056072	0.0056742
18	2	0	1	7.1161671	6.6235066	6.5847785
	4	0	1	14.705526	12.67506	13.710625
	6	0	1	20.579329	21.06223	19.20842
	8	0	1	25.093422	26.267717	23.411459
	12	0	1	31.227019	29.875252	29.078724
	16	0	1	34.848237	28.268477	32.388957
	24	0	1	38.248385	38.185853	35.451805
	36	0	1	39.693293	30.420789	36.721807
	48	0	1	39.990636	33.888005	36.974888
	72	0	1	40.064417	41.736102	37.035371
	0.25	1	0	0.0033018	0.002773	0.003212
	0.5	1	0	0.0112737	0.0107567	0.0110405
	0.75	1	0	0.021884	0.0232441	0.0215507
	1	1	0	0.0338943	0.0334713	0.0335322
	1.5	1	0	0.0594051	0.0687542	0.0591755
	2	1	0	0.0847168	0.0864715	0.0847714
	4	1	0	0.171146	0.1799073	0.1723437
	6	1	0	0.2336021	0.2357432	0.2351572
	8	1	0	0.2773166	0.2732192	0.2785906
	12	1	0	0.3255215	0.3484744	0.325226
	16	1	0	0.3406862	0.2907301	0.3383678
	24	1	0	0.3243182	0.3131108	0.318375
	36	1	0	0.2657045	0.2697179	0.256576
	48	1	0	0.2085147	0.1864733	0.1982306
	72	1	0	0.1252732	0.1054585	0.1155371
	96	1	0	0.0749167	0.058095	0.0670481
	120	1	0	0.0447875	0.0462072	0.0388975
	0.25	1	1	0.47351	0.4156392	0.4638652
	0.5	1	1	0.748496	0.8049801	0.7406519
	0.75	1	1	0.9022563	0.8974428	0.9001338

	1	1	1	0.9821694	1.1387504	0.9862335
	1.5	1	1	1.0253425	0.9796739	1.038611
	2	1	1	0.999747	0.867084	1.0172943
	4	1	1	0.7881436	0.7745452	0.8026425
	6	1	1	0.6059838	0.583237	0.6140519
	8	1	1	0.465627	0.4994617	0.4693122
	12	1	1	0.274902	0.2640843	0.2741242
	16	1	1	0.1622995	0.1836008	0.1601151
	24	1	1	0.0565713	0.0544485	0.0546264
	48	1	1	0.0023957	0.0021485	0.0021693
19	2	0	1	6.414482	6.7625345	6.5226356
	4	0	1	13.434256	10.570413	13.573083
	6	0	1	18.839797	19.849257	18.982251
	8	0	1	22.956851	21.831774	23.092169
	12	0	1	28.477516	34.663374	28.584985
	16	0	1	31.677259	34.246331	31.753868
	24	0	1	34.606696	32.205465	34.636741
	36	0	1	35.80006	34.712654	35.798764
	48	0	1	36.032411	35.163654	36.021889
	72	0	1	36.086458	37.083285	36.072959
	0.25	1	0	0.0065448	0.0069735	0.0063014
	0.5	1	0	0.022617	0.0210098	0.0216978
	0.75	1	0	0.0443544	0.0400692	0.0424219
	1	1	0	0.0692944	0.073709	0.0661036
	1.5	1	0	0.1230966	0.1401943	0.1169554
	2	1	0	0.1772387	0.1660768	0.1679162
	4	1	0	0.364888	0.3027227	0.3438986
	6	1	0	0.5020455	0.516099	0.472519
	8	1	0	0.5993182	0.5147549	0.5639743
	12	1	0	0.7107433	0.6536256	0.669582
	16	1	0	0.7523811	0.7656261	0.710373
	24	1	0	0.7364106	0.5762923	0.699739
	36	1	0	0.6351338	0.637271	0.6110442
	48	1	0	0.5280265	0.4043772	0.5151583

	72	1	0	0.3581732	0.3560969	0.3598404
	96	1	0	0.2422271	0.2575787	0.2506725
	120	1	0	0.1637869	0.1847073	0.1745989
	0.25	1	1	0.9704814	0.8859526	0.9798879
	0.5	1	1	1.5605742	1.655316	1.5670098
	0.75	1	1	1.9077166	1.8173404	1.9066428
	1	1	1	2.1000461	1.8278236	2.0907048
	1.5	1	1	2.2260166	2.0495915	2.2033172
	2	1	1	2.1881241	2.2061319	2.1576893
	4	1	1	1.7286403	1.5168153	1.6944653
	6	1	1	1.3178342	1.5350257	1.2884612
	8	1	1	1.0033327	1.1312904	0.9786858
	12	1	1	0.5815264	0.6032914	0.5646184
	16	1	1	0.337049	0.346637	0.3257363
	24	1	1	0.1132244	0.0959991	0.1084148
	48	1	1	0.0042922	0.0040377	0.0039972
20	2	0	1	6.0729593	5.9608602	6.4778633
	4	0	1	12.900712	14.02034	13.745851
	6	0	1	18.311547	20.561039	19.473176
	8	0	1	22.552319	23.41387	23.934627
	12	0	1	28.477418	29.495321	30.113147
	16	0	1	32.113873	33.559423	33.858766
	24	0	1	35.715456	40.946618	37.506061
	36	0	1	37.388734	32.889438	39.153209
	48	0	1	37.775559	44.023376	39.520195
	72	0	1	37.885658	40.047545	39.620178
	0.25	1	0	0.0025215	0.0022582	0.0025998
	0.5	1	0	0.0087341	0.0092991	0.009012
	0.75	1	0	0.0171664	0.0182946	0.0177232
	1	1	0	0.0268744	0.0280805	0.0277592
	1.5	1	0	0.047921	0.0468055	0.0495306
	2	1	0	0.0692323	0.0696264	0.0715807
	4	1	0	0.1441202	0.1462749	0.1489612
	6	1	0	0.2000458	0.2198362	0.2065288

8	1	0	0.2405452	0.2161067	0.2480448
12	1	0	0.2883253	0.3190218	0.2967434
16	1	0	0.3070891	0.3336485	0.3156991
24	1	0	0.3008752	0.3509051	0.3093136
36	1	0	0.2548937	0.2651714	0.2631538
48	1	0	0.2055829	0.2187839	0.2137656
72	1	0	0.1296442	0.1266804	0.1371763
96	1	0	0.0812257	0.085591	0.0875442
120	1	0	0.050862	0.0572723	0.0558458
0.25	1	1	0.6784612	0.6918881	0.6727659
0.5	1	1	1.0955639	1.2013795	1.0874084
0.75	1	1	1.344748	1.3340006	1.3356517
1	1	1	1.4862475	1.7504455	1.4768329
1.5	1	1	1.5876785	1.4379082	1.5779645
2	1	1	1.5724493	1.2571774	1.5621254
4	1	1	1.278882	1.4030669	1.2643252
6	1	1	1.0032988	1.0032545	0.9858925
8	1	1	0.7860401	0.7641213	0.7676704
12	1	1	0.482423	0.4592484	0.4653876
16	1	1	0.2960809	0.2159491	0.282133
24	1	1	0.1115258	0.1110702	0.1036888
48	1	1	0.0059603	0.0052252	0.0051471

Appendix 7 List of Generated Reference Pharmacokinetic Parameters for Each Subject of the Fictive Study Population

Subject No	Vd	ka	km	krent	kme	Vdm
1	49.648199	2.2143263	0.0568595	0.0460919	0.0120203	88.143406
2	60.538842	2.2464392	0.0593411	0.0393724	0.0156721	128.84997
3	42.954921	2.1812214	0.0632722	0.0509839	0.0217296	105.13021
4	55.382437	1.8263312	0.071841	0.0540087	0.0227682	100.10564
5	47.606361	2.053098	0.0780819	0.0502985	0.0208112	57.869396
6	31.772092	2.0139164	0.0654791	0.0491387	0.0200315	73.55747
7	55.189417	1.9839798	0.0525767	0.0607715	0.0199309	112.05095
8	40.019802	1.5485144	0.0774982	0.0509412	0.0216738	122.4723
9	50.467329	1.6881496	0.057533	0.0493409	0.0155923	107.56276
10	38.729882	2.0969073	0.078417	0.0454121	0.0222333	118.61088
11	66.237014	2.1039181	0.0594835	0.059939	0.0211764	143.3636
12	53.901768	2.2843861	0.0849787	0.0533181	0.0135709	64.318954
13	43.794237	2.3689302	0.063819	0.045062	0.0197759	91.629494
14	50.521355	1.9189099	0.0689846	0.0476874	0.0220922	114.62823
15	53.730846	2.1247098	0.0778883	0.048495	0.0178252	34.142565
16	45.944191	1.8847814	0.0634974	0.0531334	0.0223847	75.11871
17	42.441147	1.9311633	0.0633758	0.0627325	0.021492	130.12886
18	80.26251	1.9566554	0.0789565	0.0527863	0.0214363	123.38238
19	36.266573	1.775434	0.0871477	0.0492093	0.016305	63.22284
20	51.501648	1.7550828	0.0758012	0.0462459	0.019507	141.45837

Appendix 8 List of Iterated Pharmacokinetic Parameters for Each Subject of the Fictive Study Population

Subject No	Vd	ka	km	krent	kme	Vdm
1	49.258249	2.077793	0.0556085	0.0456774	0.0124941	83.812192
2	59.872391	2.3961377	0.0564467	0.0409059	0.0159185	122.68794
3	44.094737	2.1015418	0.0622378	0.050791	0.0215282	102.98343
4	53.366304	1.7045232	0.0743952	0.0537961	0.0224698	100.9878
5	49.979203	2.4147573	0.0732264	0.0501693	0.0190173	61.750121
6	30.627031	1.8195802	0.0719634	0.0498813	0.0203541	76.933808
7	54.43748	1.7677715	0.051437	0.0629831	0.0218437	102.44048
8	41.354764	1.7702857	0.0797551	0.0477001	0.0206195	130.06509
9	49.761046	1.7335505	0.057492	0.0493591	0.0153146	108.77829
10	38.426925	1.829782	0.0800588	0.0456491	0.0228393	114.7659
11	63.320136	2.2516023	0.0588408	0.0602108	0.019753	154.41862
12	55.860784	2.1961645	0.0835794	0.052962	0.0139831	60.272729
13	42.071247	2.0509976	0.0645954	0.0447382	0.0209457	86.477704
14	53.450564	1.8648074	0.0671662	0.0516877	0.0227802	110.22189
15	52.098817	2.160347	0.0789819	0.0471016	0.0179726	34.588562
16	43.772238	1.7802009	0.0648217	0.0531676	0.0229437	77.102148
17	41.509349	2.1114123	0.0631611	0.0641991	0.0205833	136.42332
18	78.392268	1.8497699	0.084635	0.0497871	0.0226875	129.54813
19	36.772012	1.829347	0.0879069	0.0496086	0.0150693	67.964976
20	51.581611	1.7408839	0.0755425	0.0495805	0.0187328	135.74774

Appendix 9 Evaluation of Study Data SP641

Program 14

```
**************Generation of Demographic Datasets of Study SP641**********;
************* Author: Carina Schäfer *11.02.2015************;


libname abc
'N:\Sites\Monheim\NewMedicines\GED\SP\CSchaefer\LCM\SP641\Doktorarbeit\SASd
ata\';


data crea;
 set abc.Crea;
 newPatID=input(PT,5.0);
 *drop PT;
 rename newPatID=PatID;
run;


data crea;
 set crea;
 where substr(Pt,3,1) ne '5';
run;
proc sort data=crea;
 by PatID;
run;


data hwbinfo;
  set abc.hwbinfo;
  newPatID=input(PT,5.0);
  drop PT;
  rename newPatID=PatID;
run;


proc sort data=hwbinfo;
 by PatID;
run;
```

```
data demog;
 merge crea hwbinfo;
 by PatID;
run;

data demog;
  set demog;
  where SEX_C ne .;
run;

data abc.demog;
  set demog;
run;

option nodate nonumber orientation=Portrait;

ods rtf
file='N:\Sites\Monheim\NewMedicines\GED\SP\CSchaefer\LCM\SP641\Doktorarbeit\
TablesRTF\DemographicData\demoq.rtf';

*****Listing of Demographic Parameters******;
Title 'Listing of demographic parameters';

PROC REPORT NOWINDOWS DATA=demog MISSING SPACING=3 HEADLINE HEADSKIP
SPLIT='|';
          * by PatID;
 COLUMN PatID ("- parameter -" SEX_L AGER HEIGHM HEIGHCM WEIGHKG BMIR CCR
);

DEFINE PatID      /order order=internal  left   format=6.   width=6  'Pat.|Number';
DEFINE SEX_L      /display            center format=$1.  width=4  'Sex';
DEFINE AGER       /display            center format=5.0  width=7  'Age|(years)';
DEFINE HEIGHM     /display              center format=5.2  width=6  'Height|(m)';
DEFINE HEIGHCM    /display               center format=3.2  width=6  'Height|(cm)';
DEFINE WEIGHKG    /display               center format=5.1  width=6  'Weight|(kg)';
DEFINE BMIR       /display            center format=5.3  width=6  'BMI|(kg/m2)';
```

```
DEFINE CCR       /display          center format=5.4  width=6  'CreaCL|(ml/min)';
run;

data abc.demog;
 set demog;
run;

data demog1;
 set demog;
run;

PROC transpose data=demog1 out=demog1;
 by PatID;
 var AGER HEIGHM HEIGHCM WEIGHKG BMIR CCR;
run;
data demog1;
 set demog1;
 rename _name_=parameter;
run;

PROC sort data=demog1;
 by parameter;
run;

proc univariate data=demog1 noprint;
 by parameter;
 Var col1;
 output out=demog2 mean=mean
                n=n
              std=std
              min=min
           median=med
              max=max;
run;
```

```
data demog2;
 set demog2;
 Label parameter='Parameter';
run;

proc sort data=demog2;
 by parameter;
run;
data demog2;
 set demog2;
 if parameter='AGER' then parameter='Age(years)';
 if parameter='HEIGHM' then parameter='Height(m)';
 if parameter='HEIGHCM' then parameter='Height(cm)';
 if parameter='BMIR' then parameter='BMI (kg/m2)';
 if parameter='CCR' then parameter='CreaClearance(ml/min)';
 if parameter='WEIGHKG' then parameter='Weight(kg)';
run;

Title 'Results of descriptive statistics - demographic parameters';

PROC REPORT NOWINDOWS DATA=Demog2 MISSING SPACING=3 HEADLINE HEADSKIP
SPLIT='|';
 COLUMN parameter ("- Summary Statistics -" n mean std min med max);

DEFINE parameter  /order order=internal  left   format=$12.  width=16 'Parameter';
DEFINE n      /display           center format=3.    width=3  "N";
DEFINE mean   /display           center format=6.2   width=6  "Mean";
DEFINE std    /display           center format=6.2   width=9  "Std.-Dev.";
DEFINE min    /display           center format=5.1   width=7  "Minimum";
DEFINE med    /display           center format=6.2   width=6  "Median";
DEFINE max    /display           center format=5.1   width=7  "Maximum";
run;

ods rtf close;
```

Program 15

```
**************Merging of Plasma and Urine Data**********;
************ Author: Carina Schäfer *11.02.2015***********;
libname LCM
'N:\Sites\Monheim\NewMedicines\GED\SP\CSchaefer\LCM\SP641\Doktorarbeit\SASd
ata';


data pkmod_oral;
 set LCM.Sp641_pc;
 keep DOMAIN STUDYID SUBJID ARM ARMCD ARMDOSE ARMDOSU EXTRT TIMEPT_S
PCSPEC PCSTRESN PCSTRESU PCPARM;
run;


proc sort data=pkmod_oral;
 by subjid;
run;
data pkmod_oral;
 set pkmod_oral;
 where conc>0;
run;


data pkmod_urine;
 set LCM.SP641_urine;
 keep drugname treatno timept group timeint cuma aint;
run;
data pkmod_urine;
 set pkmod_urine;
 rename drugname=PCPARM Treatno=SUBJID timept=TIMEPT_S cuma=PCSTRESN;
run;


data pkmod_urine;
 set pkmod_urine;
 if group=1 then ARM="part 1 group 1";
 if group=2 then ARM="part 1 group 2";
 if group=3 then ARM="part 1 group 3";
```

```
 if group=4 then ARM="part 1 group 4";
 if group=5 then ARM="part 1 group 5";
run;

data pkmod_urine;
  set pkmod_urine;
  keep PCPARM SUBJID TIMEPT_S timeint PCSTRESN aint ARM;
run;

Proc sort data=pkmod_urine;
by subjid ;
run;

data all;
  set pkmod_oral pkmod_urine;
run;

data all;
  set all;
  where arm ne 'part 2';
run;

data all;
  set all;
  if PCSPEC='PLASMA' then ti=1;
  else ti=0;
  if PCPARM='SPM 927' then dr=1;
  else dr=0;
run;


data LCM.dataset_01;
  set all;
run;
```

Program 16

```
****PK modeling with SP641 Data - Pharmacokinetic of LCM and its Metabolite in
Plasma as Well as Amount of LCM Drug in Urine********;

************ Author: Carina Schäfer *18.03.2015***********;

%let
inpath=N:\Sites\Monheim\NewMedicines\GED\SP\CSchaefer\LCM\SP641\Doktorarbei
t\;

%let runnr=PK06C;

libname lcm "&inpath.SASdata";


********************* preparation dataset with PK parameter ka from
PK01*******************************************;


libname abz
'N:\Sites\Monheim\NewMedicines\GED\SP\CSchaefer\LCM\SP641\Doktorarbeit\SASd
ata\PK01\';


************ H:\F\PKmodeling\SP641   *****;

data parameter01;

 set abz.Pkmod_01;

 keep SUBJID Vd ka ke tlag;

run;

proc sort nodupkey;by SUBJID;run;

data parameter01;

 set parameter01;

 rename Vd=Vds ke=kes tlag=tlags;

run;


data pkmod01;

 set lcm.dataset_01;

 where dr=0;

run;


 data pkmod01;

  set pkmod01;

  where ti=1;
```

```
run;

data pkmod02;
 set lcm.dataset_01;
 where dr=1;
run;

data pkmod;
 set pkmod01 pkmod02;
run;

data pkmod;
 set pkmod;
 rename PCSTRESN=y timept_s=time;
 dose=100;
 ggg=substr(arm,length(arm),1);
run;

proc sort;
 by SUBJID;
run;

data pkmod;
  merge pkmod parameter01;
  by SUBJID;
run;

proc sort data=pkmod;
  by SUBJID time;
run;

proc sort data=pkmod;by subjid time dr ti;run;
data pkmod;
  set pkmod;
```

```
  by subjid;
  retain subnum 0;
  if first.subjid then subnum=subnum+1;
run;

data ccr;  set lcm.Crea;  keep pt ccr;run;
data ccr; set ccr; rename pt=subjid;run;
proc sort data=ccr;by subjid;run;

data pkmod;
 merge pkmod ccr;
 by subjid;
run;
data pkmod;
  set pkmod;
 where y>0;
   krens=0.02*ccr/100;
   kmes=0.1*ccr/100;
     kms=kes-krens;
     Vdms=Vds*kes/kms;
run;

data iter_res; set _null_;run;

%macro calc(ttt);
%do sub=1 %to 32;
data xxx;
 set pkmod; where (subnum=&sub);
 call symput('krens',left(put(krens,best6.)));
 call symput('kmes',left(put(kmes,best6.)));
 call symput('Vds',left(put(Vds,best6.)));
 call symput('kms',left(put(kms,best6.)));
 call symput('tlags',left(put(tlags,best6.)));
 call symput('Vdms',left(put(Vdms,best6.)));
```

```
run;

*  ods output ParameterEstimates=Parms;

proc nlin data=pkmod method=marquardt convergeobj=0.0001;
 where (subnum=&sub);
 parms Vd=&Vds Vdm=&Vdms tlag=&tlags kren=&krens km=&kms kme=&kmes;
 ods output  ANOVA=ANOVA ParameterEstimates=Param  EstSummary=Iter;
 bounds 0<Vd<100;
 bounds km>0;
 bounds kren>0;
 bounds kme>0;
 bounds Vd>0;
 bounds Vdm>0;
 bounds tlag>0;
 _weight_=1/(y*y);
 model y=ti*dr*(dose/Vd)*ka/(ka-(km+kren))*(exp(-(km+kren)*(time-tlag))-exp(-
ka*(time-tlag)))
      -(ti-1)*kren*ka*dose/(kren+km)*(1/ka+1/((kren+km)-ka)*exp(-(kren+km)*(time-
tlag))-(kren+km)/ka/((kren+km)-ka)*exp(-ka*(time-tlag)))
      -(dr-1)*ti*100*ka*km/Vdm*(exp(-(kren+km)*(time))/(-(kren+km)+kme)/(-
(kren+km)+ka)+exp(-kme*(time-tlag))/(-kme+(kren+km))/(-kme+ka)+exp(-ka*(time-
tlag))/(-ka+kme)/(-ka+(kren+km)));
 output out=parameter parms= Vd Vdm tlag kren km kme PRED=PRED;
*  by subjid;
run;

data iter_res;
  set iter_res parameter;
run;
%end;
%mend;

%calc(1);

data parameter;
```

```
  set iter_res;
run;

proc sort data=parameter;by dr ti;run;

symbol v=dot h=0.3 i=rl c=black;

proc gplot data=parameter;
   plot pred*y;
   by dr ti;
run;

data parameter;
  set parameter;
  label time='Time[h]';
run;

proc sort data=parameter nodupkey out=paras;
by SUBJID;
run;

proc sort data=pkmod;
  by SUBJID ;
run;

data urine_parameter;
  set parameter;
  where ti=0;
  rename y=Ae_cum;
run;



data urine_parameter;
  set urine_parameter;
```

```
 label Ae_cum='Ae_cum[ug]';
run;

data Met_parameter;
 set parameter;
 where ti=1 and dr=0;
run;

data Met_parameter;
 set Met_parameter;
 rename y=Conc;
run;

data Met_parameter;
 set Met_parameter;
 label conc='Conc[ug/ml]';
run;

data Met_parameter;
 set Met_parameter;
 label time='Time[h]';
run;

data LCM_parameter;
 set parameter;
 where ti=1 and dr=1;
run;

data LCM_parameter;
 set LCM_parameter;
 rename y=Conc;
run;

data LCM_parameter;
```

```
 set LCM_parameter;
 label conc='Conc[ug/ml]';
run;

data LCM_parameter;
 set LCM_parameter;
 label time='Time[h]';
run;

***************** Statistics ******************************;

libname abc
'N:\Sites\Monheim\NewMedicines\GED\SP\CSchaefer\LCM\SP641\Doktorarbeit\SASd
ata\PK06\';

data abc.pkmod_06C;
 set parameter;
run;

data abc.Anova_C;
set ANOVA;
run;

data abc.Param_C;
set Param;
run;

data abc.Iter_C;
set Iter;
run;

*************** Saving of Tables as JPEGS ****************;

proc sort data=parameter nodupkey out=para_summary;
by SUBJID;
```

```
run;
options nodate nonumber orientation=portrait ;
title "Summary statistics of PK parameter of PK06_C";

ods rtf file="&inpath.TablesRTF\PK06\&runnr._DesStat.rtf";

goptions reset=goptions device=JPEG target=JPEG
        ftext='Arial' ftitle='Arial/bold'
        xmax=9 in ymax=7 in ;



proc summary data=para_summary print mean Std min max n median;
  var Vds ka kes tlags krens kmes kms Vdms Vd Vdm tlag kren km kme;
run;

ods rtf close;

proc sort data= para_summary;
by ARM;
run;

options nodate nonumber orientation=portrait ;
title "Summary statistics of PK parameter of PK06_C";

ods rtf file="&inpath.TablesRTF\PK06\&runnr._DesStat_group.rtf";

goptions reset=goptions device=JPEG target=JPEG
        ftext='Arial' ftitle='Arial/bold'
        xmax=9 in ymax=7 in ;

proc summary data=para_summary print mean Std min max n median;
var Vds ka kes tlags krens kmes kms Vdms Vd Vdm tlag kren km kme;
by ARM;
run;
```

```
ods rtf close;

****************** Simulations ***************************;
proc sort data= parameter nodupkey out=para;
by SUBJID ti dr;
run;
data para;
  set para;
  tlag=0;
  keep SUBJID ka Vd Vdm ke tlag kren km kme ti dr;
run;

data sim;
set para;
dose=100;
do dr=0 to 1;
do ti=0 to 1;
do time=0.5 to 96 by 0.1;
 simy=0;
 if time>tlag then simy=ti*dr*(dose/Vd)*ka/(ka-(km+kren))*(exp(-(km+kren)*(time-
tlag))-exp(-ka*(time-tlag)))
      -(ti-1)*kren*ka*dose/(kren+km)*(1/ka+1/((kren+km)-ka)*exp(-(kren+km)*(time-
tlag))-(kren+km)/ka/((kren+km)-ka)*exp(-ka*(time-tlag)))
      -(dr-1)*ti*100*ka*km/Vdm*(exp(-(kren+km)*(time))/(-(kren+km)+kme)/(-
(kren+km)+ka)+exp(-kme*(time-tlag))/(-kme+(kren+km))/(-kme+ka)+exp(-ka*(time-
tlag))/(-ka+kme)/(-ka+(kren+km)));

 output;
end;
end;
end;
run;
```

```
**********Preparation Datasets for Following Presentations***************;
data sim_oral;
 set sim;
 where ti=1 and dr=1;
 keep SUBJID time simy;
run;

data Pkmod_oral;
 set Pkmod;
 where ti=1 and dr=1;
run;
proc sort data=Pkmod_oral;by SUBJID time;run;
proc sort data=sim_oral;by SUBJID time;run;
data sim_oral;
 set sim_oral pkmod_oral;
 by SUBJID time;
run;

proc sort data=sim_oral;
 by SUBJID ;
run;

data sim_oral;
 set sim_oral;
 rename y=Conc;
run;

data sim_oral;
 set sim_oral;
 label simy='Conc[ug/ml]';
run;

data sim_oral;
 set sim_oral;
```

```
 label time=time[h];
run;

data sim_urine;
 set sim;
 where ti=0 and dr=1;
run;

data Pkmod_urine;
 set Pkmod;
 where ti=0;
run;
proc sort data=Pkmod_urine nodupkey;by SUBJID time;run;
proc sort data=sim_urine nodupkey;by SUBJID time;run;
data sim_urine;
 set sim_urine Pkmod_urine;
 by SUBJID time;
run;

data sim_urine;
 set sim_urine;
 rename y=Ae_cum;
run;

data sim_urine;
 set sim_urine;
 label simy='Ae_cum[ug]';

proc sort data=sim_urine;
 by SUBJID time;
run;

data sim_met;
 set sim;
```

```
 where ti=1 and dr=0;
 keep SUBJID time simy;
run;

data Pkmod_met;
 set Pkmod;
 where ti=1 and dr=0;
run;
proc sort data=Pkmod_met nodupkey;by SUBJID time;run;
proc sort data=sim_met nodupkey;by SUBJID time;run;
data sim_Met;
 set sim_met pkmod_Met;
 by SUBJID time;
run;

proc sort data=sim_Met;
 by SUBJID;
run;

data sim_Met;
 set sim_Met;
 rename y=Conc;
run;

data sim_Met;
 set sim_Met;
 label simy='Conc[ug/ml]';
run;

data sim_Met;
 set sim_Met;
 label time=time[h];
run;
```

```
****************** Illustrations rtf files **************************;
******** Simulated Concentration vs. Measured Concentration of LCM********;
options nonumber nodate orientation=portrait;
ods rtf file="&inpath.FiguresJPEG\PK06\&runnr.oral.rtf";
goptions reset=goptions device=JPEG target=JPEG
        ftext='Arial' ftitle='Arial/bold'
        xmax=8 cm in ymax=7 cm in ;
title "Simulated concentration vs. measured concentration of LCM";
symbol1 v=none i=join c=black l=2;
symbol2 v=dot i=none c=black;
axis2 logbase=10 order= 0.01 0.1 1 10 100 ;
axis1 order=0 to 96 by 12;
proc gplot data=sim_oral;
plot simy*time=1 conc*time=2 /overlay vaxis=axis2 haxis=axis1;
by SUBJID;
run;
ods rtf close;

options nonumber nodate orientation=portrait;

ods rtf file="&inpath.FiguresJPEG\PK06\&runnr.corr_oral.rtf";

goptions reset=goptions device=JPEG target=JPEG
        ftext='Arial' ftitle='Arial/bold'
        xmax=12 cm in ymax=10 cm in ;

title "PRED vs. conc PKmodeling with data of &runnr." font="Albany AMT";
symbol1 v=dot i=rl c=black;
proc reg data=LCM_parameter;
  model pred=conc;
  plot pred*conc;
run;
ods rtf close;
```

```
****************** Illustrations RTF Files **************************;
******** Simulated Cumulative Amount of LCM Excereted in Urine vs. Measured
Cumulative Amount of LCM Excreted in Urine **************;

options nonumber nodate orientation=portrait;
ods rtf file="&inpath.FiguresJPEG\PK06\&runnr.urine.rtf";
goptions reset=goptions device=JPEG target=JPEG
        ftext='Arial' ftitle='Arial/bold'
        xmax=8 cm in ymax=7 cm in ;
title "Simulated cumulative amount of LCM excereted in urine vs. measured
cumulative amount of LCM excreted in urine";
symbol1 v=none i=join c=black l=2;
symbol2 v=dot i=none c=black;
axis2 logbase=10 order= 0.01 0.1 1 10 100 ;
axis1 order=0 to 48 by 12;
proc gplot data=sim_urine;
plot simy*time=1 Ae_cum*time=2 /overlay vaxis=axis2 haxis=axis1;
by SUBJID;
run;
ods rtf close;

ods rtf file="&inpath.FiguresJPEG\PK06\&runnr.corr_urine.rtf";

goptions reset=goptions device=JPEG target=JPEG
        ftext='Arial' ftitle='Arial/bold'
        xmax=12 cm in ymax=10 cm in ;

title "PRED vs. amount excreted - PKmodeling with data of &runnr." font="Albany
AMT";
symbol1 v=dot i=rl c=black;
proc reg data=urine_parameter;
  model pred=Ae_cum;
  plot pred*Ae_cum;
run;
ods rtf close;
```

```
****************** Illustrations as RTF Files ***************************/;
******** Simulated Concentration vs. Measured Concentration of the Metabolite**;
options nonumber nodate orientation=portrait;
ods rtf file="&inpath.FiguresJPEG\PK06\&runnr.met.rtf";
goptions reset=goptions device=JPEG target=JPEG
        ftext='Arial' ftitle='Arial/bold'
        xmax=8 cm in ymax=7 cm in ;
title "Simulated concentration vs. measured concentration of Metabolite";
symbol1 v=none i=join c=black l=2;
symbol2 v=dot i=none c=black;
axis2 logbase=10 order= 0.01 0.1 1 10 100 ;
axis1 order=0 to 96 by 12;
proc gplot data=sim_Met;
plot simy*time=1 Conc*time=2 /overlay vaxis=axis2 haxis=axis1;
by SUBJID;
run;
ods rtf close;

options nonumber nodate orientation=portrait;

ods rtf file="&inpath.FiguresJPEG\PK06\&runnr.corr_met.rtf";

goptions reset=goptions device=JPEG target=JPEG
        ftext='Arial' ftitle='Arial/bold'
        xmax=12 cm in ymax=10 cm in ;

title "PRED vs. conc (metabolite) PKmodeling with data of &runnr." font="Albany
AMT";
symbol1 v=dot i=rl c=black;
proc reg data=Met_parameter;
  model pred=conc;
  plot pred*conc;
run;
ods rtf close;
```

```
************************* Visual Check ************************;
proc sort data=parameter nodupkey out=params;by subjid;run;
data params;
 set params;
 Vdm2=Vdm*km/(km+kren);
 ke=kren+km;
run;

symbol1 h=1 v=dot i=rl c=black;
Title 'kren vs krens';
proc gplot data=params;
 plot kren*krens;
run;

Title 'Vd vs Vds';
proc gplot data=params;
 plot Vd*Vds;
run;

Title 'Vdm vs Vdms';
proc gplot data=params;
 plot Vdm*Vdms;
run;

Title 'km vs kms';
proc gplot data=params;
 plot km*kms;
run;

Title 'kme vs kmes';
proc gplot data=params;
 plot kme*kmes;
run;
```

```
Title 'kme vs ccr';
proc gplot data=params;
  plot kme*ccr;
run;

Title 'kren vs ccr';
proc gplot data=params;
  plot kren*ccr;
run;

Title 'km vs ccr';
proc gplot data=params;
  plot km*ccr;
run;

Title 'Vdm vs Vd';
proc gplot data=params;
  plot Vdm2*Vdm;
run;

Title 'ke=(kren+km) vs kes';
proc gplot data=params;
  plot ke*kes;
run;
```

Appendix 10 Publications Used in the Present Thesis

Publication	Part Supplied by the PhD Student
Schaefer, C., Cawello, W., Waitzinger, J. and Elshoff, J. P. 2015. Effect of Age and Sex on Lacosamide Pharmacokinetics in Healthy Adult Subjects and Adults with Focal Epilepsy. Clin Drug Investig	· Writing SAS Programs · Normalization of pharmacokinetic parameters by body weight, FFM, LBW, V_d, body height · Writing the paper Percentage: 60%

X. SCIENTIFIC QUALIFICATION

iGRAD seminars at the Heinrich-Heine-University

1. Introduction to Good Scientific Practice
2. Presenting Sciene I – comprehensive competent and convincing
3. Effective Scientific Writing
4. Einführung in R

Poster with published abstract

1. Cawello, W., Andreas, JO., Schaefer, C. 2015. Immediate Steady State Concentrations in Plasma after Oral or Intravenous Administration. Neurology, 84, 4263. AES 2014, Seattle.
2. Schaefer, C., Cawello, W., Andreas, JO. 2015. High Predictability of Plasma Lacosamide and No Relevant Differences by Age and Gender Following Normalization. Neurology 84, 4261. AES 2014, Seattle.

Publications

1. Cawello, W. and Schaefer, C. 2014. A system of equations to approximate the pharmacokinetic parameters of lacosamide at steady state from one plasma sample. Epilepsy Res, 108, 1068-75.
2. Schaefer, C., Cawello, W., Waitzinger, J. and Elshoff, J. P. 2015. Effect of Age and Sex on Lacosamide Pharmacokinetics in Healthy Adult Subjects and Adults with Focal Epilepsy. Clin Drug Investig.
3. (Schaefer, C., Cawello, W. 2015. Combined Pharmacokinetic Model for Lacosamide and its Main Metabolite for Integrated Pharmacokinetic Modeling in Humans. Journal of Pharmacokinetics & Experimental Therapeutics (**SUBMITTED**))

Visited Congresses and Workshops

1. AGAH e.V. Workshop, 2013. Beyond the Guidelines – Workshop in Designing and Conducting Pharmacokinetic and Bioavailability/Bioequivalence Trials
2. 68th Annual Meeting of the American Epilepsy Society, 2014, Seattle
3. DPhG Jahrestagung, 2015, Düsseldorf
4. Pharmacometrix 2013,2014,2015