POPULATION PHARMACOKINETICS: MODEL-FREE APPROACH AND NONLINEAR MIXED-EFFECTS MODELLING

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ABSTRACT

The work is devoted to the application and further development of modern statistical methods to study pharmacokinetics of drugs. Specifically, it deals with applications and development of repeated measures analysis, so called 'population approach' methods, in the field of pharmacokinetics. In the first part of the thesis, a new, model-free approach is developed and tested. It introduces a model-free measure of patient's exposure to drugs, and then investigates the relationships between the exposure level and covariates using various statistical techniques. Classification tree models (CART) and regression analysis are used to study various subpopulations of interest. It is shown, via simulations, that the model-free method is capable to identify predictors of exposure in a wide range of variability in the data. The non-linear mixed effect modelling is used to confirm the results of the model-free investigation. Model-free approach is successfully applied to several drugs. Non-linear Mixed Effects population models developed for the same data agree with its results. Limits of the new method are also identified. Specifically, it does not allow the estimation of the variability: either the within-subject (intra-individual) variability in response, or between-subject (inter-individual) variability of the pharmacokinetic parameters in the population. The second part of the thesis is devoted to applications of the Non-linear Mixed Effect methodology to population pharmacokinetics and dose-response analysis. Population pharmacokinetic and dose-response models of several drugs are developed. Pharmacokinetic models allow for complete characterisation of the drug's pharmacokinetics and its relationships to safety and efficacy. The developed models are used to explore the relationships between the exposure (individual Bayes estimates) and demographic predictors of exposure, and safety and efficacy of the drug. Finally, the developed models are used in simulations to guide the design of new studies

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ACKNOWLEDGEMENTS

Some part of the work discussed in this thesis was done in collaboration with my colleagues. Specifically, projects discussed in Section 2.1, 2.2, and 3.1. were done in collaboration with J. Nedelman. He was a team leader for these projects providing general guidance, whereas I was responsible for the implementation, modelling, and interpretation of the results. The simulations described in Section 2.4 were done in collaboration with X. Chen who helped with S-PLUS coding and ran part of the simulations. Their contribution is gratefully acknowledged.

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DECLARATION

I certify that this work has not been accepted in substance for any degree, and is not concurrently submitted for any degree other than that of Doctor of Philosophy (PhD) of the University of Greenwich. I also declare that this work is the result of my own investigations except where otherwise stated.

LIST OF ABBREVIATIONS

Abbreviation	Definition
AGE	Age (years)
AIC	Akaike Information Criterion
ANOVA	Analysis of Variance
AUC	Area Under the concentration versus time Curve
$AUC_{0-\infty}$	AUC from time 0 to ∞
AUC_{0-48}	AUC from time 0 to 48 hours
$AUC_{0-24,ss} / AUC_{0-24,single\ dose}$	Area accumulation ratio : the ratio of the AUC over 24 hours at steady state to the AUC over the 24 hours after a single dose
$AUC_{24-\infty,n} / AUC_{0-\infty}$	Tail fraction after n weeks of dosing
AUMC	Area under the first moment curve
AUPC	Area under population curve
AUQ	Area under quartile
b_2, b_3, b_4	Exponential decay constants in the three-compartment model
bid, b.i.d., BID	Twice-a-day dosing regimen
Bid_{ij}	Indicator for bid dosing
BLQ, BQL	Drug concentration measurements below limit of quantification of an analytical assay
$C_{24,ss} / C_{24,single\ dose}$	Trough accumulation ratio: the ratio of the concentration at 24 hours post-dose at steady state to the concentration at 24 hours after a single dose
CI	Confidence interval
BSA	Body surface area
CART	Classification and regression trees methodology
Cl	Clearance
Cl/F	Apparent oral clearance
C_{max}	Maximal achieved concentration
cv_ε	Intra-individual coefficient of variation
D	Dose
DBP	Diastolic blood pressure

df	Degrees of freedom
E_{max}	Maximal effect, E_{max} model
F	Bioavailability, a fraction of the initial dose absorbed into systemic circulation
FO	First order method of NONMEM
FOCE	First order conditional method of NONMEM
IBLQ	Indicator for BLQ concentration
IBW	Ideal body weight
Log, ln, Ln	Natural logarithm
GGT	Gamma-glutamyl transferase
HGT	Height
HPRT	Indicator for hypertension
k_a	Absorption rate constant
k_e	Elimination rate constant
LDL	Lipid level
lowess	Weighted locally linear regression
NONMEM	Nonlinear mixed-effects modelling software
OBES	Indicator for obesity
OLS	Ordinary least squares method
PATI	Indicator for patient study
PIBW	Percent above Ideal Body Weight
PK	Pharmacokinetics
PRED	Typical population prediction
q.d., qd, QD	Once-a-day dosing regimen
Qd_{ij}	Indicator for once a day dosing
SD	Standard deviation
SE	Standard error
SEX	Gender
SGOT	Serum glutamic oxaloacetic transaminase
SGPT	Serum glutamic pyruvic transaminase
SMOKE	Indicator for smoking
sqrt	Square root
SSE	Sum-of-squared-errors
$t_{1/2}$	Terminal elimination half-life
t_{max}	Time to achieve the maximal concentration

V	Apparent volume of distribution
V_{ss}/F	Apparent volume of distribution at steady state of an oral dose
WGT	Weight
WRES	Weighted residuals
ε_{ij}	Residual intra-patient random error
η	Inter-individual random error
θ	Fixed–effect parameter of a nonlinear mixed- effects model
σ^2	Variance of residual intra-patient random error
Ω	Variance-covariance matrix of inter-individual random effects
ω^2	Variance of inter-individual random effect

1 INTRODUCTION

The work is devoted to application and further development of modern statistical methods to study pharmacokinetics of drugs. Due to the dual (statistical and biological) nature of the work, it requires some introduction to the field and definition of pharmacokinetic terms. Let us begin with such an introduction.

1.1 Overview Of Principles Of Pharmacokinetics

When a drug is given (*administered*) orally to a human or an animal, it first enters the *systemic circulation* (a blood stream) through complex absorption mechanisms [Rowland & Tozer, 1995]. Following absorption, it is distributed to different tissues in the body. On passage through organs of elimination (e.g., liver, kidneys, etc.) it is eliminated (*cleared*) from the body. The amount of drug in each tissue is not constant. It rises following administration of the drug, then decreases, and eventually is cleared completely. Figure 1 depicts the typical *pharmacokinetics* or *time course* of the drug (i.e., time dependence of the amount of the drug in an organ) in different tissues following a single oral dose of a drug.

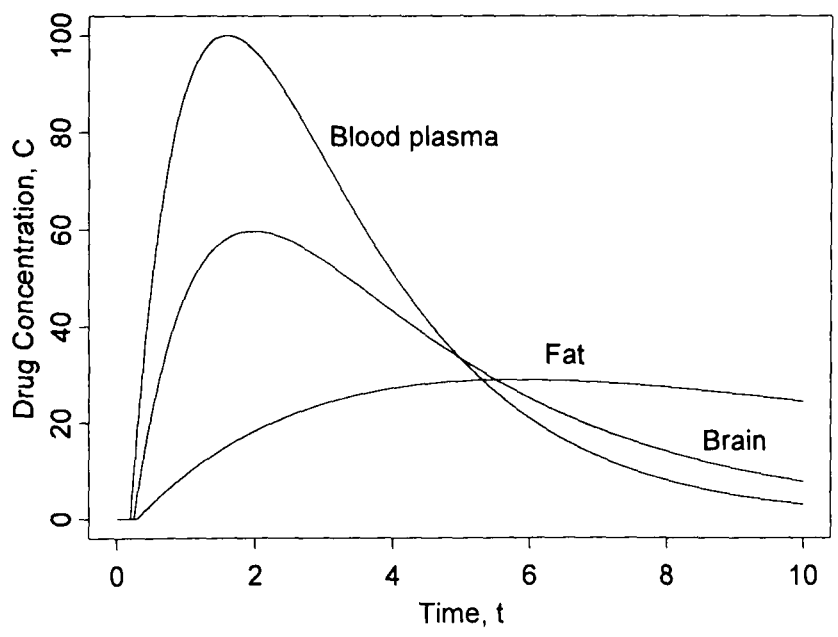

Figure 1. Typical drug pharmacokinetics after a single oral dose.

Pharmacological action of a drug, positive (*efficacy*) or negative (*toxicity*) depends on the amount of the drug at the site of action. Therefore, for *optimal* therapy (therapy that balances desired and side effects of the drug) understanding of the kinetics of the drug is crucial. However, most internal organs in humans are difficult to access, and the amount of the drug in the tissue can not be measured directly. Instead one typically

measures the drug concentration in blood or blood plasma (also in urine, feces, milk, etc). From this *profile* (a time course) one can characterise pharmacokinetics of the drug in the body. The field of science that study the time course of absorption, distribution and elimination of drugs in the body is called *pharmacokinetics (PK)* [Gibaldi & Perrier, 1982].

Several *pharmacokinetic parameters* are commonly used to characterise drug pharmacokinetics. The most important are:

- Area under the concentration versus time curve (AUC);
- Maximal achieved concentration (C_{max});
- Time to achieve the maximal concentration (t_{max}) ;
- *Clearance* (Cl), defined as the proportionality coefficient between the rate of drug elimination from the body and the drug concentration in plasma. Clearance represents the volume of plasma that is cleared of drug per unit of time;
- *Apparent volume of distribution* (V), defined as a proportionality coefficient between the amount of drug in the body and drug concentration in plasma;
- *Bioavailability* (F), defined as fraction of the dose absorbed into systemic circulation;
- *Half-life* ($t_{1/2}$) that is the time that takes to lower plasma concentration of the drug in half.

Repeated administration of a drug eventually (after several doses) yields *steady-state* concentrations of the drug in different tissues. These concentrations typically fluctuate periodically, with the period of dosing. The pharmacokinetic parameters at steady state may differ from those following a single dose. The relationship between single and multiple-dose pharmacokinetics is an important feature of the drug kinetics.

1.2 Compartmental Methods

Mathematical models that describe pharmacokinetics may be purely empirical or may have a physiological meaning. The most widely used pharmacokinetic models are the so-called compartmental models. They assume that the body consists of several 'compartments' storing the drug, as shown schematically in Figure 2. The drug

transfers between the compartments and is eliminated following some functional relationships. The central compartment 1 may, for example, represent blood that transfers the drug to all the other tissues, presented by peripheral compartments 2 and 3.

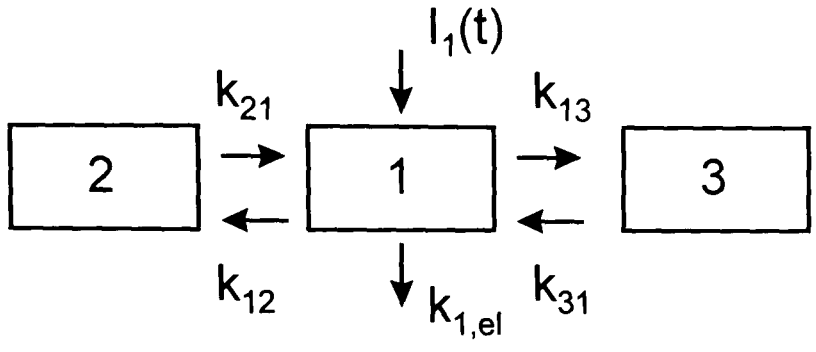

Figure 2. Three-compartment model with input into and elimination from the central compartment.

The compartment models aim to describe the disposition of the drug (e.g., concentration time course of the drug) in any compartment given concentration measurements from an individual at known times and the history of dosing. They can be described by systems of ordinary differential equations of the form;

$$\frac{dX_i}{dt} = -\sum_{\substack{j=1 \\ j\neq i}}^{n} k_{ij} X_i + \sum_{\substack{j=1 \\ j\neq i}}^{n} k_{ji} X_j + I_i(t) - k_{i,el} X_i , \qquad i = 1,\ldots, n, \qquad \text{Eq. 1}$$

where X_i represents the amount of the drug in the i-th compartment, I_i (t) is an input function into the i-th compartment from outside the system, k_{ij} and $k_{i,el}$ are the rates of transfer between the compartments and rates of loss of drug, respectively, and n is the number of compartments in the model.

Usually, the transfer and the elimination rates, k_{ij} and $k_{i,el}$, are assumed to be constant. Then the system is linear, and the solution is described by a sum of several exponential terms. Combinations of the rate constants then describe all the pharmacokinetic parameters of the drug.

The scope of compartmental modelling is to define functional relationships between the compartments and to estimate the parameters that describe the data. Estimation of unknown parameters of compartmental models is usually performed with the nonlinear regression.

1.3 Variability In Pharmacokinetics

Different people respond differently to the same drug, and the same individual may have different responses (drug concentrations) on different occasions. Many factors can contribute to the *inter-individual* (between subjects) and *intra-individual* (within a subject) variability. Factors such as genetics, diseases, age, weight, and gender contribute to inter-individual variability, while drugs given concomitantly, environmental factors, non-compliance, food, time of the day and season can contribute to intra-individual variability. Determining subpopulations with altered kinetics has the implication for the choice of an appropriate *dosing regimen* (that is, the way of administering the drug, such as once or twice a day, orally or intravenously, etc.).

1.4 Two-Stage Approach

The traditional way to deal with variability is to use the *two-stage* method. First, the kinetics is described individually for a number of subjects from a homogeneous population (holding all factors contributing to variability constant), and pharmacokinetic parameters are derived for each subject. Then the population values of each parameter (mean and variance or coefficient of variation) are computed from the empirical distribution of individual estimates of the parameter. To define the influence of a specific factor on the drug kinetics, several populations that differ only in that factor should be compared with respect to their parameters (e.g., young versus elderly, fed versus fasted, etc.) [Rowland & Tozer, 1995].

This approach is widely used in pharmacokinetic studies, and until recently it has been the only method used. However, it has many limitations as discussed by Beal and Sheiner [1985], and Sheiner [1984].

Firstly, the two-stage method can be applied only to small pharmacokinetic studies under restrictive inclusion criteria. These studies are usually short and well controlled. They employ few dosing regimens and small number of usually healthy subjects who do not take other drugs. Many measurements are taken from each person allowing the description of the kinetics in each individual. To have enough power for comparisons,

these studies are designed to compare kinetics with respect to only few factors. Thus, they cannot be used to study several correlated influential factors.

Secondly, in order to distinguish between inter- and intra-individual variability, traditional studies have to employ artificial and complex designs. These designs are not representative of clinical practice and usually can not be carried out in studies that involve real patients.

1.5 Population Approach

In patient studies (*population studies*), where the primary objective is the investigation of the drug safety and efficacy, the optimal pharmacokinetic designs are neither feasible nor desirable. Design of patient studies is dictated by the therapeutic goals. From the pharmacokinetic prospective these studies have non-experimental (*observational*) design. Only a few measurements are usually available per individual. The timing and number of measurements may differ between subjects, dosing regimens may also differ. This type of data is called *sparse* data. The population included in such studies is much broader and less homogeneous. Many factors can contribute to pharmacokinetic variability of a particular drug in a patient population. The number of homogeneous subpopulations can also be very large (and unknown *a priori*). The two-stage approach is not appropriate in dealing with such data.

A more recent approach for analysing sparse kinetic data from a population (called *population approach*) was first proposed by Sheiner *et al.* [1972]. Its first published application was five years later [Sheiner & Rosenberg, 1977] and the first software for analysing data in this manner, NONMEM, was released in 1980 [Beal & Sheiner, 1980]. Since then, the population approach has been an area of active research [Beal, 1998; Grasela & Sheiner, 1991; Sheiner & Grasela, 1991]. The approach uses the *Nonlinear Mixed Effects* regression *Model* to analyse the data pooled over all individuals (see an overview by Sheiner & Ludden [1992]).

The population model combines a pharmacokinetic model, called the *structural model* (for example, a compartmental model) and a statistical model. The basic idea behind the population model is that the same mathematical equation describes the response for any particular individual, but the underlying structural parameters of this

equation vary from individual to individual. The overall variability in the measured response reflects the inter-subject variability in kinetics and the residual variation. The latter includes the intra -individual variability and a measurement error.

Individual structural (pharmacokinetic) parameters are modelled in terms of *fixed* and *random* effects. Fixed effects account for inter-individual differences in the values of individual *covariates* (age, sex, liver function, severity of a disease or other demographic or laboratory data). Random effects of the first type account for unexplained inter-individual variability in the pharmacokinetic parameters. Random effects of the second type account for residual variability. The full set of assumptions and models on (i) pharmacokinetic structural relationships, (ii) inter-individual variation and (iii) residual error variance build a "pharmacostatistical" population model.

A form of the Nonlinear Mixed-Effect Model sufficiently general for our purposes is given by the equation:

$$y_{ij} = f_{ij}(D_{ik}, t_{D\,ik}, t_{ij}; \varphi_i) + \varepsilon_{ij}(D_{ik}, t_{D\,ik}, t_{ij}; \varphi_i), \qquad \text{Eq. 2}$$

where the index i=1,...,I denotes the subject (I is the number of subjects), the index j=1...,J_i denotes an observation (J_i is the number of observations for the subject i), and k=1,…,K_i denotes a dose administration (K_i is the number of doses administered to the subject i). The observed plasma concentration y_{ij} (or it's transformed value, such as log concentration) is a noise-corrupted realisation of the expected value for the j-th observation on the i-th subject. This model assumes the existence of some parametric function of time $f_{ij}(D_{ik}, t_{D\,ik}, t_{ij}; \varphi_i)$ (a structural model) that describes the expected response (e.g., plasma concentration) in a subject. The vectors D_{ik} and $t_{D\,ik}$ describe the dosing history of an i^{th} subject, and the vector t_{ij} describes times of the observations. The vector φ_i is the vector of pharmacokinetic parameters for the i^{th} subject. The random noise ε_{ij} that perturbs the expected value of y_{ij} is represented in (Eq. 2) as a function to indicate that its distribution (e.g., variance) may depend on dose, time and pharmacokinetic parameters.

The vector of parameters φ_i vary randomly among the subjects. It is a function

$$\varphi_i = h(\underline{x}_i; \underline{\theta}, \underline{\eta}_i) \qquad \text{Eq. 3}$$

of the vector of parameters $\underline{\theta}$ (the fixed effects that characterise the population), on the collection $\underline{x}_i$ of covariates, and on the vector of random effects $\underline{\eta}_i$. The random effects $\underline{\eta}_i$ and ε_{ij} are assumed to have zero expectations

$$E(\underline{\eta}_i) = 0, \; E(\varepsilon_{ij}) = 0, \qquad \text{Eq. 4}$$

and be statistically independent

$$Cov(\underline{\eta}_i, \varepsilon_{ij}) = 0 \; . \qquad \text{Eq. 5}$$

The variance-covariance matrix of inter-individual random effects and variance of intra-individual random effects are denoted as Ω and σ^2, respectively, i.e.,

$$Cov(\underline{\eta}_i) = \Omega, \quad Var(\varepsilon_{ij}) = \sigma^2, \quad i=1,\ldots,I; \quad j=1,\ldots,J_i \; . \qquad \text{Eq. 6}$$

(In a more general model, ε_{ij} may be a vector; its variance-covariance matrix is then denoted as Σ).

Thus, the pharmacokinetics of the drug is completely described within the given model by (i) vector of the population parameters $\underline{\theta}$, (ii) vectors of individual random effects $\underline{\eta}_i$ (or its variance-covariance Ω), and (iii) variance of residuals σ^2 (or the variance-covariance matrix Σ).

The simplest method for estimation of unknown parameters is the so-called *First – Order method* [Sheiner, *et al.*, 1972]. It approximates the nonlinear model with a model that is linear in all random effects by using a first-order Taylor expansion in all random effects around zero [Beal, 1984]. To illustrate the method let us rewrite (Eq. 2) and (Eq. 3) in a more general form:

$$y_{ij} = M_{ij}(\theta, X_{ij}, \eta_i, \varepsilon_{ij}), \qquad \text{Eq. 7}$$

including into X_{ij} the covariates $\underline{x}_i$, the dosage histories D_{ik}, $t_{D\,ik}$, and the sampling time histories t_{ij}. Then the first-order model can be written as

$$y_{ij} = M_{ij}(\theta, X_{ij}, 0, 0) + \frac{\partial M_{ij}}{\partial \eta_i}(\theta, X_{ij}, 0, 0)\eta_i + \frac{\partial M_{ij}}{\partial \varepsilon_{ij}}(\theta, X_{ij}, 0, 0)\varepsilon_{ij}. \quad \text{Eq. 8}$$

The estimates of the model parameters $\underline{\theta}$, Ω and Σ are then obtained by the *extended least squares* method [Beal, 1984]. Under the assumption of normality of random effects, the extended least squares yields maximum likelihood estimates for the first - order model [Beal, 1984].

The first -order method produces estimates of the population parameters $\underline{\theta}$, Ω and Σ, but it does not obtain estimates of the random inter-individual effects $\underline{\eta}_i$. An estimate of $\underline{\eta}_i$, conditional on the first order estimates for $\underline{\theta}$ and Ω (at zero value of Σ) can be obtained by maximising the empirical Bayes posterior density of $\underline{\eta}_i$, given the vector y_{ij} for the i^{th} individual [Beal & Sheiner, 1998]. Since the estimate $\underline{\eta}_i$ is obtained after the population estimates, it is called the *posthoc* estimate.

The first-order method was implemented in the software NONMEM, and is referred to as FO method.

In contrast to the first-order method, *conditional estimation methods* (also implemented in NONMEM [Beal & Sheiner, 1998]) produce estimates of the population parameters $\underline{\theta}$, Ω and Σ and, simultaneously, estimates of the random inter-individual effects η_i. They maximise the likelihood for all the data with respect to $\underline{\theta}$, Σ, Ω and $\underline{\eta}_i$. Different methods use different approximations to the likelihood. These methods are very time-consuming and prone to problems. Therefore, they are used only when the FO method produces biased estimates.

The NONMEM software that implements FO and conditional estimation methods has become a standard for nonlinear mixed effect modelling in the pharmaceutical industry. The alternatives include other Gaussian maximum likelihood algorithms based on different linearisations of the model [Lindstrom & Bates,1990; Vonesh, 1992; Vonesh & Carter, 1992; Wolfinger, 1993], semi-nonparametric maximum

according to observed interim response, are used more and more often. The mixed-effects methodology is the only option for deriving dose-response relationships in such studies.

1.6 Model-Free Approach

Nonlinear mixed-effects modelling is a very powerful technique. However, it has its own limitations. Firstly, it is a very time intensive method [Steimer, *et al.*, 1994]. Secondly, it requires an answer to the following question: how do the structural and covariate models, f_{ij} and h, depend on their arguments? Seldom, if ever, does theory provide *a priori* answers to these questions. Exploratory diagnostic techniques have been developed to guide the selection of model form f_{ij} and covariate dependencies h [Ette & Ludden, 1995; Mandema, *et al.*, 1992]. The success of these exploratory methods led to the idea of using nonparametric "exploratory" data analysis methods developed by Chambers *et al.* [1983]. Such an analysis is especially useful when the data has a fairly simple structure, e.g., in the situation of steady state dosing with the same dose given to all the individuals. These nonparametric exploratory methods are essentially a mix of graphical and statistical techniques (see [Pollak, 1990] for a general survey of exploratory methods).

Motivated by Ebelin *et al.* [1992] and Laplanche *et al.* [1991], where exploratory analysis were made primal, a nonparametric, *model-free*, approach to pharmacokinetic population analysis has been developed [Gibiansky, *et al.*, 1997, 1999; Nedelman, *et al.*, 1995,1996]. The basic idea of the model-free approach is to categorise patients into groups according to their exposure, using graphical algorithms, and then use various statistical techniques to explore association of these groups with the covariates.

The approach involves partitioning observed plasma concentrations into several regions (observation levels) taking into account time of concentration measurements. Patients are then partitioned into 'exposure levels' depending on which observation level their concentrations fall into. Exposure level serves as a new response - an ordered factor that characterises the exposure to the drug. It can be explored for an association with covariates. Depending on the goals of the investigation a variety of statistical techniques can be used: from univariate measures of association to elaborate

multivariate classification and regression tree (CART) analysis [Breiman, *et al.*, 1984]. Quantitative measures of exposure, individual (Area Under Quartile or AUQ) and population (Area Under Population Curve or AUPC) can also be derived. This allows for comparisons of exposure for subpopulations. Both, the exposure level and the individual AUQ, can also be used as a covariate in pharmacodynamic models, models that relate drug effect to pharmacokinetic parameters.

The method has been evolving over time. First, it was mostly a qualitative method, designed to serve as a screening tool for parametric modelling, the aim was to reduce the number of variables in the model building process. It later developed into an elaborate statistical technique able to stand on its own.

In the present work the aforementioned techniques are developed and applied to several drugs under development. The structure of the work is the following.

1.7 Organisation of the thesis

Chapter 2 starts with a simpler, model-free approach. It describes the evolution of the approach as it is applied to three projects, three different drugs. In the first two sections (Sections 2.1 and 2.2), model-free approach served as a screening tool: results were to be incorporated into model building of the nonlinear mixed-effects model [Nedelman *et al.*, 1995, 1996]. Therefore, the most interest was in qualitative results. In the third section (Section 2.3) the model-free approach was meant to be the only technique used for the analysis of the data. This necessitated a considerable refinement of the method: use of a wider spectrum of modern statistical techniques and development of quantitative measures of exposure for subpopulations [Gibiansky *et al.*, 1997]. The fourth section of Chapter 2 (Section 2.4) supports the model-free approach by an extensive simulation [Gibiansky *et al.*, 1999].

Chapter 3 is devoted to two applications of the Nonlinear Mixed Effect methodology. In the first section (Section 3.1), a population pharmacokinetic model for one of the drugs described in Chapter 2 is developed. To find a form of the structural model, individual pharmacokinetic models are first developed for data from phase I pharmacokinetic studies using compartmental modelling [Gibiansky, 1995; Nedelman *et al.*, 1997a]. These data and patient data used in Chapter 2 are then used for the

development of the population model [Nedelman *et al.*, 1996]. Initial values of the population parameters are obtained by the two-stage method [Gibiansky, 1994]. After model development is completed, simulations are used to assess the bias and precision of the model parameters. The developed model is used to help design subsequent studies for the drug [Gibiansky, 1996].

In the second section of Chapter 3 (Section 3.2), the Nonlinear Mixed Effects Methodology is used to develop a population dose-response model of a drug. The drug was given to hypertensive patients to reduce their diastolic blood pressure (DBP). If a patient did not respond (i.e. his/her blood pressure did not drop below a pre-specified threshold after a pre-specified time), the dose for that patient was increased or a dose regimen was changed. Thus, different patients received different doses of the drug during the trials. Only the patients most resistant to the therapy received the highest doses. In this chapter development of the population model of change in DBP depending on dose is described. During the trials more cardiovascular adverse events were seen among African-American patients than among Caucasians. Therefore, these subpopulations are thoroughly investigated in covariate models. Structural model relationships were sought among step, linear and sigmoid [Gabrielsson & Weiner, 1997] models. The best structural models turned out to be different for different races.

Finally, Chapter 4 concludes the work by summarising results of all investigations described in Chapters 2 and 3. The results of the model-free approach of Chapter 2 and model-based approach of Chapter 3 are compared. Differences and similarities of these approaches are discussed.

1.8 Tools

Software is an essential tool in this work. The main software packages used in the work include SAS® [SAS Institute Inc., 1990], S-PLUS [1997] and NONMEM [1992]. SAS was used throughout the work for data management and conventional statistics. It was also used for the development of the spline-partitioning technique described in the Sections 2.2, 2.3 and 2.4 of Chapter 2, and for the compartmental modelling and simulation of Section 3.1 of Chapter 3. S-PLUS is a very powerful tool for modern statistical techniques, exploratory graphics and visualisation of data. It was used for tree-based modelling, robust regression and simulation in the Sections 2.3

and 2.4 of Chapter 2, for exploratory graphics and statistical computing in the Section 3.2 of Chapter 3. NONMEM today is a gold standard in the pharmaceutical industry for the nonlinear-mixed effect modelling. It was used for model development in the projects of Chapter 3.

2 MODEL-FREE POPULATION PHARMACOKINETICS

One of the goals of pharmacokinetics is to characterise the relationship between the pharmacokinetic parameters of a drug and covariates (such as demographic, disease-related, etc.) that alter patient's drug exposure (e.g., AUC). In many situations, finding such factors and quantifying the differences in exposure in subpopulations is the main goal of the investigation. A model-free approach deals with such situations [Gibiansky, *et al.*, 1997; Nedelman, *et al.*, 1995, 1996]. The basic idea of the approach is to classify patients into groups according to their exposure and then use various statistical techniques to explore association of these groups with covariates. In the following three sections this model-free approach is applied to three situations, each time the method is more refined and modified to the needs of each project. In the fourth section the developed technique is tested on simulated data.

2.1 Anxiolytic Compound

2.1.1 BACKGROUND

As part of the development of a new anti-anxiety drug, there was a need to estimate the systemic exposure to the drug (i.e. AUC of the drug in plasma) from *phase III* clinical trials (large-scale safety and efficacy trials in patients). In the trials, patients received the drug three times a day for six weeks. Plasma samples were drawn once a week at times chosen by the patients. Evaluating pharmacokinetics from such sparse, arbitrarily timed plasma samples is known as a *pharmacokinetic screen* [Steimer, *et al.*, 1994].

2.1.2 DATA

The data was obtained from two phase III trials in patients with generalised anxiety disorder. The patients received their medication orally at home three times a day, but not on a rigid 8-hour schedule. Patients were randomised to different treatment groups and were *titrated up* (i.e. dose was slowly increased) to the target dose (within a given treatment) over the first few days of the study. The final daily doses ranged from 3 mg to 22.5 mg across treatment groups. Figure 3 displays the distribution of daily doses in the two studies.

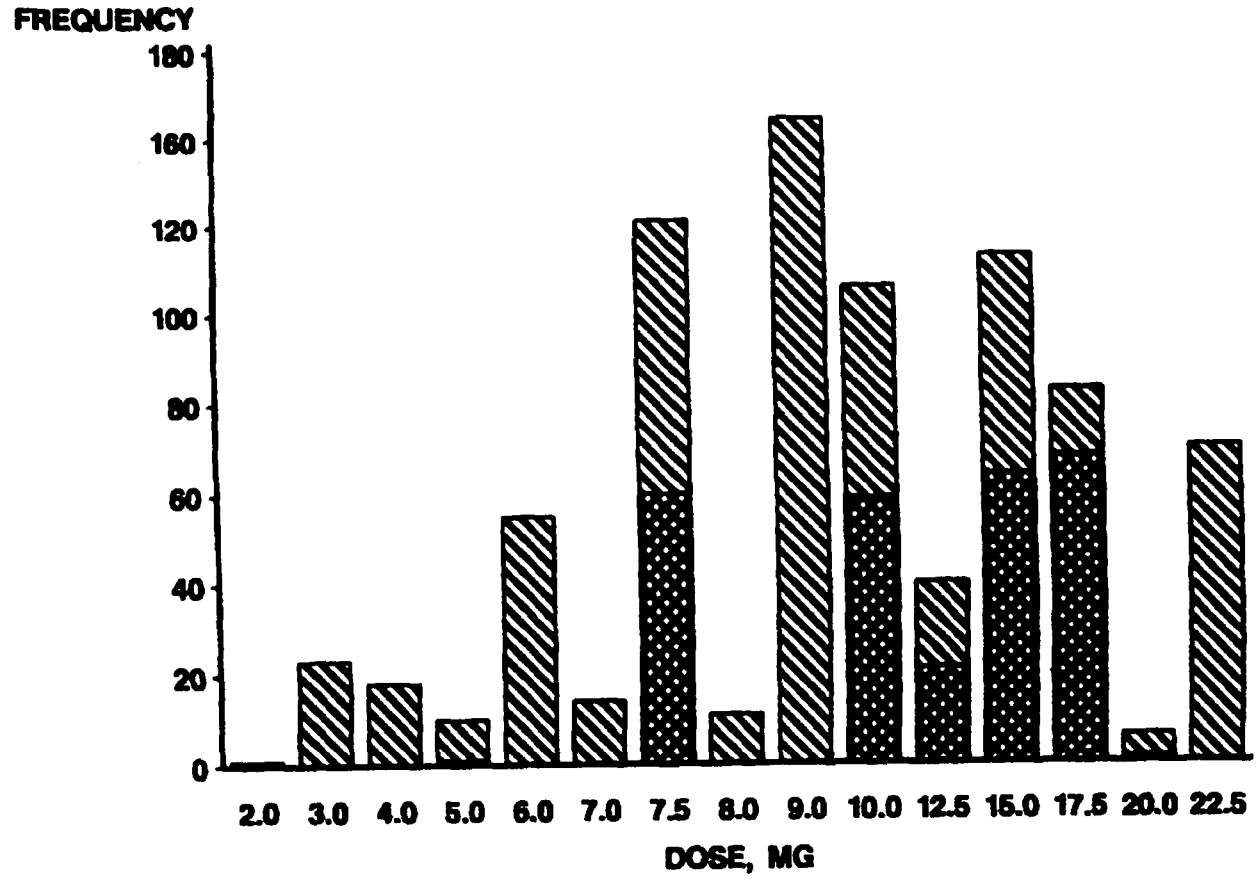

Figure 3. Frequency distributions of prescribed daily doses, after titration and during the time when blood draws were performed, in the two patient studies, Study I ▪▪▪▪, and Study II ▨▨▨.

Some of the daily doses were non-uniformly distributed between the three administrations. For example, a frequent daily dose of 17.5 mg was divided into 5 mg in the morning, 5 mg in the afternoon, and 7.5 mg in the evening. The patients took their medication at home, and maintained a diary recording the doses they took at each administration, but did not record the time.

Patients returned to their physicians for an evaluation once a week for 6 weeks. During the weeks 3-6, after the titration period, a blood sample was taken during the patient's visit to the clinic. The time of the visit and the time interval since the last administration of the drug (*time post-dose*) were not controlled but rather were determined by the patient's choice of when to take the drug and visit the clinic. When blood was sampled, patients were asked how long it had been since their most recent administration of the drug and since the second most recent. Thus, for each patient, in addition to a weekly diary record of dosages, a report from memory of the times of the two most recent drug administrations was available. Table 1 displays the numbers of patients and numbers of blood samples available for analysis.

Table 1. Numbers of patients and plasma samples

Study	Number of patients	Number of samples
I	87	274
II	170	562

2.1.3 OBJECTIVES

The goal of this investigation was to characterise the average exposure to the drug and relate it to demographic predictors, i.e. identify covariates that affect the exposure to the drug. Demographic covariates chosen for exploration of their relationships to exposure were age, gender, race, weight, height, body surface area and smoking.

2.1.4 METHODS

The method is based on partitioning observed plasma concentrations into several regions, called *observation levels*, taking into account time of concentration measurements. Patients are then partitioned into *exposure levels* depending on which observation level their concentrations fall into. Exposure level serves as a new response, an ordered factor that characterises the exposure to the drug. It can be explored for an association with covariates.

First, observed plasma concentrations are partitioned into quartiles. This partitioning involves several steps:

1. Concentrations are normalised for dose, using weighted average dose WDOSE. Because it was common to have non-uniform dosage regimens with a cycle of three dose levels during a day, WDOSE accounted for three doses prior to blood sampling: D1 - the last dose, D2 - second-to-last dose, and D3 - third-to-last dose. Weights were chosen to give more importance to more recent doses:

$$WDOSE = (4D1 + 2D2 + D3)/7. \qquad \text{Eq. 9}$$

Previous pharmacokinetic studies [Krause, 1991; Krause, *et al.*, 1990] of the drug had indicated that concentrations vary proportionally to dose. Dose-normalisation permitted us to combine observations from many dose levels.

2. A scatter plot of dose-normalised concentrations versus time post-dose was considered. Most concentrations were obtained within 0 to 8 hours post dose; few concentrations obtained later than 8 hours were excluded from the analysis. The time axis was divided into one-hour time intervals from 0 to 8 hours post-dose.

3. Within each one-hour interval, the quartiles of the dose-normalised concentrations were determined. Figure 4 shows four piecewise constant functions that within each one-hour interval take on the values of the four quartiles. These functions thus divide the scatter plot of points into four areas, which are called *observation levels*.

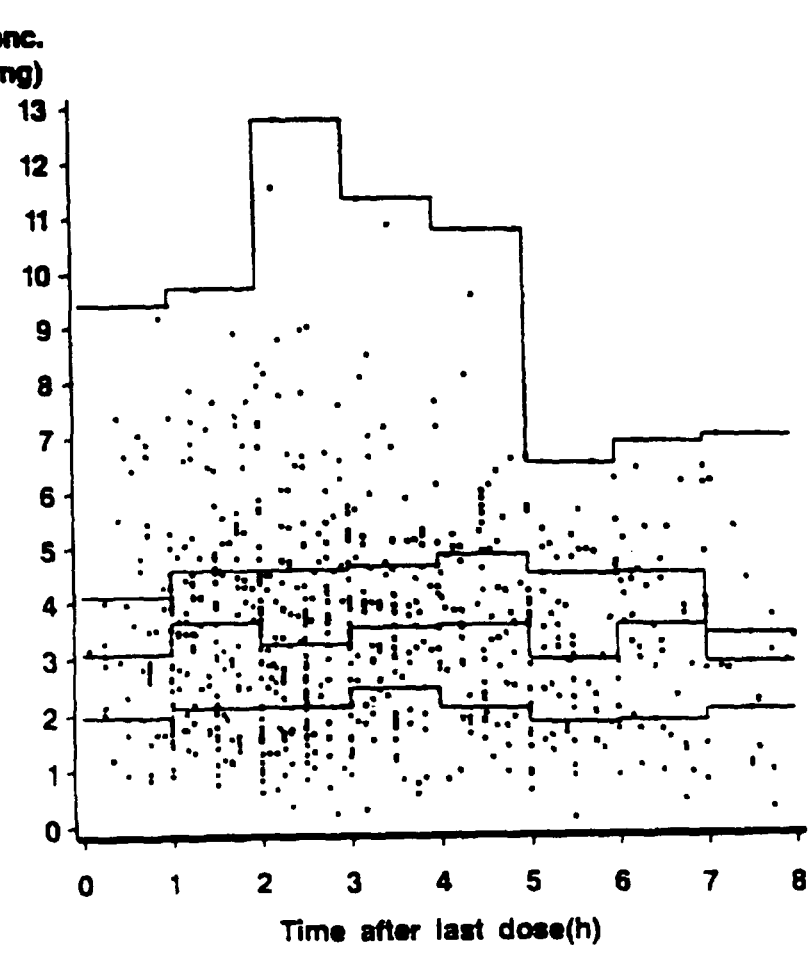

Figure 4. Dose-normalised plasma concentrations from the two patient studies, with piece-wise constant functions of hourly quartiles of observations. Regions between step functions are defined to be observation levels.

Then patients are partitioned into exposure levels depending on which observation levels their dose-normalised concentrations fell into. This process is schematically depicted in Figure 5. One can distinguish four different situations:

A. If all of a patient's observed dose-normalised concentrations fall into one observation level, the patient is then called an 'all-in-one' patient and is assigned to the corresponding exposure level (Figure 5A).

B. Suppose that a patient's dose-normalised concentrations fall into two adjacent observation levels. Let u out of n observations for the patient, be in the upper of the two adjacent levels and n-u in the lower. Let $d_1, \ldots, d_u$, be the distances from the points in the upper level to the common boundary, and let $d_{u+1}, \ldots, d_n$ be the distances for the points in the lower level. If

$$d_1 + \ldots + d_u > d_{u+1} + \ldots + d_n ,$$

then the patient is assigned to the exposure level corresponding to the upper observation level; otherwise, the reverse.

In Figure 5B, $n = 4$, $u = 2$, and since the two points in the third observation level are farther from the common boundary than are the two observations in the second level, the patient is assigned to the third exposure level.

C. If a patient's dose-normalised concentrations fall into either the first to third observation levels or else the second to fourth observation levels, such 'three-adjacent' patient is assigned to the exposure level corresponding to the middle of the three observation levels (Figure 5C).

D. If a patient has dose-normalised concentrations spanning the first and fourth observation levels, then the patient is called an 'all-four' patient and is considered not to represent a stable type. Such patient is left unclassified as to exposure level (Figure 5D).

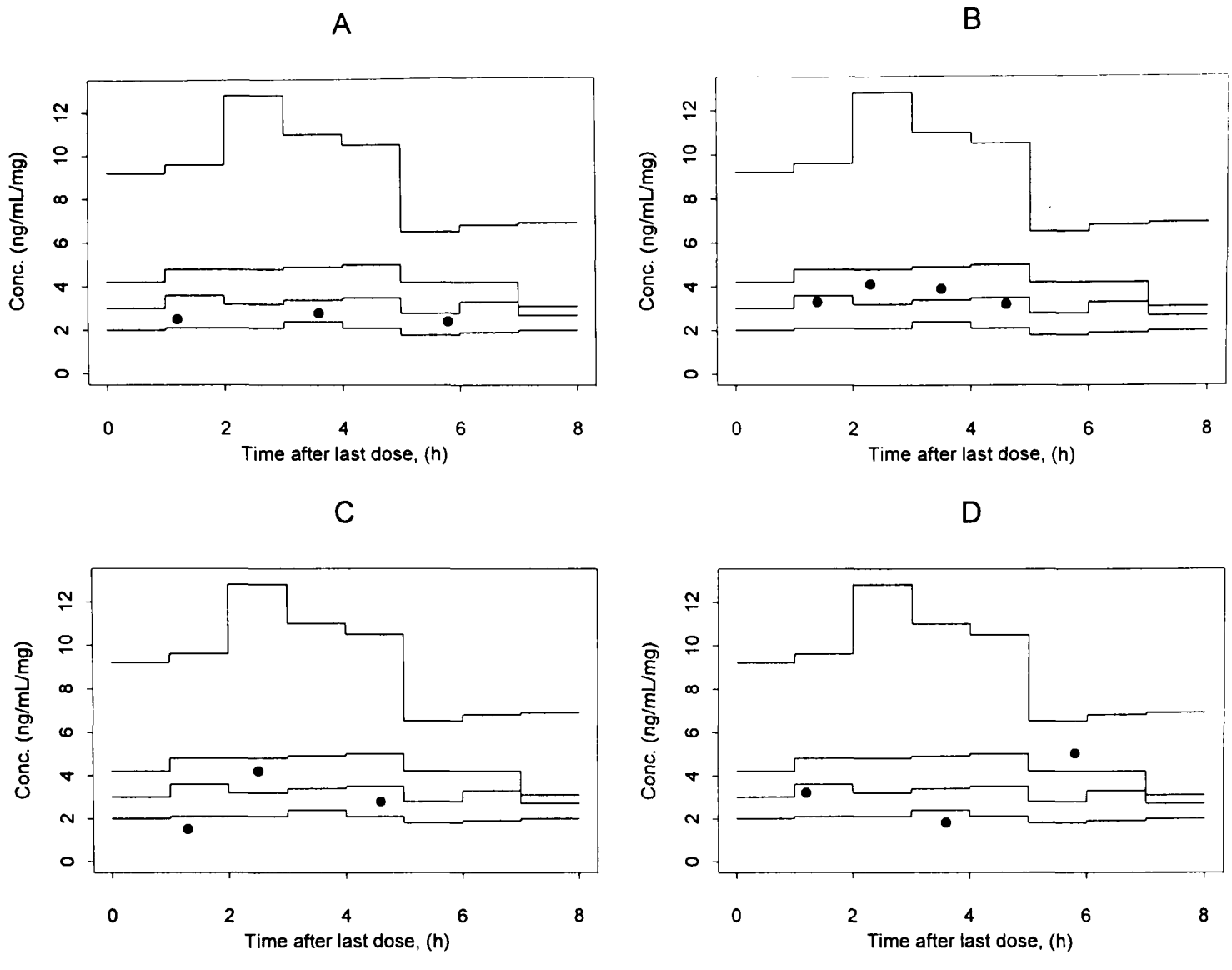

Figure 5. Dose-normalised plasma concentrations for fictitious patients: illustration of assignment algorithm. Patient observations fall in A - one observation level, B – two adjacent regions, C –three adjacent regions, D – four observation levels.

This classification provides a crude estimate of exposure for each patient; the exposure level to which a patient is assigned is an ordinal measure of the patient's dose-normalised exposure to the drug.

The choice of the observation levels and the exposure measure in the model-free approach is somewhat arbitrary. The four observation levels used in this work were chosen by the analogy with four quartiles commonly used in the statistical analyses. One can use an ordinal measure based on more observation levels or create a continuous measure. For example, some average (over the subject's measurements) of standardised distances of the subject's measurements from the average population concentrations within the respective time intervals may serve as such a measure. The

rule of subject's assignment to the exposure level is not unique as well. One can imagine more elaborate schemes. As one will be able to see, the present choice made it possible to obtain meaningful results. More experiments may be needed to find the best possible exposure measure within the model-free framework. This work focuses on obtaining practical results and proving of method's concept with the chosen exposure measure rather than experimenting with various possible alternatives.

To find predictors that affect exposure, exposure levels are related to demographic covariates by standard statistical techniques: contingency-table analysis for the categorical covariates such as smoking, gender, and race; ANOVA for the continuous covariates such as age and measures of body size.

For comparison of model-free and model-based results, discussed later, a quantitative measure of an individual exposure, a quartile-based analogue of the AUC, was created. It is called the area under the quartile, or AUQ.

To compute an AUQ for each patient during each week, a number called a dose-normalised AUQ is first associated with each exposure level. Figure 6 shows how such a number is computed for the third exposure level. The shaded area is the area under the function that defines the middle of the third observation level, the fifth octile. For the first, second and fourth exposure levels, the first, third and seventh octiles are used, respectively.

An AUQ for a given patient in a given week is then computed by multiplying the patient's average weighted dose WDOSE times the dose-normalised AUQ for the patient's exposure level.

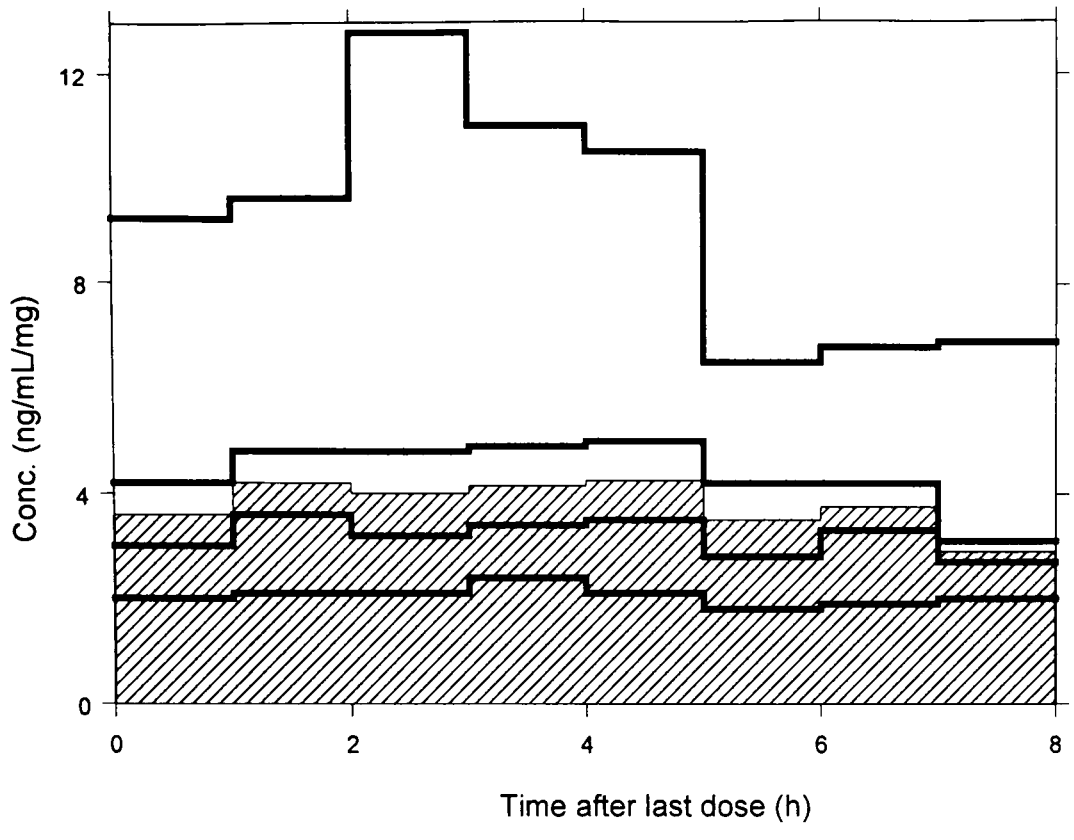

Figure 6. Definition of the dose-normalised area under the quartile for the exposure level. Shaded is AUQ for he third exposure level. Thick solid lines denote boundaries of the exposure levels.

2.1.5 RESULTS

Table 2 shows the frequency distributions of the four types of patients in each study. The observation levels were determined from the data from the two studies combined; the purpose of the separation in Table 2 is to check whether there is any large difference between the studies with respect to frequencies of types. The frequencies show that 80 percent of subjects were in the two most stable types, all-in-one and two-adjacent. Only 3 per cent were not classified because of having dose-normalised concentrations in both the first and fourth observation levels. Results confirm that patients were similar in two studies with respect to their types; there were no large differences in the frequencies of types in these studies.

Table 2. Frequencies of patient types

Type	Frequency (Col %)		
	Study I	Study II	Total
All-in-one	41	68	109
	47%	40%	42%
Two-adjacent	26	69	95
	30%	41%	37%
Three-adjacent	17	28	45
	20%	16%	18%
All-four	3	5	8
	3%	3%	3%
Total	87	170	257

Table 3 shows the distribution of patients among exposure levels. Due to the way that exposure levels are constructed, there is no constraint that the patients partition uniformly among them. Despite this, the distribution across the four levels of classified subjects is fairly uniform. Furthermore, patients were similar in the studies; there are no large differences between the studies in frequencies of assigned exposure levels.

Table 3. Frequencies of exposure levels

Exposure level	Frequency (Col %)		
	Study I	Study II	Total
Unclassified	3	5	8
	3%	3%	3%
1	17	37	54
	20%	22%	21%
2	16	48	64
	18%	28%	25%
3	30	45	75
	35%	27%	29%
4	21	35	56
	24%	21%	22%
Total	87	170	257

Table 4 contains the main results of the project. Specifically, it summarises the results of the univariate statistical analysis. Each covariate was tested separately. For categorical covariates the null hypothesis of no difference was tested against a two-sided alternative hypothesis of a difference in exposure level depending on the level of covariate. For continuous covariates the null hypothesis was the hypothesis of no difference in means of the covariate between different exposure level groups. Testing was performed at the 95% significance level. The contribution of each covariate to the exposure level is presented in terms of the p-values, with $p < 0.05$ being regarded as significant, $p < 0.01$ more significant, and $p < 0.001$ regarded as highly significant. The direction of the influence is also shown. As can be seen, smoking, gender and age are found to be significantly related to exposure; with smoking being the most important factor followed by gender, and then by age. Smoking decreased exposure levels, whereas age increased the levels. Females tended to be in higher levels. Neither race nor any measure of body size was significantly related to exposure level.

Table 4. Relationships between demographics and exposure.

Covariate	Contribution	Direction of effect on exposure level
Smoking	+++	↓
Gender (female)	++	↑
Age	+	↑
Race	-	
Weight	-	
Height	-	
Surface Area	-	

- $p \geq 0.05$, + $p < 0.05$, ++ $p < 0.01$, +++ $p < 0.001$.

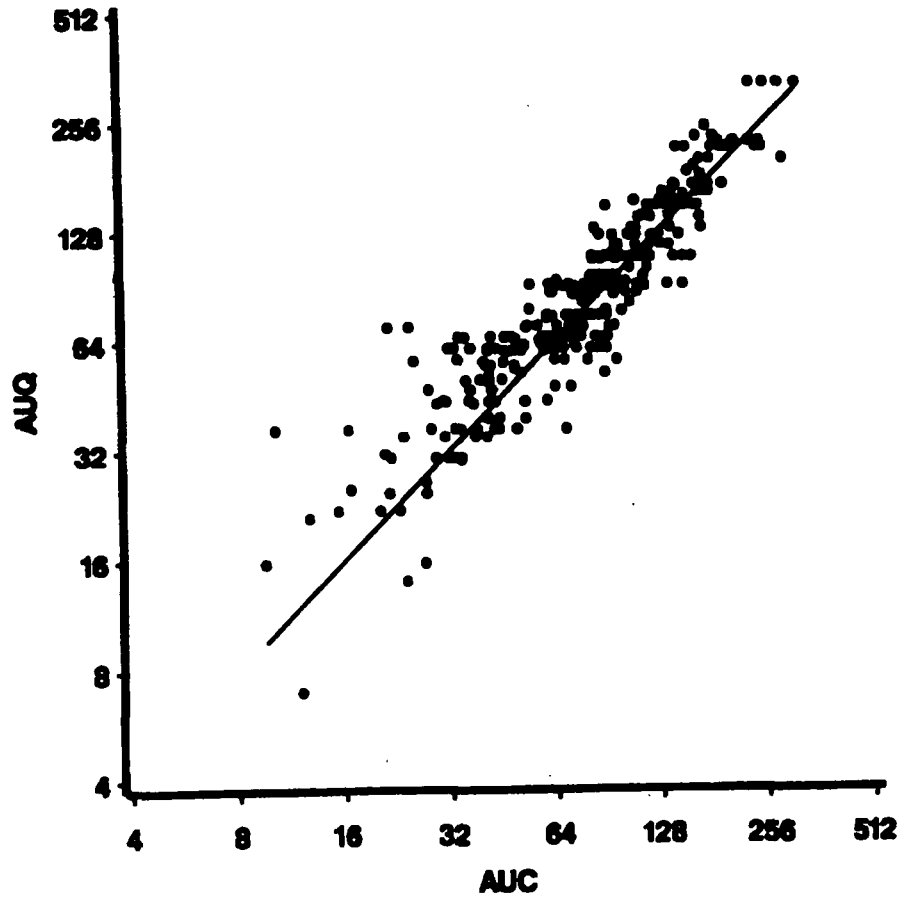

Figure 7 Model-free AUQs versus model-based AUCs.

These results are reported in Nedelman *et al.* [1995], where they were used in the model-based analysis of the drug's pharmacokinetics. The covariates found to be important by the model-free method were incorporated into the nonlinear mixed effect

model. The model (not described in this thesis) confirmed the findings of the model-free approach. Apparent oral clearance increased in smokers, and decreased in females and with increasing age. The contribution of these covariates followed the same order as in model-free approach. Figure 7 shows a strong linear correlation between model-free AUQs and model derived AUCs.

2.2 Antifungal Compound

2.2.1 BACKGROUND

The second project is devoted to an anti-fungal drug. Pharmacokinetic studies have revealed that this drug has a prolonged half-life ranging from 4 to 22 days [Faergemann, *et al.*, 1991; Jensen, 1989, 1990]. This long half-life may cause marked accumulation of the drug over the 6 to 24 weeks of administration. Long-term exposure to high levels of the drug might alter the risk/benefit ratio of treatment with this agent. Consequently, the investigation was undertaken to identify demographic predictors of its exposure and to explore whether increased exposure or demographic predictors of increased exposure were associated with altered safety or efficacy in patients.

2.2.2 DATA

Data was obtained from two efficacy and safety studies (P1 and P2) in patients with onychomycosis. Patients were directed to take one tablet daily, either placebo or the drug. Dosing continued for 24 weeks in Study P1 and 12 weeks in Study P2. In each study, there were three treatment arms. One arm received placebo for the entire duration of dosing. The second arm received active drug at 250 mg/day for the entire duration of dosing. A third arm received active drug at 250 mg/day for the first half of the dosing period (12 weeks in Study P1 and 6 weeks in Study P2) followed by placebo for the second half. Sparse pharmacokinetic samples were obtained in these efficacy studies. Maximally three plasma samples were collected per patient during the study. One sample per patient was drawn when the patients visited their physicians at weeks 4, 12, and 24 in Study P1, and weeks 4, 6, and 12 in Study P2. The times during the day of the patients' appointments, and consequently, the times post dose of the blood samples, were not specified in the protocol but rather were determined solely by the patients' and investigators' convenience - i.e., they were, in the sense of population screens, "random". Patients kept diaries recording the times of doses taken on the two evenings prior to blood sampling. Investigators recorded the times of the blood samples.

In both studies, the times post dose at which the blood samples were drawn generally fell into three major groups: around 15 hours, 1000 hours, and 2000 hours post dose

as shown in Figure 8. Indeed, since doses were taken in the evening before the day of the visit to the physician, blood samples were usually taken around 15 hours after the tablet was ingested. The second cluster at around 1000 hours comes from the blood samples at 12 weeks from those patients in Study P1 who received the drug for only the first six weeks. The third cluster at around 2000 hours comes from the blood samples at 24 weeks from those patients in Study P2 who received the drug for only the first 12 weeks.

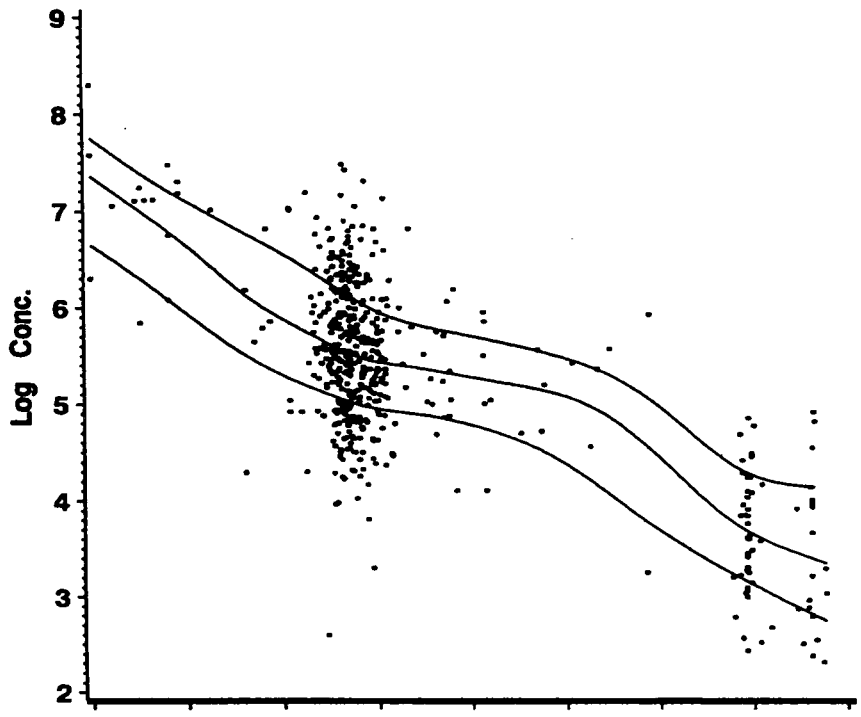

Figure 8. Concentration versus time data for studies P1 and P2 (points) on the log scales. Superimposed are three splines that define partition boundaries.

In total, 545 plasma concentrations were available: 327 observations from 130 patients in Study P1 and 218 observations from 89 patients in Study P2. Among them, 29 samples had zero concentrations, i.e. concentrations below the quantification limit of the bioanalytical assay (BQL). They were excluded since a log scale for concentrations was used. Two more observations were eliminated because they were obvious outliers. The resulting data set had 514 observations.

2.2.3 OBJECTIVES

As before, the goals of the model-free population pharmacokinetic analysis were to partition patients into exposure levels, and subsequently explore the relationship

between the covariates and exposure levels. It was decided in advance to restrict consideration to the following covariates:

Demographic:	age, sex, race, weight, body surface area, and smoking status;
Lipid levels:	LDL (low-density lipoprotein cholesterol) concentration;
Medical conditions:	hypertension, peripheral vascular disease.

2.2.4 METHODS

Unlike the previous project, the data was very non-uniformly distributed across time. There were clusters of many points and there were extended intervals with few or no points. Consequently, use of a piecewise constant function at equal intervals, to partition plasma concentrations into quartiles, was not possible. Any other arbitrary chosen division of time intervals (for example, intervals with equal numbers of points) was also questionable. Due to the temporal variations of the plasma concentration data, lumping together distant points was also inappropriate. As a result, interpolation of the data was used. In particular, nonparametric smoothing splines were used to separate observation regions instead of piecewise constant functions (see DeBoor, 1978 for a detailed description of smoothing splines or a brief Remark below).

Remark:

Suppose one has a scatterplot of n pairs (x_i, y_i). *Among all functions* $f(x)$ *with two continuous derivatives, a smoothing spline minimises a penalised residual sum of squares*

$$\sum_{i=1}^{n}\{y_i - f(x_i)\}^2 + \lambda\int_a^b\{f''(t)\}^2 dt, \qquad \textit{Eq. 10}$$

where λ *is a fixed constant, called a smoothing parameter, and* $a \le x_1 \le \ldots \le x_n \le b$. *The solution is a natural cubic spline with knots at the unique values of* x_i *(i.e.* $x_i \neq x_j$ *for any pair of knots* x_i, x_j*) . The smoothing parameter* λ *controls the fit. At the one extreme, as* $\lambda \to 0$, *the penalty term dominates, forcing* $f''(x) = 0$ *everywhere, and thus the solution is the least-squares line. At the other extreme, as* $\lambda \to \infty$, *the penalty term becomes unimportant and the solution tends to an interpolating twice-differentiable function. The*

smoothing spline is a powerful and flexible form of non-parametric regression technique based on strictly interpolating splines [Silverman, 1985].

To partition plasma concentrations into observation levels a nonparametric cubic smoothing spline is fitted through the scatter plot of concentration versus time for all patients, all visits and studies together, as in Figure 8. The resulting curve estimates "typical" plasma levels as a function of time, dividing the scatter plot into two parts, a higher and a lower. Then the same nonparametric smoothing is applied separately to each of the two parts. The resulting curves estimate "typical" lower and upper concentrations as functions of time. The three curves, three *partition boundaries*, divide the points into four regions, four observation levels. Each of these four regions do not necessary contain 25% of the observations, as in the previous project. The less uniform the concentrations are distributed at each time interval, the further the regions are from the quartiles.

Fitting a smoothing spline involves an arbitrarily assigned value of the smoothing parameter λ. The greater the parameter the smoother the fitted curve is. Several values of λ were used, based on experimentation with the smoothing algorithm.

The smoothing was to be applied to a scatter plot of concentration versus time post dose. However, both concentrations and times post dose ranged over several orders of magnitude, so the linear scales of concentration and time might not be the most convenient. Log-transformed concentrations were used on the y-axis. On the x-axis, both log-transformed and untransformed raw times were tried.

The duration of treatment from the first dose until the plasma sample was drawn ranged from 4 to 24 weeks. It was suspected that the drug might accumulate in the blood over such periods. In this case, in order to standardise the concentration values during different weeks of dosing, the concentrations should be adjusted for the expected accumulation. Both variants, with and without adjustment, were tried. In one variant, the concentrations were left unadjusted for accumulation. In the other variant, they were adjusted by dividing concentrations by a pseudo-accumulation factor,

$$C_{adj} = \frac{C}{1 - e^{-24d*b}}, \qquad \text{Eq. 11}$$

where d is the number of days of dosing prior to the blood sample, and b is an estimate of a parameter that characterises a half-life. Two values of b were used: 0.00165 and 0.0019. This parameter comes from the compartmental modelling and it is described later in Chapter 3 (where it was denoted b_4).

Not all possible combinations of the preceding options regarding smoothing parameter, axis scales, and adjustment for accumulation were used. Complete analyses were conducted using five different combinations of options. Table 5 exhibits those combinations.

Table 5. Combinations of parameters used for partition

Combination	Transformation of time	b_4	Smoothing parameter
1	none	0.0019	10,000,000
2	log	0.0019	0.1
3	log	0.0019	1.0
4	log	none	1.0
5	log	0.00165	1.0

After observation levels are determined, patients are assigned to the exposure levels according to the same algorithm as in the previous project. Thus, in the modified partitioning method piecewise constant boundaries of the observation levels are replaced by smooth functions of time, namely smoothing cubic splines.

A statistical analysis was performed to explore the association between the exposure levels and the covariates. The categorical covariates used in the analysis were gender, age (divided at 40 years from the previous experience with the drug), race, smoking, history of hypertension, and history of peripheral vascular disease. The continuous covariates were age, weight, body surface area and LDL cholesterol level. Age was used both as a continuous and categorical covariate.

For each categorical covariate, frequency tables were generated and the Fisher's exact test was applied. The null hypothesis of no difference was tested against a two-sided alternative hypothesis of a difference in exposure level depending on the level of the covariate. Testing was performed at a 95% significance level.

For continuous covariates, the distribution of the covariate by exposure level was summarised by means and standard deviations. Furthermore, the mean of each continuous covariate was compared across the exposure levels by an analysis of variance (ANOVA), in which the null hypothesis of equality of the means was tested against an alternative that the means either increased or decreased linearly with the exposure levels.

It is important to note that not all of the covariates are independent. It is known that weight and body surface area differ for men and women; interaction of cholesterol level and age in the studies with gender could also be suspected. For a continuous response variable a natural choice of analysis would be to perform a two-way ANCOVA, with gender and a covariate in the model. The exposure level, however, is not a continuous variable. Therefore, in order to account for possible confounding of the effect of the covariate on the exposure level by gender, the two-way ANOVA with gender and the exposure level as the main effects of the model was performed for all continuous covariates. The interaction term was also included.

Also, to account for the fact that cholesterol generally increases with age [Braunwald, *et al.*, 1987] a two-way ANOVA was performed for cholesterol level with age, the exposure level, and their interaction included in the model. As with the categorical covariates, testing was performed at a 95% significance level.

The partition algorithm, described above, was implemented in SAS and SAS/IML language [SAS Institute Inc., 1989a]. The SAS/IML function SPLINEC was used for spline fitting. The statistical analysis was implemented using SAS/STAT [SAS Institute Inc., 1989b].

2.2.5 RESULTS

Figure 8 shows the scatter plot of log concentrations versus log time with the partition boundaries resulting from combination 4 of the parameters (see Table 5). The other combinations produced similar plots.

Table 6 displays the percentage of observations assigned to each of the four observation levels by each Combination of options. As can be seen, five Combinations yield similar partitions. As expected, the method did not partition the observations into four equally sized groups; the first and the fourth observation levels have slightly less observations than the second and the third levels.

Table 6. Frequencies of observation levels

Combination	Observation Level			
	1	2	3	4
1	22%	28%	29%	21%
2	21%	27%	29%	23%
3	21%	28%	29%	22%
4	23%	30%	27%	21%
5	21%	28%	29%	22%

Table 7 displays the distribution amongst the exposure levels generated by each of the five combinations. The five distributions are similar, with combination 4, where no adjustment for accumulation was made, being the most different. Only 4% of patients have not been assigned to the exposure levels because they had plasma levels in both the first and fourth observation levels. As with the observation levels, the distributions of patients are not uniform over the four exposure levels. The differences between the first and fourth exposure levels relative to the second and third are more pronounced than with the observation levels. This suggests that many patients had occasionally but not consistently high or low plasma levels.

Table 7. Frequencies of exposure levels

Combination	Exposure level				
	1	2	3	4	Unassigned
1	16%	34%	29%	17%	4%
2	15%	35%	30%	16%	4%
3	15%	35%	30%	16%	4%
4	18%	33%	31%	14%	4%
5	15%	35%	30%	16%	4%

Table 8 displays the distributions of patients according to the spread of their plasma levels among the four observation levels. The results are generally similar, with combination 4 again differing most from the others. On average, 35% of the patients were in the most consistent All-in-One category, and another 41% were in the Two-Adjacent category.

Table 8. Frequencies of patient types

Combination	Patient Type			
	All-in-One [a]	Two-Adjacent [b]	Three-Adjacent [c]	All-Four [d]
1	33%	42%	21%	4%
2	32%	41%	23%	4%
3	31%	43%	21%	4%
4	47%	35%	15%	4%
5	31%	44%	21%	4%

a) All concentrations in one observation level

b) All concentrations in two adjacent observation levels

c) All concentrations in three adjacent observation levels

d) Concentrations span four observation levels

Table 9. Frequencies of gender and race versus exposure level

Exposure level	Total	Gender (Row %)[a]		Race (Row %)[a]			
		Male	Female	Caucasian	Black	Oriental	Other
Unassigned	9	89%	11%	78%	0%	0%	22%
1	33	85%	15%	97%	0%	0%	3%
2	76	84%	16%	91%	0%	0%	9%
3	65	85%	15%	88%	2%	2%	9%
4	36	89%	11%	89%	3%	3%	6%

a) Computed as percent of a cell frequency to the Total in the corresponding exposure level

Table 10. Frequencies of age versus exposure level

Exposure level	Total	Age in years							
		40 and under				over 40			
		Total under 40		Male	Female	Total over 40		Male	Female
		N	%[a]	%[b]	%[b]	N	%[a]	%[c]	%[c]
Unassigned	9	4	44%	75%	25%	5	36%	100%	0%
1	33	19	58%	79%	21%	14	42%	93%	7%
2	76	27	36%	78%	22%	49	64%	88%	12%
3	65	15	23%	80%	20%	50	77%	86%	14%
4	36	4	11%	100%	0%	32	89%	88%	13%

a) Computed as percent of a cell frequency to the Total in the corresponding exposure level;

b) Computed as percent of a cell frequency to the Total under 40 in the corresponding exposure level;

c) Computed as percent of a cell frequency to the Total over 40 in the corresponding exposure level.

Table 9 - Table 15 display the distributions of covariates by exposure levels: Table 9 - Table 11 show frequencies of categorical covariates; Table 12 - Table 15 display means and standard deviations of quantitative covariates. From the results presented for categorical covariates, it is evident that gender, race and a history of vascular disease does not influence the assignment to the exposure level: the percentage of

males, Caucasians or patients with vascular disease does not change with the exposure level. Age, smoking and a history of hypertension appear to have an effect on exposure: percentage of patients over 40 years old, non-smoking patients and patients with the history of hypertension increases with the exposure level. From the tables for the continuous covariates, mean age increases with the exposure level. There is also a slight increase in LDL for women. Means of weight and body surface area do not change across the exposure levels.

The exposure level assignments in the Table 9 - Table 15 are from combination 3. The results of the other combinations are similar.

Table 11. Frequencies of smoking, vascular disease and hypertension versus exposure level

Exposure level	Total	No. of cigarette packs per day				Vascular disease		Hypertension	
		0	<1	1-2	>2	No	Yes	No	Yes
Unassigned	9	100%	0%	0%	0%	100%	0%	89%	11%
1	33	73%	12%	15%	0%	97%	3%	100%	0%
2	76	80%	14%	5%	0%	99%	1%	93%	7%
3	65	83%	8%	8%	2%	98%	2%	88%	12%
4	36	97%	3%	0%	0%	97%	3%	78%	22%

Table 12. Means and standard deviations of age versus exposure level

Exposure level	Age (years)					
	Total		Male		Female	
	Mean	Std	Mean	Std	Mean	Std
Unassigned	44.9	16.1	46.6	16.3	31.0	0
1	41.9	12.0	42.6	12.1	37.8	12.0
2	46.1	11.9	46.3	12.4	44.6	8.4
3	50.5	11.8	50.2	11.4	51.8	14.5
4	55.7	11.8	55.5	12.4	57.3	5.1

Table 13. Means and standard deviations of weight versus exposure level

Exposure level	Baseline Weight (kg)					
	Total		Male		Female	
	Mean	Std	Mean	Std	Mean	Std
Unassigned	80	16	83	14	58	0
1	82	18	84	15	68	29
2	77	13	80	11	65	17
3	83	16	87	14	62	7
4	81	16	83	16	65	14

Table 14. Means and standard deviations of body surface area versus exposure level

Exposure level	Body surface area (m^2)					
	Total		Male		Female	
	Mean	Std	Mean	Std	Mean	Std
Unassigned	2.0	0.2	2.0	0.2	1.7	0
1	2.0	0.3	2.1	0.2	1.7	0.4
2	1.9	0.2	2.0	0.2	1.7	0.2
3	2.0	0.2	2.1	0.2	1.7	0.1
4	2.0	0.2	2.0	0.2	1.7	0.2

Table 15. Means and standard deviations of low-density lipoprotein cholesterol (LDL) versus exposure level

Exposure level	Baseline LDL level (mg/mL)					
	Total		Male		Female	
	Mean	Std	Mean	Std	Mean	Std
Unassigned	3.1	0.6	3.2	0.6	2.6	0
1	3.2	0.7	3.2	0.7	2.6	0.8
2	3.3	0.9	3.3	1.0	3.1	0.9
3	3.5	1.2	3.4	1.2	3.6	0.9
4	3.3	1.0	3.3	1.0	3.8	0.2

Table 16 contains the main results of the analysis. Specifically, it summarises the results of inferences regarding relationships between the exposure level and the covariates for each of five combinations. Each covariate was tested separately as described in the Methods (Section 2.2.4). Contribution of each covariate to the exposure level is shown in the table in terms of the p-values with $p < 0.05$ being regarded significant, $p < 0.01$ more significant, and $p < 0.001$ regarded as highly significant. The direction of the influence is also shown.

As can be seen, the five combinations agreed on the importance of smoking and age as correlates of the exposure level. Four of five combinations also recognised hypertension as a significant covariate. Sex, race, history of vascular disease, lipid level (LDL), and two measures of body size (weight and surface area) were all found not to be significantly associated with the exposure level. Smokers generally had lower plasma levels; older patients and patients with hypertension (of which there were only 24) generally had higher plasma levels.

Table 16. Relationships between demographics and the exposure level for five combinations.

Covariate	Contribution					Direction of effect on exposure level
	Combination					
	1	2	3	4	5	
Smoking	+	+	+	++	+	↓
Age (>40)	+++	+++	+++	+++	+++	↑
Age	+++	+++	+++	+++	+++	↑
Hypertension	+	+	++	-	++	↑
Gender	-	-	-	-	-	
Race	-	-	-	-	-	
Vacular disease	-	-	-	-	-	
Weight	-	-	-	-	-	
Surface Area	-	-	-	-	-	
LDL	-	-	-	-	-	

- $p \geq 0.05$, + $p < 0.05$, ++ $p < 0.01$; +++ $p < 0.001$.

The results of this analysis were used in the model-based analysis of the drug discussed in Section 3.1.3 of Chapter 3. The covariates found to be important by the model-free method were incorporated there into the nonlinear mixed effect model.

2.3 Antiplatelet And Vasodilative Agent

2.3.1 BACKGROUND

The third analysis deals with a drug indicated for treating the symptoms of intermittent claudication. The drug increases the distance that patients can walk before pain prevents their motility.

The aim of the pharmacokinetic analysis of phase III safety and efficacy studies, was to identify covariates affecting patients' exposure to the drug and to quantify the influence of these covariates.

Analysis of phase I pharmacokinetic data of the drug showed proportional increases in AUC, and less than proportional increases in C_{max}, following single doses across the dose range of 50-200 mg. The time to plasma maximum concentration (t_{max}) and the terminal half-life were approximately 3 hours and 12 hours respectively, and were similar across the doses. Following multiple administration of a 100 mg dose, twice-a-day (b.i.d.), steady-state plasma concentrations were achieved within 4-5 days. When administered under fed conditions, there was an increase of approximately 50% in the C_{max} and an increase of 25% in the AUC.

Plasma concentration-time profiles after a single dose or after discontinuing a multiple-dose regimen had irregular secondary peak(s), as shown in the Figure 9 at 20 - 25 hours post dose for approximately 70% of subjects.

A nonparametric approach for the population analysis was further developed and applied to the data of the project.

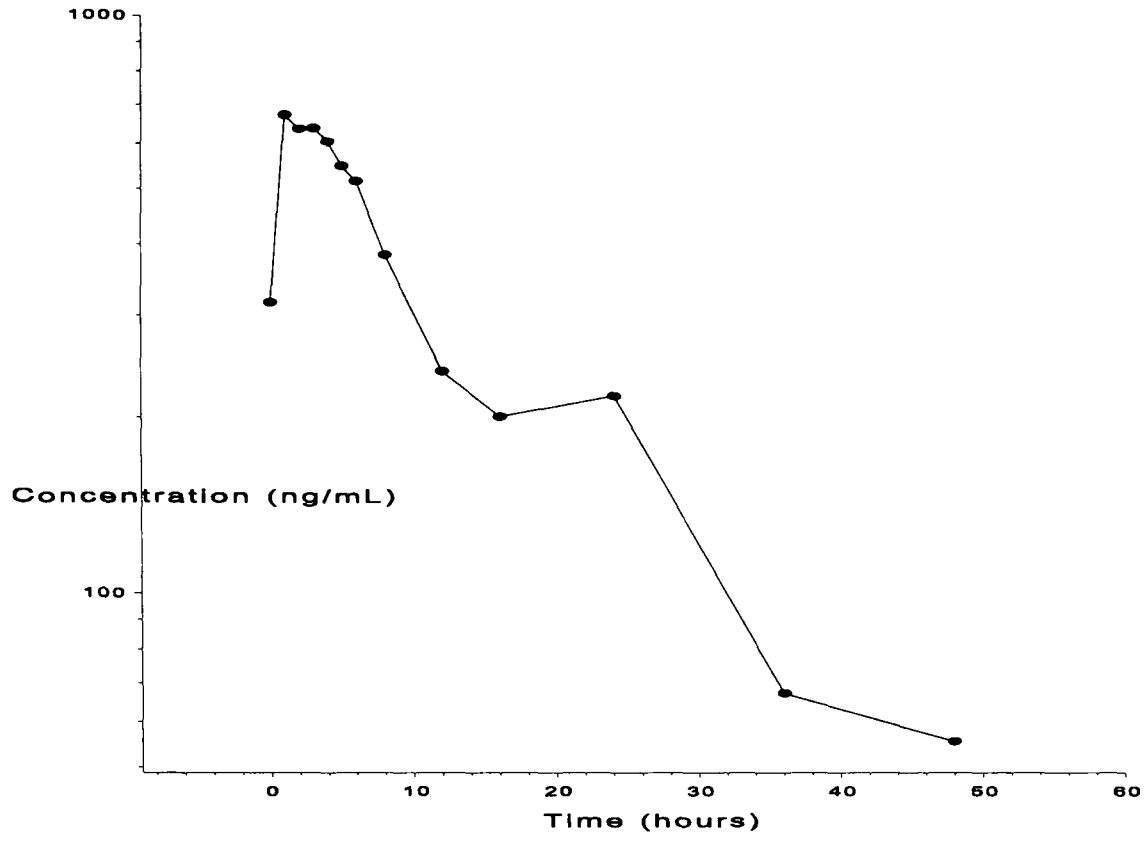

Figure 9. Plasma concentration versus time after last dose for a typical subject after eight days of dosing.

2.3.2 DATA

Data come from four randomised, double blind, efficacy and safety studies (I, II, III and IV) in patients with intermittent claudication. Walking distance at baseline measured on a treadmill, was one of the main criteria for inclusion in the studies. Drug (100 mg daily) or placebo was administered for 12, 16 or 24 weeks twice-a-day (bid), once in the evening and once in the morning, half an hour before the meal. Patients were evaluated every two to four weeks during their visit to the clinic. They had to skip their morning dose at the day of the visit (for morning visits), or take their dose early morning before the afternoon visit. Patients had to come to the clinic for 'trough' (at the end of dose interval, right before the next dose) evaluation always around the same time. At some visits in some of the studies, 'peak' evaluations were also performed. For 'peak' evaluation, patients had to take the drug immediately after the 'trough' evaluation and be evaluated 2-4 hours later. The number of patients from each study on a 100 mg dose, the duration of treatment, and the number of plasma samples are listed in Table 17.

Table 17. Number of patients, samples and treatment duration.

Study	No. patients on 100 mg dose	Treatment duration (weeks)	Samples per patient up to	
			'Trough'	'Peak'
I	171	24	8	----
II	95	12	5	2
III	133	24	3	----
IV	119	16	3	2

Criteria for inclusion and exclusion of patients were similar across the studies except for some differences in treadmill set-up and in the baseline walking requirements (See Appendix A). In addition, patients in the study II had to complete 3 weeks of low fat, low cholesterol diet prior to the study, and had to adhere to the diet during the study.

Blood samples of non-compliant patients were excluded from the analysis. Non-compliance was defined prospectively in the clinical protocol. A patient was considered non-compliant if he/she took less than 75% of the prescribed drug on 2 or more successive visits or had undetectable plasma levels on 2 or more successive occasions.

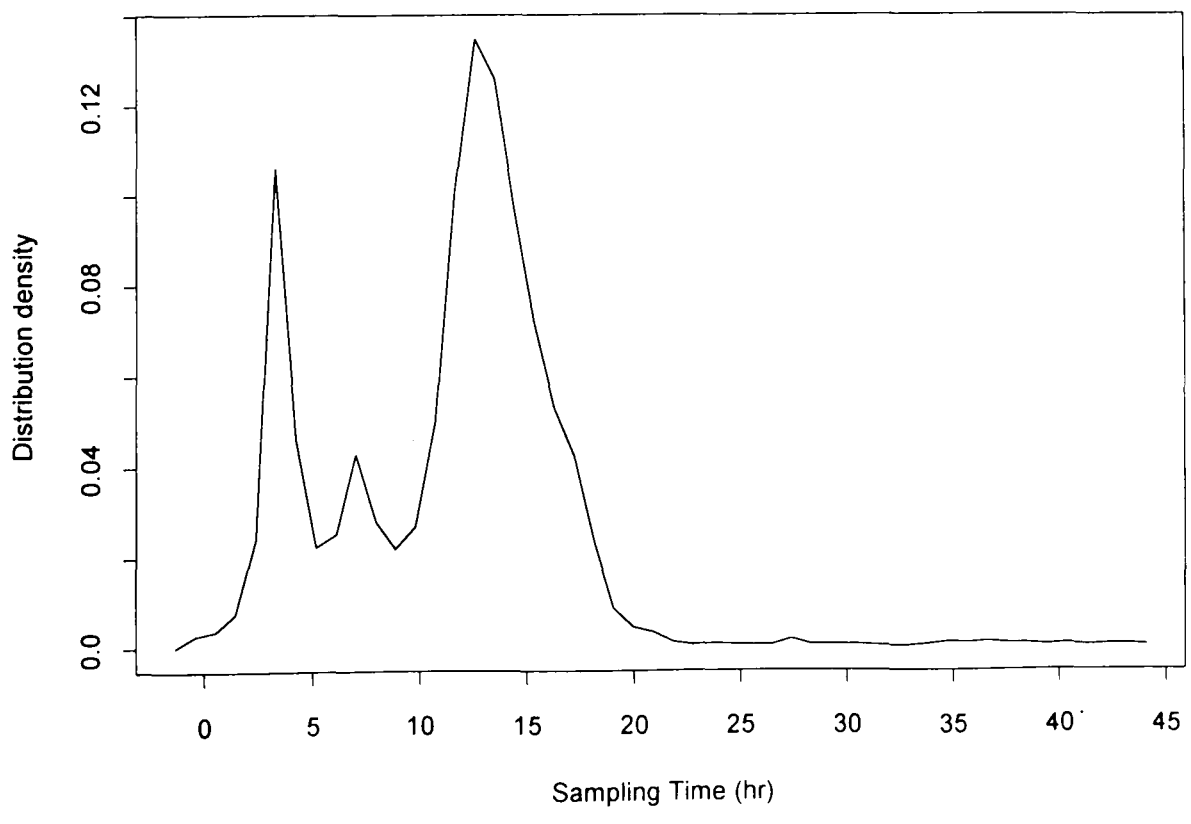

Figure 10. Distribution of sampling times between 0 and 50 hours.

Samples taken later than 20 hours after the last dose were also excluded. There were 28 samples between 20 and 50 hours, which was insufficient to meaningfully define the observation regions (this is discussed later in the Methods, Section 2.3.4). Figure 10 shows the distribution of sampling times between 0 and 20 hours. In addition, there were 39 samples spread from 50 to 3108 hours, which were also excluded from the analysis. The details concerning the excluded observations are described in Appendix B.

In total, 2161 plasma concentrations from 462 patients were used in the analysis.

2.3.3 OBJECTIVES

As before, the objectives of the model-free population pharmacokinetic analysis were to partition patients into exposure levels, and then, explore relationships between the covariates and exposure levels. The list of covariates included:

Demographics:	age, gender, race, weight, body surface area, and obesity;
Lifestyle:	alcohol and smoking habits;
Medical history:	myocardial infarction, cerebro-vascular event, and diabetes;
Disease state:	duration of disease and walking impairment at baseline;
Concomitant medications and medical conditions:	drugs and therapeutic subclasses of drugs used by at least 25 patients.

2.3.4 METHODS

The analysis, as described earlier in Section 2.2.4, was based on partitioning plasma concentrations into observation levels, and assigning each patient an exposure level. An investigation of the relationships between the exposure level and covariates was then conducted. Plasma concentrations were partitioned into observation levels and patients were assigned to exposure levels following the same procedure as in Section 2.2.4. Further steps dealing with the relationships between the exposure and the covariates, were considerably refined and expanded as presented in the following sections.

It was assumed that there was no accumulation of the drug during the studies and the disease progression or drug's pharmacological effects did not affect pharmacokinetics of the drug. The assumption was supported by phase I studies, where steady state was reached by Day 4 of twice-a-day dosing. The earliest plasma concentrations were taken after 2 weeks of dosing in phase III studies, so steady-state should have been reached by the first evaluation.

In Step I, as before, three nonparametric cubic smoothing splines were fitted through the scatter plot of concentration versus time, to estimate partition boundaries as functions of time after the last dose. The span of times after the last dose was not as large as in the previous project, so there was no need for transformation of times. Also, all patients had the same dose throughout all the studies, therefore dose normalisation of concentrations was not necessary. Raw concentrations, not their log-transformations were used for partitioning. Log-transformation of the concentrations in a pharmacokinetic analysis is a customary practice, based on the observation that plasma concentrations are often log-normally distributed in the population. Transforming the data therefore allows one to make mean-based comparisons using the normal theory assumptions. The partitioning algorithm does not use the assumption of normality, so there is no theoretical advantage in using transformed data. There were no reasons to expect that the results depend on whether log-transformed or raw data were used.

The determination of splines depends on the choice of a smoothing parameter. Since the choice of this parameter is somewhat arbitrary, three different values of the smoothing parameter were used. The set of four regions obtained for each value of the smoothing parameter will be further referred to as a Partition set (not a combination as in Section 2.3, since only one parameter influenced the partition).

In Step II, patients were assigned an exposure level according to the algorithm described earlier. It was done for several Partition sets.

The next step, Step III, relates the exposure levels and variability to covariates. Two types of responses were investigated for association with covariates: 1) For patients classified into one of four exposure levels, exposure level represented an ordered categorical response; 2) High and Low variability was another response variable, a

categorical response. Patients assigned to one of the exposure levels represented the Low variability group, patients from the 'unclassified' group represented the High variability group.

Table 18 lists all the covariates, their types and levels (for categorical covariates) that were investigated for association with the exposure level and the variability type. Two measures of obesity (Obesity, OBES, and %above ideal body weight, PIBW) and body surface area (BSA) were computed for each patient from their weight, height and gender as follows [Bayley & Briars, 1996; Rowland & Tozer, 1995]:

$$\text{Ln(BSA)} = -3.751+0.422*\ln(\text{HGT})+0.515*\ln(\text{WGT}), \qquad \text{Eq. 12}$$

where HGT is height (cm), WGT is weight (kg), and BSA is measured in cm^2;

$$\text{IBW} = \begin{cases} 50 + 2.3/2.5* \max(0, \text{HGT} -152) & \text{for males} \\ 45 + 2.3/2.5* \max(0, \text{HGT} -152) & \text{for females,} \end{cases} \qquad \text{Eq. 13}$$

and

$$\text{PIBW} = \quad 100\,(\text{WGT} - \text{IBW}) / \text{IBW}, \qquad \text{Eq. 14}$$

where IBW denotes ideal body weight (kg) and PIBW is percent above ideal body weight.

Obesity (OBES) was defined as 0, if PIBW < 20, and 1, otherwise.

Concomitant medications were considered in two ways: grouped by their generic name (irrespective of the dose and manufacturer) and grouped by subclasses of major therapeutic classes. To be used as covariates, the concomitant medications and groups of medications had to be used by more than 25 patients. The cut-off of 25 patients was decided prospectively as approximately 5% of initial 518 patients, before cleaning the database.

Table 18 Description of covariates

	Covariate	Type
Demographics	gender, race, obesity (>20% above ideal body weight)	Factor
	age, weight, body surface area (BSA) , % above ideal body weight	Continuous
Lifestyle	smoking(never/ previously/current), alcohol (never/ previously/current)	Ordered categorical
Medical history	diabetes, myocardial infarction, cerebro-vascular event	Factor
Disease state	duration of disease (0.5 to 1; 1 to 5 ; 5 to 10; >10 years), baseline walking impairment [a] (Mild/ Moderate/ Severe)	Ordered categorical
Concomitant medications and medical conditions	drugs and therapeutic subclasses of drugs used by at least 25 patients (Yes/No) Individual drugs: acetaminophen, nifedipine, combination vitamins and minerals, lisinopril, nitroglycerin, lovastatin, glyburide, enalapril maleate, atenolol, furosemide, combination diuretics, verapamil hydrochloride, digoxin, gemfibrozil, levothyroxine sodium, vitamin e, diclofenac sodium, potassium chloride, ranitidine hydrochloride, isophane insulin suspension; Groups by therapeutic class: antihistamine drugs, sympathomimetic (adrenergic) agents, cardiac drugs, antilipemic agents, hypotensive agents, vasodilating agents, nonsteroidal anti-inflammatory agents, antidepressants, benzodiazepines, replacement preparations, diuretics (except potassium sparing diuretics), antacids and adsorbents, cathartics and laxatives, misc. GI drugs, insulins, sulfonylureas and thyroid agents	Factor (Yes/No)
Study design	study	Factor

a) Mild - > 200 m; Moderate - from 100 to 200 m; Severe - < 100 m.

To count the number of patients on a particular medication or a group of medications, every patient was assumed to be on a drug, if he/she had at least one plasma sample while on that medication. For each drug/group of drugs that was used by 25 or more patients, the indicator variable of whether a person was on that medication at the time of sampling was recorded for each blood sample.

In total, there were 21 individual drugs and 16 therapeutic subclasses of drugs used by 25 or more patients.

Multivariate Classification Tree-based analysis (CART)

To account for possible confounding by correlated covariates, a binary classification tree was grown by CART methodology using S-Plus (Version 3.3) [Venables & Ripley, 1994]. The attractiveness of the tree approach includes the ability to handle categorical and continuous variables, interaction between variables and missing values of covariates. Also, the tree is invariant to monotone transformations of variables, thus relaxing the distributional requirements for independent and dependent variables [Breiman *et al.*, 1984]. Following in the Remark is the brief description of the methodology:

Remark:

Constructing trees is a modelling technique especially suitable for modelling of a categorical response function of several categorical, factor or continuous variables. Tree based models seek to partition the space of observations into the groups (leaves) that are as homogeneous (with respect to response) as possible within the groups, and as heterogeneous as possible between the groups. The resulting model consists of a partition of the space of observations into a set of leaves and a probability distribution over the levels of response variable for each leaf. The splitting rules uniquely define the leaves. The tree construction process starts with the tree with just one leaf that includes all the observational space. The procedure takes the maximum reduction in deviance (objective function used for the tree modelling, see [Ciampi et al., 1987]) over all allowed splits of this initial leaf to choose the first split. The procedure is repeated until the number of observations in each leaf or its deviance is small. The tree grown by this procedure may overfit the data; i.e. may describe the training data set well while not adequately

describing a new data set. The pruning procedure [Breiman et al., 1984], a methodology analogous to model selection in regression, obtains an optimal subtree by minimising a cost-complexity measure (a sum of the deviance and a term proportional to the tree size) of a sequence of subtrees. An even better way is to grow the tree on one set of data and test it on a different set of data (external validation) or to split the data to use different data for building and predicting (internal validation). A detailed description of the tree-based modelling methods can be found, for example, in [Clark & Pregibon, 1992].

Separate trees were grown for each of three partition sets. Each tree was then pruned and cross-validation was performed [Venables & Ripley, 1994]. For cross-validation, the data set was randomly divided into 10 subsets, the tree was grown for each $9/10^{th}$ of the data, and the sequence of pruned trees was tested on the remaining $1/10^{th}$. Averaging over ten trees for each pruning size gave a cross validated plot of deviance as a function of the tree size. The tree size that corresponded to a minimum deviance was considered to be optimal. An *overparametrised* model (i.e., the model with 1-2 more terminal nodes than in the optimal tree) was considered for further exploration of subpopulations. The goal of allowing 1-2 more covariates than in the optimal tree was to check that those covariates (less important according to the tree) would not be significant in further explorations. This would ensure that the tree model captured all the important covariates.

Univariate analyses

For the covariates identified as significant by the tree models, nonparametric Spearman rank correlation analysis [Snedecor & Cochran, 1980] and subgroup analysis of association between the covariates and response were performed. Subgroups were defined by the tree models and by the correlation between the covariates.

Table 19 describes the types of nonparametric association tests used for different types of variables. The purpose of this analysis was two-fold: first, it was aimed to formally confirm the results of the tree-based analysis of association of covariates with the exposure level for patients classified to an exposure group. Second, for all patients, the analysis was to test the association between patient's variability type (High/Low) and the covariates.

Table 19. Univariate analysis

Data used	Response		Covariate type	Analysis	Reference
	Type	Variable			
All patients except unclassified	Ordinal	Exposure level	Categorical	van Elteren test	Lehmann, 1975
			Continuous	Jonckheere's test	Hollander, Wolfe, 1973; Morris, Dietz, 1989
All patients	Categorical	Variability type	Categorical	Fisher exact test	Kendall, Stuart, 1979; Mehta, Patel, 1983
			Continuous	tables of means and standard deviations, side-by-side box plots	

After the tree models identified influential covariates it was important to estimate the clinical, not statistical importance of these covariates. Therefore, it was important to quantify the effect of covariates. This was done in Step IV.

In Step IV, scatter plots of concentrations were examined for identified subpopulations to obtain quantitative information about differences between those subpopulations. Concentrations from all patients, not only from patients classified into one of four 'exposure levels' were used. Population curves were obtained by lowess regression (S-Plus, version 3.3) [Venables & Ripley, 1984]) fitted to subpopulations. Lowess regression is an iterative robust algorithm that fits weighted locally linear regression to the data. The result is a smooth curve through the data that downweights outliers [Chambers & Hastie, 1992].

Areas under population plasma concentration-time curves (AUPC) were computed and compared for subpopulations. In this case a population curve is a purely empirical

curve, a smoothed 'average' of the data. Therefore the curve can not be extrapolated over the boundaries of the data. This means that the computed area under the curve would depend on the time of the first and the last data point used to compute the curve. Thus, to be able to compare the areas for subpopulations these areas should be computed using the same start and stop time. Area Under the Curve (AUC) is a strictly defined parameter in pharmacokinetics, with defined time intervals (0 to infinity for a single dose or dosing period for a steady state multiple dosing). Time intervals of AUPC would not agree with the traditional definition and would depend on the data points for the subpopulations. Therefore, instead of presenting absolute values of AUPCs, only a comparison of AUPCs for the subpopulations of interest is reported.

Quantification of the differences by comparing AUPCs is a univariate procedure: it accounts for one variable at a time. Correlation analysis of the covariates identified by the tree models was used to determine the appropriate subpopulations for AUPC comparisons.

2.3.5 RESULTS

In total, 2161 plasma concentrations from 462 patients were used in the analysis. Figure 11 shows scatter plots of all available samples for each study. Though timing and amount of data differed across the studies, the range of concentrations was approximately the same for all the studies.

Demographics

In order to combine four studies in one analysis, the study population should be similar. The data in Table 20 - Table 24 describe the distributions of all the covariates across the studies. Table 20 exhibits the distribution (counts and percentages) of categorical demographic covariates. Statistically significant differences (Chi-square test) are marked with the asterisk (*) and p-values are shown for those variables.

Table 21 shows the distribution (counts and percentages) of patients on concomitant medications or therapeutic subclasses of medications. Thirty seven individual drugs or groups were used by 25 or more patients. Of them, 11 were distributed differently

between the studies ($p < 0.05$ in 2-tail Fisher's exact test). Only these medications are presented.

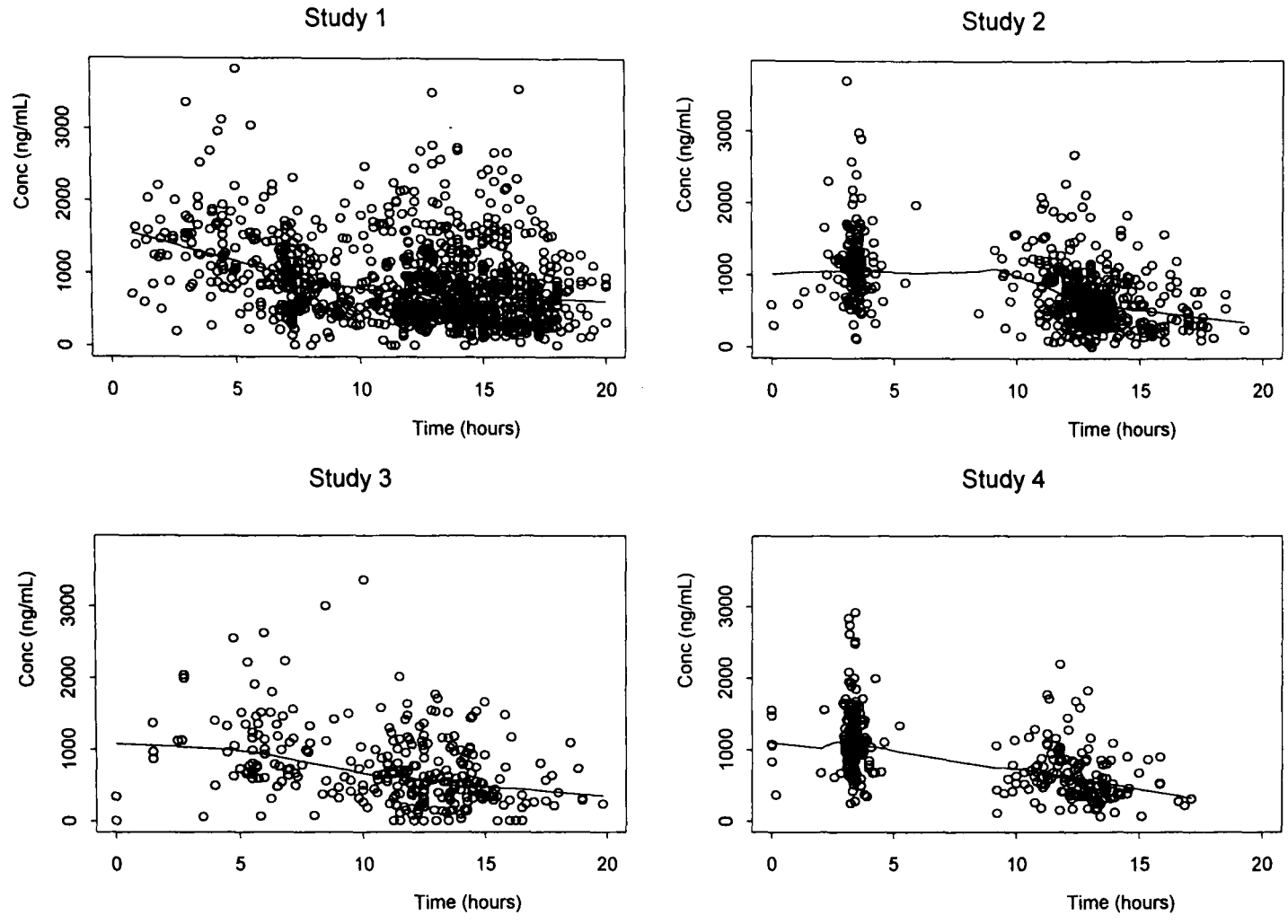

Figure 11. Plasma Concentration versus time after last dose for four studies.

Table 22 - Table 24 show the distributions of continuous variables: age, % above ideal body weight, weight and body surface area across the studies.

Table 20. Distribution of categorical demographic covariates across the studies.

Covariate/ levels (p-value)[a]	Frequency N (%)				
	Study				Total
	I	II	III	IV	
# patients (N)	157	88	116	101	462
Gender:					
Males	119(75.8)	76 (86.4)	88 (75.9)	79 (78.2)	362 (78.4)
Females	38(24.2)	12 (13.6)	22(19)	28 (27.7)	100 (21.6)
Race:					
Caucasian	137(87.3)	77 (87.5)	106 (91.4)	91 (90.1)	411 (89.0)
Other	20(12.7)	11(12.5)	10 (8.6)	10 (9.9)	51 (11)
Obesity					
No	83 (52.9)	49 (55.7)	63 (54.3)	52 (51.5)	247 (53.5)
Yes	74(47.1)	39 (44.3)	53(45.6)	49 (48.5)	215 (46.5)
Current smoking:					
No	97(61.9)	52(59.1)	56(48.3)	56(55.5)	261(56.5)
Yes	60(38.2)	36(40.9)	60(51.7)	45 (44.5)	201 (43.5)
Alcohol consumption:	22(14)	14 (15.9)	21(18.1)		80 (17.3)
Never	44(28)	26 (29.5)	25(21.5)	NA	117 (25.3)
Previous	91 (58.0)	48(54.6)	70 (60.3)		265 (57.4)
Current					
Amount of alcohol:					
Seldom	26 (28.5)	19(39.6)	27(38.6)	19 (33.9)	91 (34.3)
Sometimes	35 (38.5)	21(43.8)	17(24.3)	20(35.7)	93(35.1)
Daily	30 (33.0)	8(16.7)	26(37.1)	17(30.4)	81(30.6)
Disease state *** (0.001):					
Mild	15(9.6)	50(56.8)	11(9.5)	39 (38.6)	115 (24.9)
Moderate	79(50.3)	24(27.3)	52(44.8)	42(41.6)	197 (42.6)
Severe	63(40.1)	14(15.9)	53(45.7)	20(19.8)	150 (32.5)

Covariate/ levels (p-value)[a]	Frequency N (%)				
	Study				Total
	I	II	III	IV	
# patients (N)	157	88	116	101	462
Duration of illness:					
6MO to 1YR	19(12.1)	6 (6.8)	5 (4.3)	6 (5.9)	36 (7.8)
1YR to 5YRS	66(42.0)	42(47.7)	60(51.7)	48(47.5)	216 (46.8)
5YRS to 10YRS	45(28.7)	21(23.9)	30(25.9)	28(27.7)	124 (26.8)
>10YRS	27(17.2)	19(21.6)	21(18.1)	19(18.8)	86 (18.6)
Diabetes:					
NO	115(73.2)	72 (81.8)	87(74.0)	77(76.2)	351(75.9)
YES	42(26.8)	16(18.2)	29(25.0)	24(23.8)	111(24.0)
Myocardial infarction:					
NO	122(77.7)	78(88.6)	95(81.9)	80(79.2)	375(81.1)
YES	35(22.3)	10(11.4)	21(18.1)	21(20.8)	87 (18.8)
Cerebro-vascular event**(0.002):					
NO	148(94.2)	73(82.9)	92(79.3)	88(87.1)	401(86.8)
YES	9(5.73)	15(17.05)	24(20.69)	13(12.87)	61(13.2)

a) indicator of significance: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Table 21. Use of concomitant medications

Concomitant medication or therapeutic subclass (p-value)[a]	Frequency N (%)				
	Study				Total
	I	II	III	IV	
Acetaminophen*** (0.0005)	46 (29.3)	14 (15.9)	41 (35.3)	15 (14.9)	116 (25.1)
Lovastatin*** (0.0003)	14 (8.9)	0 (0.0)	18 (15.5)	10 (9.9)	42 (9.1)
Verapamil hydrochloride (0.0001)	21 (13.4)	1 (1.1)	2 (1.7)	4 (4.0)	28 (6.1)
Gemfibrozil* (0.011)	9 (5.7)	0 (0.0)	8 (6.9)	10 (9.9)	27 (5.8)
Ranitidine hydrochloride (0.0000)	16 (10.2)	0	12 (10.3)	0	28 (6.1)
Diclofenac sodium* (0.041)	9 (5.7)	4 (4.6)	11 (9.5)	1 (1.0)	25 (5.4)
Potassium chloride*** (0.0006)	8 (5.1)	5 (5.7)	17 (14.7)	1 (1.0)	31 (6.7)
Diuretics *** (0.0007)	92 (58.6)	31 (35.2)	69 (59.5)	46 (45.5)	238 (51.5)
Sympathomimetic agents *** (0.0000)	37 (23.6)	0	43 (37.1)	24 (23.8)	104 (22.5)
hypotensive agents *** (0.0001)	20 (12.7)	3 (3.4)	19 (16.4)	2 (2.0)	44 (9.5)
cathartics and laxative* (0.032)	17 (10.8)	10 (11.4)	27 (23.3)	14 (13.9)	68 (14.7)

a) indicator of significance: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Table 22. Distribution of age and % above Ideal Body Weight across the studies

Study	N	Age				% above Ideal Body Weight			
		Median	Mean	Std	Range	Median	Mean	Std	Range
All	462		64.6	9.4	40-91		20.0	20.8	-31-93
I	157	65	64.2	8.4	42-85	18	18.4	21.6	-31-84
II	88	70	67.3	8.9	43-85	17.5	18.8	17.7	-28-63
III	116	64	63.0	10.4	40-85	17	20.2	19.8	-18-81
IV	101	65	64.7	9.5	46-91	19	23.5	23.1	-25-93

Table 23. Distribution of Weight across the studies

Study	Males					Females				
	N	Median	Mean	Std	Range	N	Median	Mean	Std	Range
I	119	82.7	82.7	14.8	45-115	38	64.3	67.6	14.5	41.8-95.8
II	76	83	84.0	14.4	47-126	12	61	62.9	15.1	36-89
III	88	83.5	83.9	13.3	58-118	28	70	70.2	13.9	48-99
IV	79	84	86.8	15.8	54-130	22	69.5	72.6	15.6	42-100

Table 24. Distribution of Body Surface Area by study

Study	Body Surface Area									
	Males					Females				
	N	Median	Mean	Std	Range	N	Median	Mean	Std	Range
I	119	2.0	2.0	0.2	1.5-2.4	38	1.7	1.7	0.2	1.4-2.1
II	76	2.0	2.0	0.2	1.5-2.6	12	1.7	1.7	0.2	1.2-2.0
III	88	2.0	2.0	0.2	1.7-2.5	28	1.8	1.8	0.2	1.5-2.2
IV	79	2.0	2.1	0.2	1.6-2.7	22	1.8	1.8	0.2	1.3-2.2

The data in the tables show that the distributions across the studies were similar for most covariates. The exceptions were Disease State, the history of cerebro-vascular event and several concomitant medications. Disease State was measured as a baseline walking distance on a treadmill test until a patient could walk no longer. It was recorded as follows: Mild - > 200 m; Moderate - from 100 to 200 m; Severe - < 100 m. Studies I and III had more moderate and severe patients than studies II and IV. These differences could be attributed to the differences in treadmill set-up between the studies (See Appendix A) The number of patients with the history of cerebro-vascular event ranged from 9 (6%) in the Study I to 24 (21%) in the Study III.

In total, there were 21 individual drugs and 16 therapeutic groups of drugs used by 25 or more patients. Of them, 7 individual drugs and 4 groups were distributed differently between the studies. Some of the differences were due to differences in exclusion criteria for concomitant medications.

Partitioning

Table 25 displays the percentage of observations assigned to each of the four observation levels by each partition set. As can be seen, all three partition sets had similar frequencies of observations in the respective observation levels with fewer observations in high groups.

Table 25. Frequencies of observations in observation levels.

Partition set	Value of smoothing parameter	Observation level [a]			
		1	2	3	4
1	1	28%	30%	27%	15%
2	10	28%	30%	27%	15%
3	100	28%	30%	27%	15%

a) 1 is the lowest, 4 is the highest observation level

Table 26 displays the distribution of patients among exposure levels generated by each of three partition sets. All the distributions were similar. About 8% of patients were not assigned to exposure levels because they had plasma concentrations in both the

first and fourth observation levels. The smaller numbers in the fourth exposure level suggest that many patients had occasionally, but not consistently, high plasma levels.

Table 26. Distribution of patients among exposure levels

Partition set	Value of smoothing parameter	Exposure levels				
		1	2	3	4	Unclassified [a]
1	1	23%	37%	23%	9%	8%
2	10	23%	37%	23%	10%	8%
3	100	23%	37%	23%	9%	8%

a) Concentrations span four observation levels

Table 27 displays the distribution of patients according to the spread of their plasma concentrations among the four observation levels. The results were similar between the partition sets. On average, 29% of the patients were in the most consistent All-in-one category, and another 38% were in the Two-adjacent category.

Table 27. Distribution of patients among patient types

Partition set	Patient type			
	All-in-one [a]	Two adjacent [b]	Three adjacent [c]	All four [d]
1	28%	39%	26%	8%
2	29%	38%	25%	8%
3	30%	37%	26%	8%

a) All concentrations in one observation level;

b) All concentrations in two adjacent observation levels;

c) All concentrations in three adjacent observation levels;

d) Concentrations span four observation levels.

For each of the three partitions, two earlier studies, I and II, had a greater percentage of patients in All-four category thus assigned to the unclassified group.

Tree Models

Cross-validation of the tree models showed that the trees of size 2-3 (depending on the partition set) had the minimum deviance with the next increase at size 5. Figure 12 shows the plot of deviance versus tree size for one partition set. Sequence of pruned trees for the whole data set confirmed it (Figure 13), consequently trees with 5 terminal nodes were chosen as the final models. Tree models for all three partition sets identified the same covariates: concomitant use of diltiazem, current smoking, age (split at 49.5 or 50.5), and concomitant use of nitroglycerin (Figure 14 - Figure 16). Partition set I differed from partition sets II and III in whether current smoking or age was split first. In the partition set I smokers were split at age 49.5, whereas in partition sets II and III elderly patients (age >50.5) were split according their current smoking status.

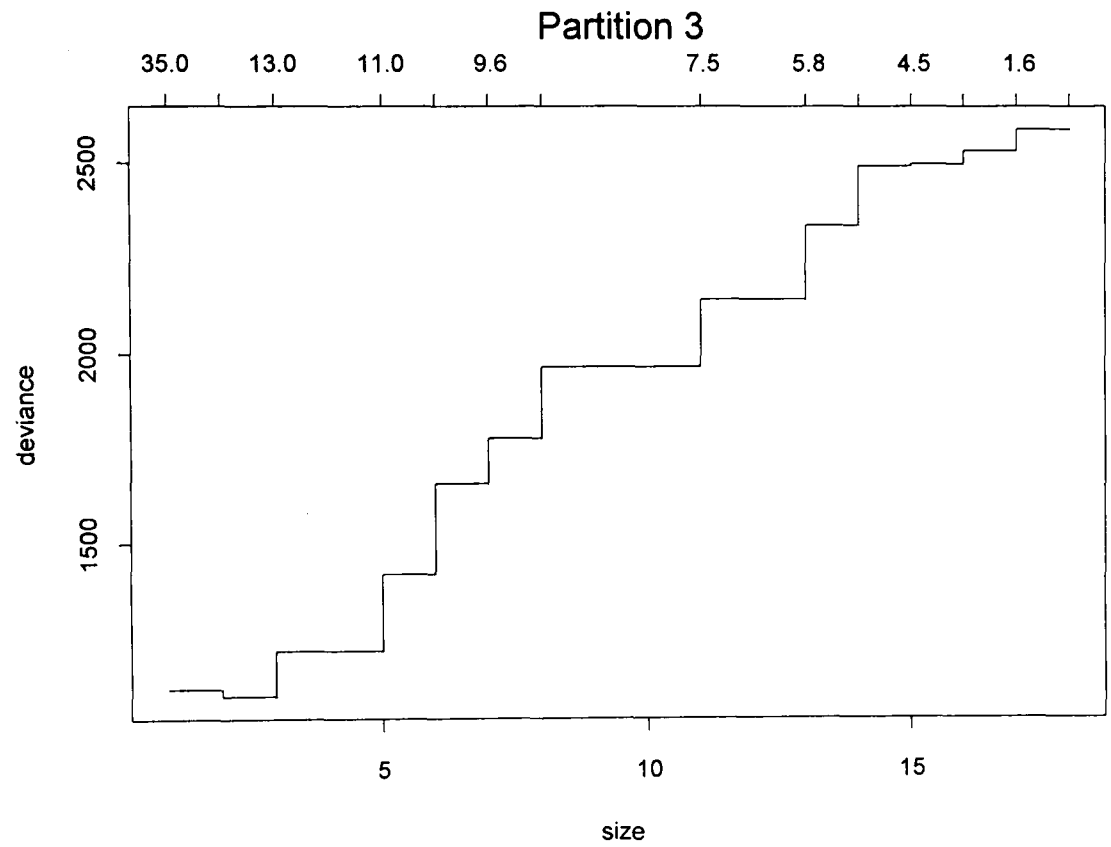

Figure 12. Cross-validation: deviance versus tree size for Partition 3.

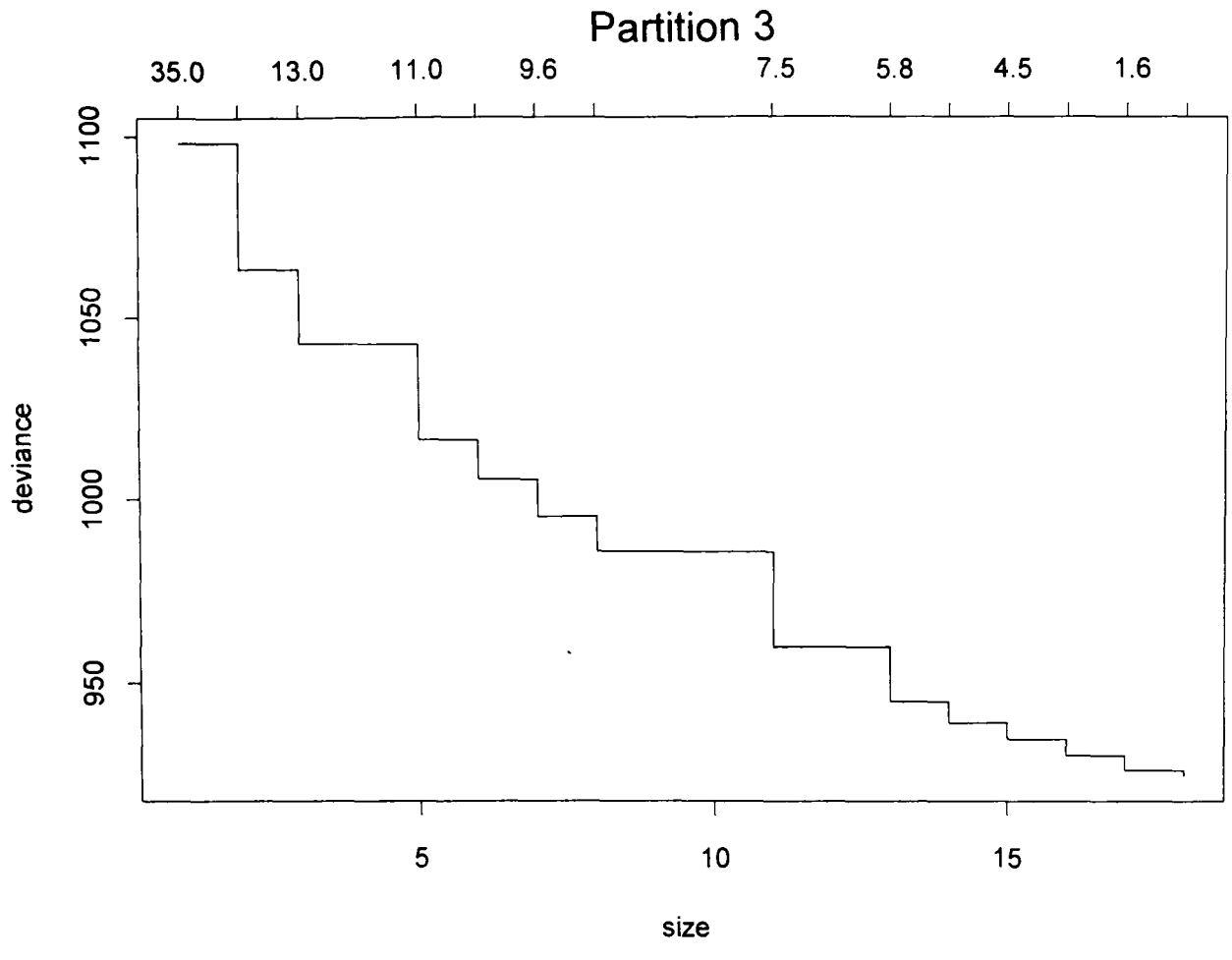

Figure 13. Whole data set: deviance versus tree size for Partition 3.

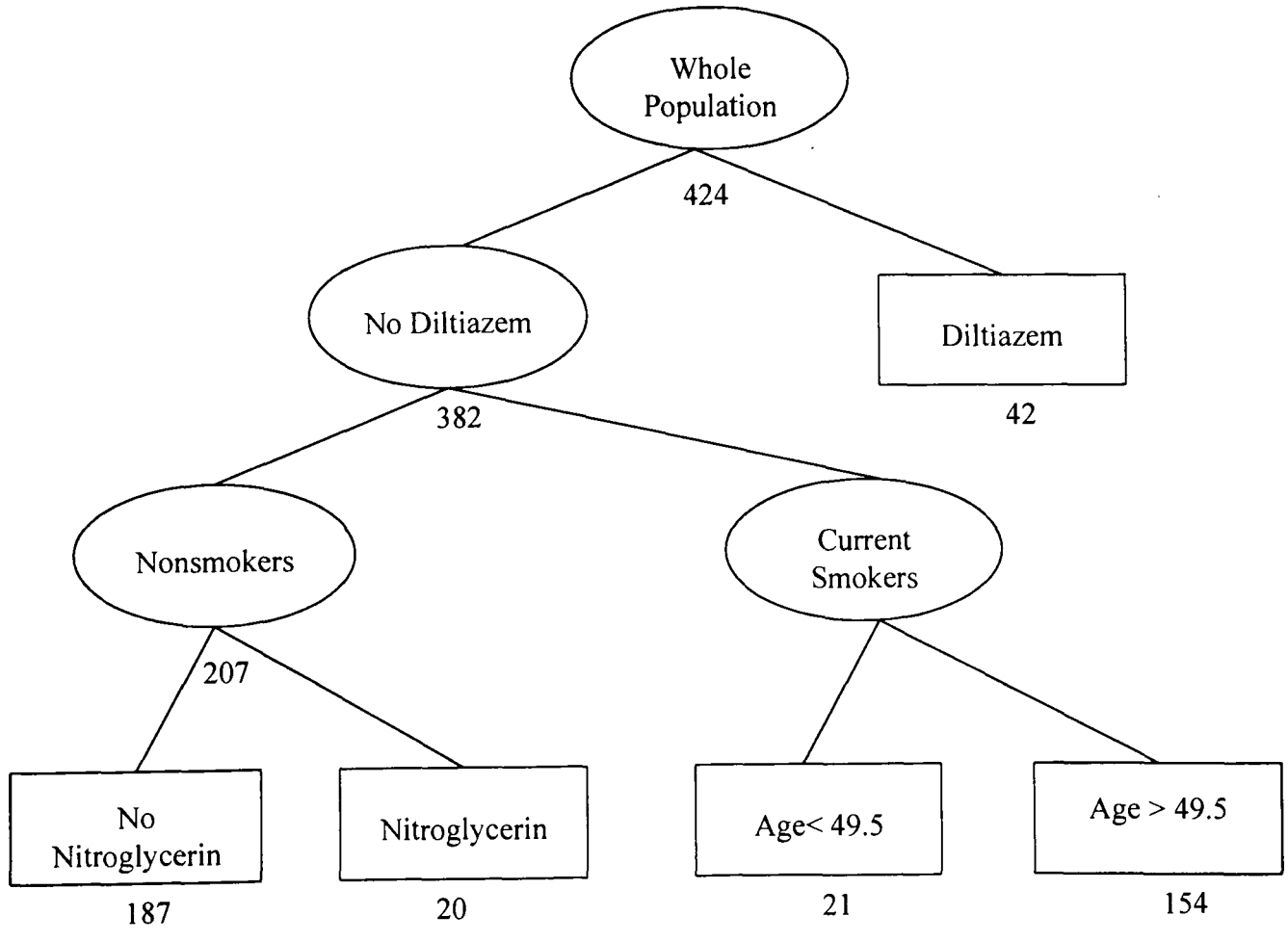

Figure 14. Final tree model for Partition set 1.

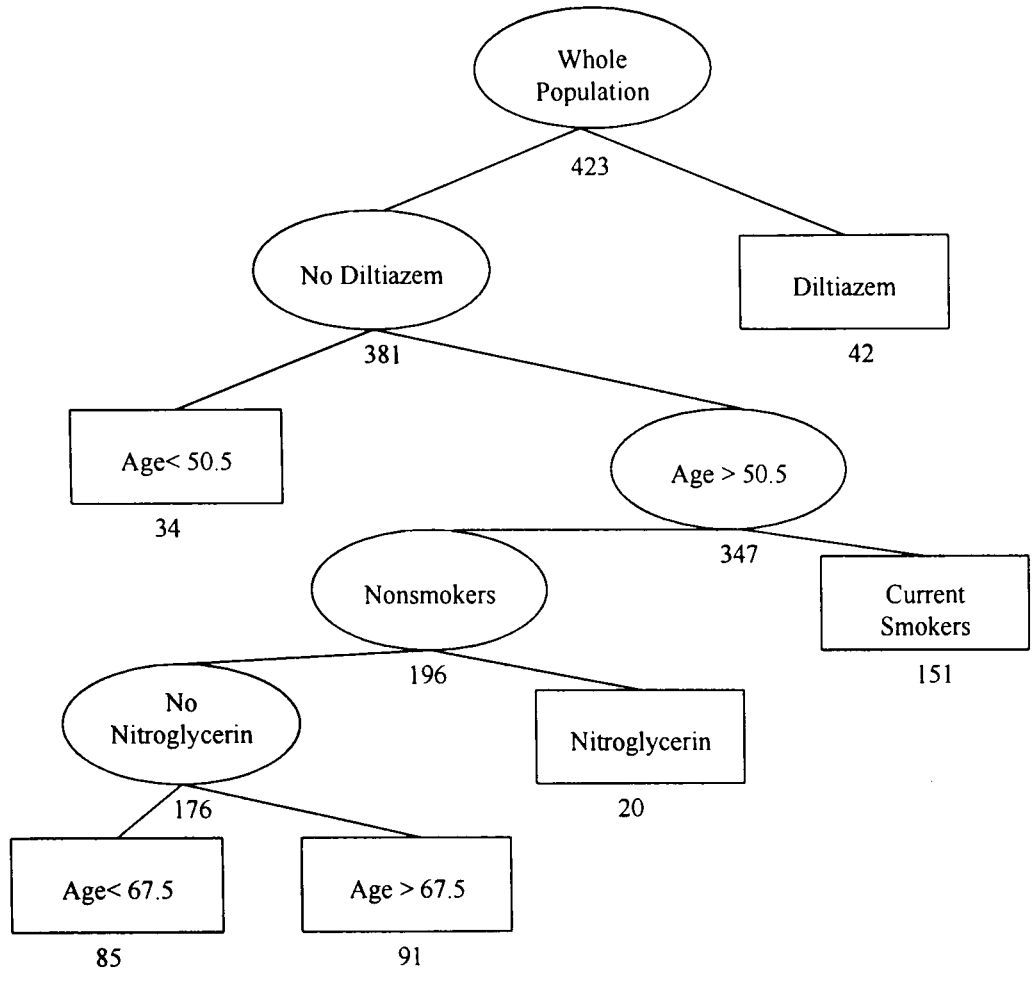

Figure 15. Final tree model for Partition set 2.

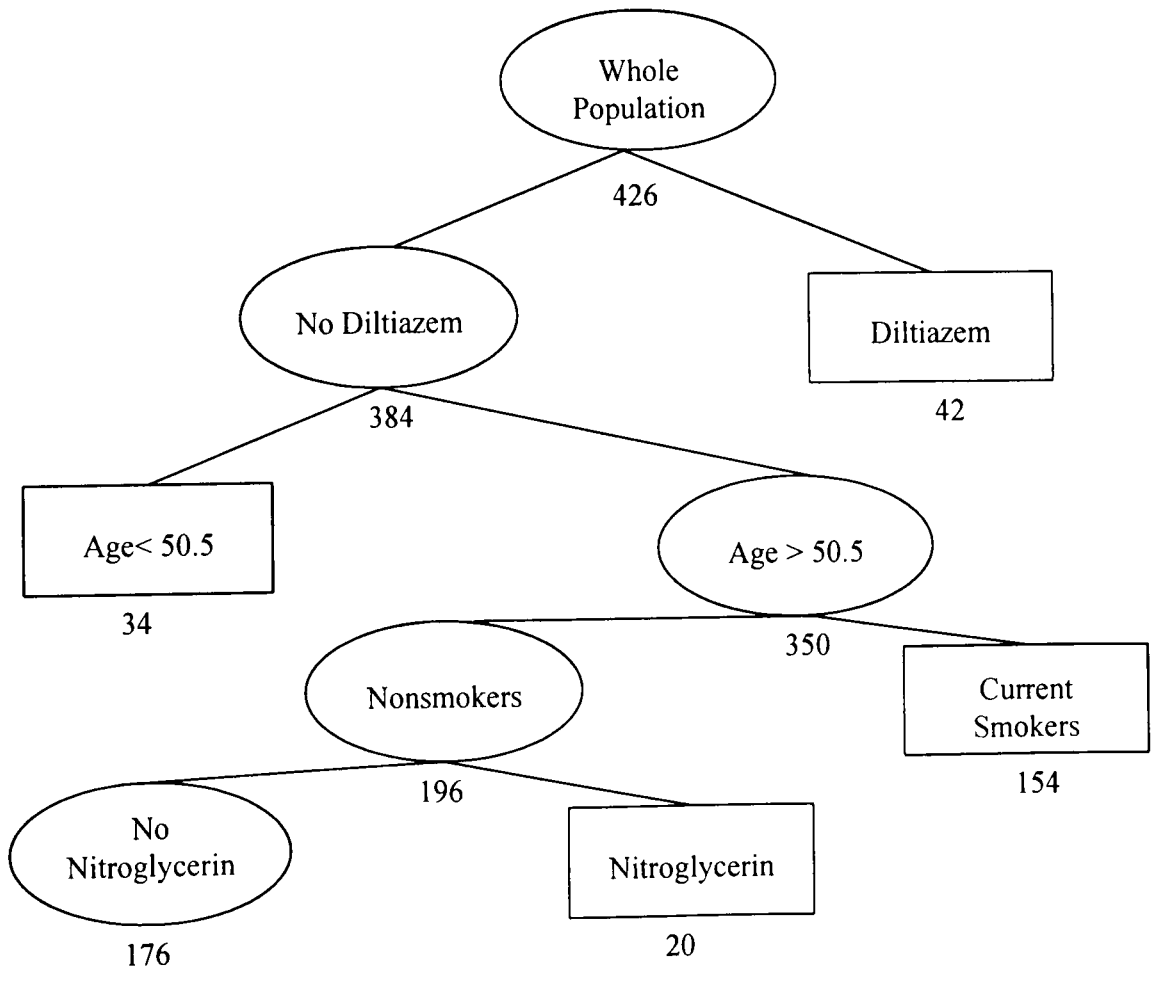

Figure 16. Final tree model for Partition set 3.

Analysis of subpopulations

Correlation analysis (nonparametric Spearman correlation) for the variables identified by the trees showed that current smoking and age were highly negatively correlated (r=-0.38, p=0.0001); smoking was also negatively correlated with diltiazem (r=-0.10, p=0.0001). Nitroglycerin was correlated with all three, positively with diltiazem (r=0.18, p=0.0001), negatively with smoking and age (p=0.004 and p=0.02, respectively). This means that younger patients tended to smoke more than older patients did. Patients not taking diltiazem or nitroglycerin smoked more than patients on these medications did. Patients taking nitroglycerin were more likely to co-administer diltiazem as well, and a greater percent of younger patients were taking nitroglycerin as compared to the elder patients. All of these correlations, except the negative correlation of nitroglycerin and age, were expected. Therefore the following subpopulations were considered for further exploration:

1. Patients taking diltiazem versus patients not taking diltiazem;
2. Current smokers versus non-smokers among patients without diltiazem;
3. Current smokers versus non-smokers separately for young (<=50) and elderly (>50), among patients without diltiazem;
4. Young (<=50) versus elderly (>50);
5. Young (<=50) versus elderly (>50) for current smokers and non-smokers separately, among patients without diltiazem;
7. Patient taking nitroglycerin versus patients not taking nitroglycerin among non-smokers without diltiazem;
8. Patient taking nitroglycerin versus patients not taking nitroglycerin among elderly (>50) non-smokers without diltiazem.

Table 28 exhibits the results of the comparison of Areas Under Population Curves (AUPCs) and statistical inferences regarding relationships between exposure level and covariates for these subpopulations. Figure 17 - Figure 19 show concentration-time plots for subpopulations with population curves superimposed.

Comparison of AUPCs and statistical tests of association were performed based on the order of the covariates in the tree model and correlation between them. The concomitant use of diltiazem and age were compared in the whole population, smoking - in patients not taking diltiazem, and nitroglycerin - in non-smokers not

taking diltiazem. These comparisons showed a 53% AUPC increase in patients on diltiazem, 20% increase in patients older than 50 years old, 18% decrease in smokers, and essentially no change in patients on nitroglycerin.

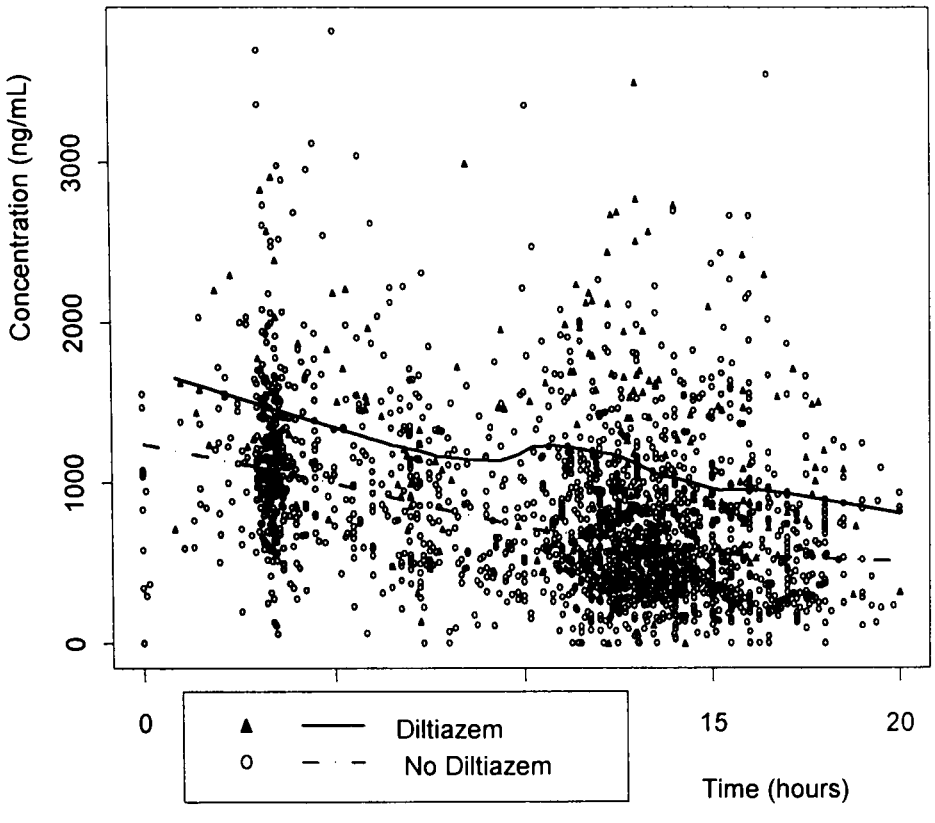

Figure 17. Observed plasma concentrations and population curves: patients taking diltiazem versus no diltiazem

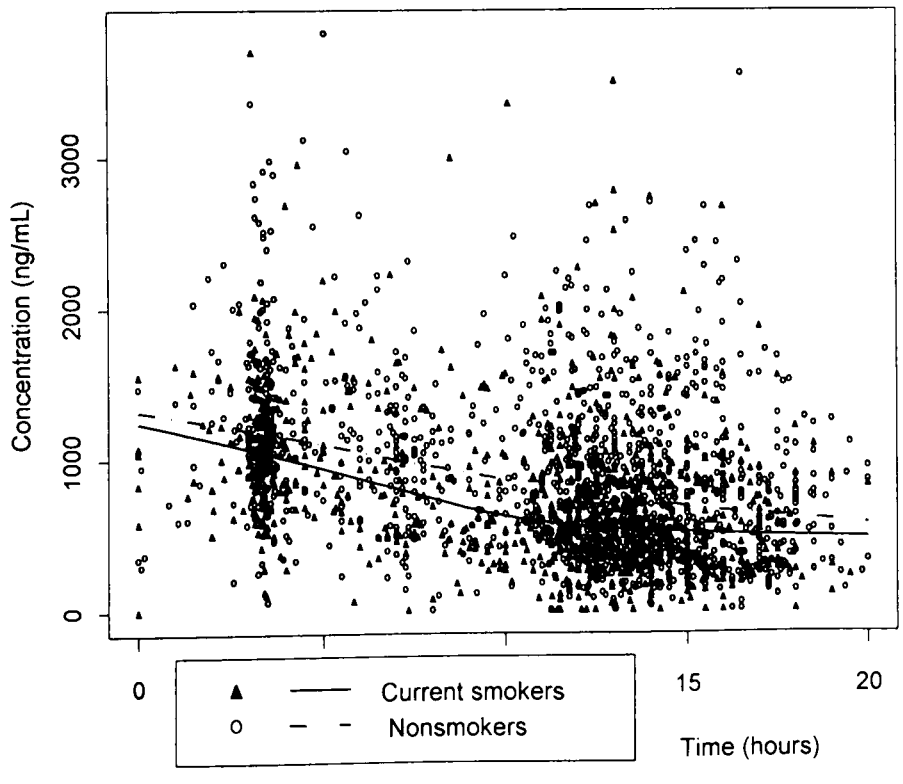

Figure 18. Observed plasma concentrations and population curves for patients not taking diltiazem: smokers versus non-smokers.

Table 28. Percent change in AUPCs and p-values of van Elteren or Jonkheer's tests, for categorical and continuous variables, respectively, subpopulations

Subpopulation			Variable (levels)	%change AUPC [a]	Significance [b,c]
All			Diltiazem (yes/no)	53	***
			Age (≤50/>50)	20	***
			Age (continuous)	Not applicable	**
No diltiazem			Current smoking (yes/no)	-18	***
	elderly (> 50)		Current smoking (yes/no)	-14	***, **, **
	young (<=50)		Current smoking (yes/no)	Not enough data for valid inference (13 non-smoking young patients)	
	smokers		Age (<=50/> 50)	Not enough data for valid inference (21 smoking young patient)	
	smokers		Age (continuous)	Not applicable	-
	non-smokers		Nitroglycerin (yes/no)	-0.7	-
	non-smokers	elderly (> 50)	Nitroglycerin (yes/no)	-1.3	-

a) % change of AUPC of the first level comparing to the second level;

b) van Elteren test for categorical variables, asymptotic Jonkheer's test for continuous variables; indicator of significance:
- $p \geq 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$;

c) Three sets of indicators correspond to partition sets I, II, and III. One set indicates the same significance for all three partition sets.

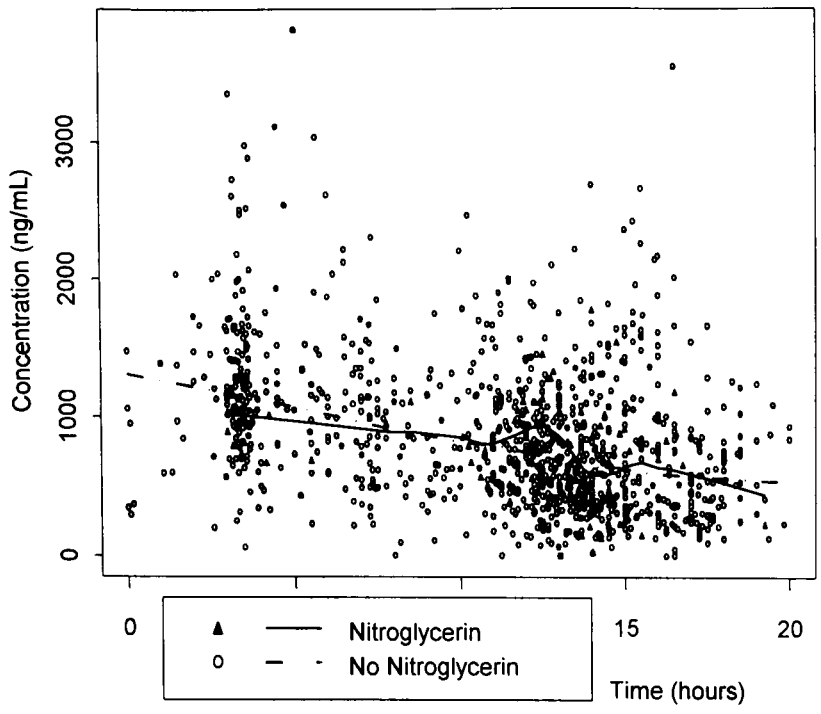

Figure 19. Observed plasma concentrations and population curves for non-smokers not taking diltiazem: nitroglycerin versus no nitroglycerin

The tree models identified age as one of the predictors, with a split at approximately 50 years. Age was highly negatively correlated with smoking. Because of this high correlation, the small differences in assigning patients to the exposure levels in different partition sets led to interchangeable effects of age and smoking in the tree models. Not surprisingly, the univariate test of the effect of age showed an increase in exposure with age and approximately the same as for smoking percent change in AUPC for young (< 50 years) versus older (> 50 years) patients. However, for smokers neither the test of significance nor the box plots (Figure 20) were able to detect an influence of age as a continuous variable.

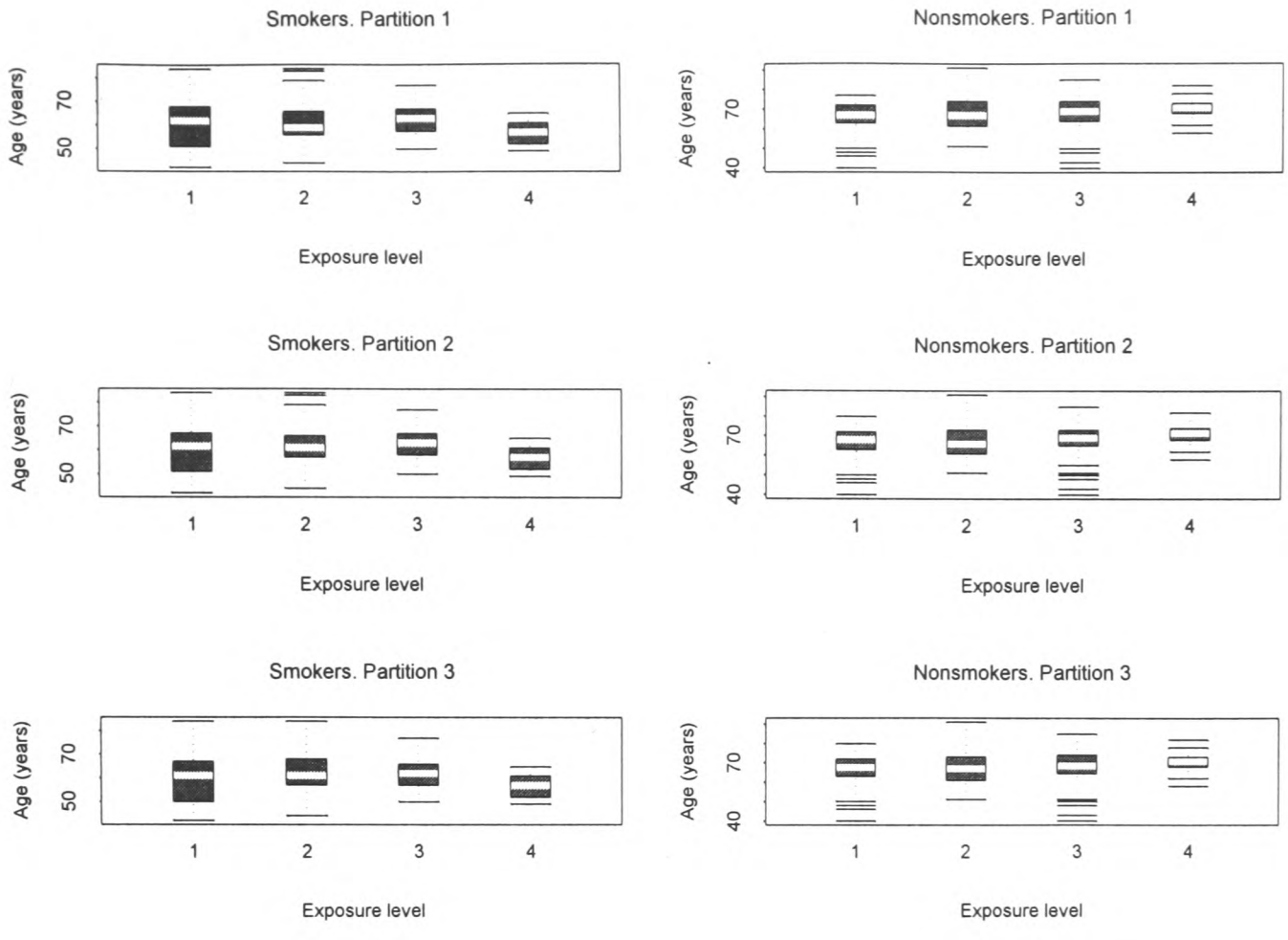

Figure 20. Age versus exposure level for smokers and non-smokers.

The tree models were chosen to be overparametrised, i.e. to have more terminal nodes (covariates) than the optimal trees. The expectation was to show that the least important covariates in the final tree model would not be judged significant by AUPC comparisons and univariate analysis. Thus, no difference for the concomitant use of nitroglycerin confirmed that all the important covariates were captured in the tree models.

As can be seen from the above table, influence of the covariates estimated by the AUPCs comparisons was in agreement with that given by the statistical inferences regarding the importance of the covariates. Both analyses were performed on the same subpopulations, but using different methods. The population curves were constructed by smooth regression on the scatter plot of all the concentrations. This corresponds to the naïve pooled approach: all concentration data points are viewed as being independent, i.e. as if coming from one individual. Statistical procedures did not use this assumption. They used one response point (the exposure level) for each person and the response variable was constructed to reflect the population nature of observations. However, the response variable was categorical, not continuous, thus

reducing the informational content of the concentration data. Thus, both approaches have their weak points. They complement each other, and their agreement adds confidence to the results.

The Fisher exact test was used to test the independence of the variability type (Low: classified versus High: unclassified patients) on the covariates. None of the covariates identified as significant predictors of the exposure by the tree model showed differences between classified and non-classified patients. This result indicates that the exclusion of high variability group of patients from the analysis did not have an impact on identification of important covariates.

2.3.6 CONCLUSIONS

On the basis of this analysis the following conclusions were made:

1. Concomitant administration of diltiazem increased the drug exposure by 53%;
2. Smoking decreased exposure by 18%;
3. No other covariates explored were predictors of the drug exposure.
4. The nonparametric method developed was shown to be capable of identifying predictors of exposure and quantifying the influence of important covariates.

2.4 Model-Free Population Pharmacokinetics: Simulation Study

2.4.1 BACKGROUND

The objective of the work described earlier was to develop a robust nonparametric technique, helpful in elucidating the structure of the data. In particular, the goal was to identify the covariate predictors of total exposure, the metrics related to AUC or clearance, the most important pharmacokinetic parameters. In the series of data analysis projects the method grew from crude qualitative one to a complex quantitative technique that used a variety of modern statistical instruments.

The developed method is a purely empirical technique with no theoretical support. The questions of bias and precision of the AUPC estimates, of sensitivity and robustness of assignment algorithm and tree models needed to be addressed.

This project attempts to answer some of these questions. It is achieved by performing a series of simulations to test the performance of the developed technique under different conditions and to compare the estimates with the true AUPCs.

2.4.2 OBJECTIVES

The general objective of this investigation was to study utility and robustness of the method, that is:

1. to assess its ability to identify subpopulations with altered exposure;
2. to investigate AUPC as a measure of exposure of the subpopulation.

Specifically, the effect of variability, relative contributions and distributions of covariates, and the choice of smoothing parameters on the outcome was studied.

2.4.3 METHODS

Generation of the demographic data

Firstly, demographic data for 1000 subjects was simulated. The real demographic data from the last project of the previous chapter (anti-platelet drug), frequencies of the covariates and correlations between them, was taken to be the basis for this simulation. Each simulated individual had five covariates: four categorical and one continuous. The categorical covariates mimicked the distributions of concomitant

diltiazem (Conmed), smoking (Smok), concomitant disease (ConDisease), and gender (Gender) of the real data. The continuous covariate (Age) did not follow the empirical distribution. Rather, to study the influence of its distribution on the results, it was simulated twice: to be uniformly distributed in one simulation, and normally distributed in the other.

Conmed, Smok, ConDisease, and Gender had values of 0 and 1, Age was a continuous variable between 40 and 80. Conmed and ConDisease followed the Bernoulli distribution with the probabilities 0.0996 and 0.0952, respectively. Smoking followed the Bernoulli distribution with the probability 0.66 if Age was less than 60 and 0.3, otherwise. Gender followed the Bernoulli distribution with a probability of 0.5. Age was uniformly or normally (with mean 60 and standard deviation of 6.67 years) distributed. The correlation between Age and Smok was equal to -0.34, and the correlation between Conmed and ConDisease was 0.18.

Generation of the pharmacokinetic data

Secondly, plasma concentration-time data was generated for each individual using the following models:

Typical population clearance Cl_{pop} was modelled to be a function of Age, Conmed, ConDisease and Smok as follows:

$$Cl_{pop} = Cl_0 \{1 - \theta_{Age}[\frac{Age - \min(Age)}{\max(Age) - \min(Age)}] - \theta_{Conmed} * Conmed - \theta_{ConDisease} * ConDisease + \theta_{Smok} * Smok\}, \quad \text{Eq. 15}$$

where Cl_0 is the typical clearance for a non-smoker with no Conmed or ConDisease at the lowest age in the population of simulated individuals. Its value was set to be equal to the mean clearance of the anti-platelet agent, Cl_0 = 13.6 L/h. Several *weighting schemes* (combinations of θ's) were used to study the effect of relative contributions of covariates. They were:

$$
\begin{aligned}
1:&\quad \theta_{Age} = 0.45, \quad \theta_{Conmed} = 0.2, \quad \theta_{\text{Smok}} = 0.15, \quad \theta_{\text{ConDisease}} = 0.35 \\
2:&\quad \theta_{Age} = 0.4, \quad \theta_{Conmed} = 0.2, \quad \theta_{\text{Smok}} = 0.1, \quad \theta_{\text{ConDisease}} = 0.4 \\
3:&\quad \theta_{Age} = 0.3, \quad \theta_{Conmed} = 0.3, \quad \theta_{\text{Smok}} = 0.15, \quad \theta_{\text{ConDisease}} = 0.4 \\
4:&\quad \theta_{Age} = 0.05, \quad \theta_{Conmed} = 0.2, \quad \theta_{\text{Smok}} = 0.15, \quad \theta_{\text{ConDisease}} = 0.35 \\
5:&\quad \theta_{Age} = 0.05, \quad \theta_{Conmed} = 0.3, \quad \theta_{\text{Smok}} = 0.1, \quad \theta_{\text{ConDisease}} = 0.5
\end{aligned}
\qquad \text{Eq. 16}
$$

Note that Gender variable was not used as a predictor of clearance.

Individual clearance Cl_i and volume V_i were modelled to be log-normally distributed in the population (proportional inter-individual error):

$$
\begin{aligned}
Cl_i &= Cl_{pop} \exp(\eta_{Cl,i}), \qquad \eta_{Cl} \sim N(0, cv_\eta^2), \\
V_i &= V_{pop} \exp(\eta_{V,i}), \qquad \eta_V \sim N(0, cv_\eta^2),
\end{aligned}
\qquad \text{Eq. 17}
$$

with the realistic and rather large coefficient of variation cv_η of 30%. Population volume of distribution V_{pop} was taken to be equal to that of the anti-platelet drug, V_{pop} = 171.5 L.

Predicted individual concentrations were computed according to the one compartment model, with the first order absorption and the first order elimination following a single dose [Gibaldi & Perrier, 1982], according to the equation:

$$
C_{ij,pred}(t_j) = \frac{k_a}{k_a - k_{e,i}} * \frac{D * F}{V_i} * (e^{-k_{e,i}t_j} - e^{-k_a t_j}) \ , \qquad \text{Eq. 18}
$$

where individual elimination rate constant $k_{e,i}$ was

$$
k_{e,i} = \frac{Cl_i}{V_i} \ , \qquad \text{Eq. 19}
$$

absorption rate constant k_a = 0.5 1/h (as for the anti-platelet drug), and the absorbed dose was D*F =1 (an arbitrary scale factor).

Proportional intra-individual error was assumed for observed concentrations, i.e.

$$C_{ij}(t_j) = C_{ij,\,pred}(t_j) * exp(\varepsilon_{ij}), \qquad \varepsilon \sim N(0, cv_\varepsilon^2) \quad . \qquad \text{Eq. 20}$$

To study the effect of variability, a range of cv_ε from 0 to 50% was investigated, concentrations with cv_ε = 0%, 10%, 15%, 20%, 30%, 50% were generated.

For each individual, 1 to 8 time points were randomly selected from the following set of times between 0 and 20 hours: 0, 0.0833, 0.1667, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.25, 2.5, 2.75, 3, 3.25, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.25, 9.5, 9.75, 10, 10.25, 10.5, 10.75, 11, 11.5, 12, 12.5, 13, 13.5, 14, 15, 16, 18, 20. Plasma concentrations were computed in the chosen points according to (Eq. 15) - (Eq. 20).

Not all combinations of all possible options, described above, were implemented. In the first three columns, Table 29 lists the combinations of options used to generate the data. In total, 16 data sets with concentration and demographic data were created, 4 of them with the uniform and 12 with normal distribution of the continuous covariate.

Table 29. Combination of options used for data generation and for analysis.

Intra-subject variability (%)	Distribution of Age	Weighting scheme (case number)	Value of smoothing parameter[a]
0	uniform	1	1, 10, 100
		2	
		3	
		4	10
0, 10, 15, 20, 30, 50	normal	1	10
		5	

a. Values of a smoothing parameter used for generating observation level boundaries.

Analysis

The first goal of the analysis was to qualitatively assess the ability of the partitioning and tree modelling methodology to identify the influential covariates. Therefore, each of the created data sets was subjected to model-free population analysis as described earlier.

Namely, a scatter plot of observed concentrations versus time was divided into four observation levels, using smoothing splines for partitioning. Each patient was then assigned a patient level according to position of his observations in regard to the observation level boundaries. Influence of covariates on the patient level was then studied by CART as described earlier. As before, cross-validation was used as the model discrimination criterion in search of the optimal tree size.

To study dependence of the results on a value of the smoothing parameter, three values of 1, 10, and 100, were used (See Table 29). Partitioning was performed with each of these values, and resulting trees were compared.

Ability of the tree models to identify the covariates was qualitatively compared for data sets with different sets of θ's, different intra-subject variability, and different distributions of the continuous covariate Gender (normal and uniform distributions were studied).

The second goal was to assess accuracy of AUPC as a measure of exposure for identified subpopulations, or more precisely, accuracy of relative change in AUPC for subpopulations as a measure of difference in exposure for the respective groups.

To do this, area under the curve between 0 and 20 hours ($AUC_{0\text{-}20}$) was analytically computed for each subject according to

$$AUC_{0-\tau,i} = \frac{k_a}{k_a - k_{e,i}} * \frac{D * F}{V_i} * \left(\frac{1}{k_{e,i}} - \frac{e^{-k_{e,i}\tau}}{k_{e,i}} - \frac{1}{k_a} + \frac{e^{-k_a\tau}}{k_a}\right), \qquad \text{Eq. 21}$$

where $\tau = 20$.

Mean or median values of $AUC_{0-\tau,i}$ over all individuals in a subpopulation were regarded to be true AUPC ($AUPC_{true}$), $AUPC_{true,\,mean}$ and $AUPC_{true,\,med}$. Empirical

AUPCs for subpopulations were computed as described earlier, by fitting weighted locally linear regression (lowess) curve to concentrations of the respective groups. True and empirical percent changes in AUPCs between subpopulations were then compared for data sets with different numbers of subjects, different sets of θ's, different residual variability, and different distributions of the continuous covariate.

In total, 40 different partition sets were analysed: tree models were created for them, true and empirical AUPCs for subpopulations were computed and compared.

2.4.4 RESULTS

No intra-individual variability

Six data sets with no intra-individual variability employed four weighting schemes for covariates with uniformly distributed Age, and two weighting schemes with normally distributed Age. Three of those with uniformly distributed Age were analysed three times each, with different values of smoothing parameters.

In all the cases tree models identified the correct set of covariates. Conmed, ConDisease, Age and Smok always appeared in the models, and Gender was never chosen. Order of categorical covariates in the models (closeness to the root) always corresponded to the weighting scheme: the greater the change in AUPC for respective subpopulations, the closer was the covariate to the root of the tree. For the continuous covariate, the result was not the same. Age was chosen as the first splitting point in all the cases where it was uniformly distributed, no matter the weight of Age. In the data set with normally distributed Age (where Age was not the most influential covariate), Age was chosen as a second splitting variable, though all the categorical covariates had greater weights (Case 5).

The value of the smoothing parameter had no influence on the tree models. It slightly changed the quartile assignment results (Table 30), but changes did not propagate further.

In all the cases change in AUPC was a very good approximation of change in true AUPCs in subpopulations (see Table 31 and Table 32).

Table 30. Percent of patients assigned to exposure levels. No intra-subject variability, weighting scheme 1.

Smoothing parameter	Exposure level				
	1	2	3	4	Unassigned
1	19.1	32.7	27.4	14.4	6.4
10	19.9	32.3	26.9	14.2	6.7
100	20.1	33.3	25.9	12.8	7.9

Table 31. Comparison of change in AUPC with change in $AUPC_{true}$. No intra-subject variability, weighting scheme 1, normally distributed Age.

Subpopulation	Variable[a]	% change AUPC	% change $AUPC_{true}$	
			Mean	Median
Whole population	Age (>56/≤56)	22.0	21.0	21.6
	Conmed	18.6	14.7	14.2
	ConDisease	32.5	29.1	29.4
	Smok	-11.1	-11.5	-10.3
Age<=56	Conmed	24.5	26.8	29.6
Age<=56 ConDisease=0	Conmed	12.3	14.5	16.3
Age>56	ConDisease	23.1	20.6	22.1
56≤Age≤58	Smok	-14.6	-11.0	-11.6
59≤Age≤65	Smok	-11.2	-10.3	-10.6
59≤Age≤63 Smok=1	Conmed	4.2	2.0	-1.1
Age>65 ConDisease=0	Conmed	26.0	24.8	24.1
Age>65 Conmed=0 ConDisease=0	Smok	-5.5	-4.9	-4.2

a. Comparison for categorical covariates: Covariate=1 versus =0 (Yes/No).

Table 32. Comparison of change in AUPC with change in $AUPC_{true}$. No intra-subject variability, weighting scheme 5, normally distributed Age.

Subpopulation			Variable[a]	% change AUPC	% change $AUPC_{true}$	
					Mean	Median
Whole population			Age (>56/≤56)	14.9	14.9	16.1
			Conmed	26.2	23.0	20.1
			ConDisease	43.8	41.7	45.6
			Smok	-6.7	-7.2	-5.5
ConDisease=1			Age (>56/≤56)	10.5	8.4	8.5
ConDisease=0			Age (>56/≤56)	14.8	15.3	15.0
ConDisease=0	Age≤56		Conmed	15.8	17.7	18.4
	57≤Age≤65		Conmed	15.3	9.8	11.3
	Age>66		Conmed	26.3	22.9	23.6
	Age>66	Conmed=0	Smok	-5.3	-5.4	-3.7

a. Comparison for categorical covariates: Covariate=1 versus =0 (Yes/No).

Intra-individual variability

Ten analysed data sets with intra-individual variability employed two weighting schemes and five variability levels, 10 to 50%. In all the data sets Age was normally distributed, and one value of the smoothing parameter was used.

Table 33 shows the distributions of patient assignment to exposure levels for the data of weighting scheme 5, with different intra-subject variability. As one might expect, the percentage of unassigned patients increases as variability increases. It reached 25% at $cv_\varepsilon = 50\%$.

The capability of the tree models to detect influential covariates decreases with variability. Table 34 exhibits the covariates and their order in the tree models for

weighting scheme 5 and different intra-subject variability. At the 0% and 10% variability level, all the covariates were detected, and except for Age, the tree models correctly ordered their influence. Starting at a 15% variability level, Smok was no longer detected. At a variability level of 50%, Conmed was not detected, rather Gender was observed in the model.

Table 33. Percent of patients assigned to exposure levels. Different intra-subject variability, weighting scheme 5.

Coefficient of variation (%)	Exposure level				
	1	2	3	4	Unassigned
0.1	19.5	34.6	26.3	11.3	8.3
0.15	17.8	36.7	25.2	9.5	10.8
0.2	17.6	35.9	25.4	7.8	13.3
0.3	15.8	36.5	22.8	6.2	18.7
0.5	15.8	35.7	18.8	4.4	25.3

Table 34. Summary of tree models for weighting scheme 5 and normally distributed Age

Coefficient of variation (%)	Detected covariates ordered according to the tree model			
0	ConDisease	Age	Conmed	Smok
10	ConDisease	Age	Conmed	Smok
15	Age	ConDisease	Conmed	
20	Age	ConDisease	Conmed	
30	ConDisease	Age	Conmed	
50	Age	ConDisease	Gender	

Note, that for the weighting scheme depicted in the table $\theta_{Smok} = 0.1$ and $\theta_{Conmed} = 0.3$. This means that smoking increased clearance by less than 10% (exactly 10% for a

person of smallest age with Conmed=0 and ConDisease=0), and Conmed decreased it by less than 30%. As can be seen in Table 35, Smok decreased AUPCs (true and empirical) by approximately 8%, i.e. half of intra-subject variability of 15% where Smok could not be detected. Also, a change of 7% would not be considered as clinically important. Conmed increased AUPC by approximately 25%, again half of the variability level where it could not be detected. Gender wrongly entered the tree model at the 50% variability level, but the mistake was caught at the next stage, AUPC comparison, where it changed AUPC by 0.3 percent (Table 36).

Table 35. Percent change in AUPCs and in $AUPC_{true}$ for subpopulations. Weighting scheme 5, normally distributed Age, and CV=20%.

Subpopulation		Variable[a]	% change AUPC	% change $AUPC_{true}$	
				Mean	Median
Whole population		Age (>56/≤56)	18.4	15.4	16.9
		Conmed	25.4	24.2	22.0
		ConDisease	45.2	43.0	46.6
		Smok	-8.2	-7.3	-5.8
Age≤53		ConDisease	38.5	37.1	37.4
Age≤53	ConDisease=0	Conmed	23.3	25.5	25.7
54≤Age≤58		ConDisease	29.9	28.3	27.2
59≤Age≤64		ConDisease	22.2	21.9	22.6
Age>65		ConDisease	39.3	38.7	41.5
Age>65	ConDisease=1	Conmed	24.7	17.3	20.1

a Comparison for categorical covariates: Covariate=1 versus =0 (Yes/No).

Comparison of the percent change in AUPC versus $AUPC_{true}$ showed a good agreement between these two measures. Table 35 and Table 36 show percent change in AUPC and $AUPC_{true}$ for each covariate in the whole population and in various subpopulations identified by the tree models, for data sets with the coefficient of intra-subject variability of 20% and 50%, respectively. Changes in AUPC and $AUPC_{true}$ are very close in all subpopulations for 20% variability, and the difference between the changes is of the same order of magnitude as the difference between mean and median

AUC_{true} changes. In the high variability case (Table 36), the difference between changes in AUPC and $AUPC_{true}$ was larger (reaching 10% for ConDisease in the whole population). However, in comparisons made for the appropriate subpopulations (Age was the first splitting variable, so comparisons for ConDisease should be made in the respective Age subgroups), the difference partially went away, with a maximum difference of 6%.

Table 36. Percent change in AUPCs and in $AUPC_{true}$ for subpopulations. Weighting scheme 5, normally distributed Age, and CV=50%.

Subpopulation	Variable[a]	% change AUPC	% change $AUPC_{true}$	
			Mean	Median
Whole population	Age (>68/≤68)	26.8	19.1	23.1
	Conmed	25.0	25.0	24.7
	ConDisease	33.7	44.2	50.2
	Smok	-7.7	-6.3	-5.4
Age≤53	ConDisease	45.1	39.2	38.8
54≤Age≤67	ConDisease	23.9	26.2	27.4
54≤Age≤67 ConDisease=1	Gender	0.3	2.3	3.1
Age>67	ConDisease	39.0	40.4	43.8

a Comparison for categorical covariates: Covariate=1 versus =0 (Yes/No).

2.4.5 CONCLUSIONS

This simulation study illustrated that the developed model-free method is able to identify predictors of exposure in a wide range of variability in the data. In the presence of 30% inter-subject variability in pharmacokinetic parameters the method was able to detect the covariates whose contribution to exposure was half the intra-individual variability level.

The simulation also showed that percent change in AUPC for subpopulations identified by the tree models is a good approximation of change in exposure for the respective populations. The maximum difference between the estimated and true

change in AUPC in the worst case (50% intra-individual variability and 30% inter-individual variability in parameters) did not exceed 6%.

The choice of smoothing parameters in the partitioning algorithm, relative contributions of categorical covariates, and correlation between them do not influence the capability and accuracy of the method. However, the tree models overestimate the contribution of the continuous covariate. This should be kept in mind when choosing subpopulations for AUPC curves based on the tree models.

3 APPLICATIONS OF NONLINEAR MIXED EFFECTS MODELLING

3.1 Population Pharmacokinetics Of An Antifungal Compound

3.1.1 OBJECTIVES

One of the objectives of the clinical studies and analyses presented here was to fully investigate the multiple-dose pharmacokinetics of the drug, characterising total exposure to the drug, clinical covariates of exposure, and the relationships between exposure, safety and efficacy. Earlier studies had indicated that the terminal half-life was long. A long half-life might contribute to marked accumulation and prolonged exposure following the extended duration of treatment. Consequently, extent and duration of exposure were the emphasis of this investigation. The primary objectives of this project were:

1. To describe the multiple-dose pharmacokinetics of the drug in order to provide a clear understanding of the duration and extent of systemic exposure following at least two weeks of dosing;
2. To characterise the relationship between plasma levels and safety in the patient population;
3. To characterise the relationship between efficacy outcomes, plasma levels and the demographic predictors of plasma levels.

The first objective was met by building a mathematical model consistent with observed multiple-dose pharmacokinetic behaviour of the drug. First, the multiple-dose pharmacokinetics of the drug was investigated by fitting compartmental models to intensely sampled pharmacokinetic data obtained from healthy volunteers. The developed model was then incorporated into a nonlinear mixed-effect model for the drug. The latter model was investigated by its application to sparse data obtained from patient studies. The model was then used to compute summary measures of duration and extent of systemic exposure, and to explore the associations between demographic covariates and pharmacokinetic parameters.

The second objective was met by exploring the relationships between indicators of safety (namely, frequencies of adverse events and above-normal laboratory values), and model - derived measures of extent of exposure. The associations between safety

indicators and demographic variables identified as related to pharmacokinetic response, were also explored.

The third objective was met by exploring the associations between efficacy outcomes and demographic variables identified as related to pharmacokinetic response.

An additional objective of the investigation was to use the developed model to guide the design of a new study, where higher doses of the drug would be employed.

Finally, the secondary objective was to compare the model-free population PK method, described earlier, with the nonlinear mixed modelling.

3.1.2 INDIVIDUAL COMPARTMENTAL MODEL

3.1.2.1 Data

Data from three pharmacokinetic studies in healthy volunteers were included in this analysis. Pharmacokinetic assessments in these studies included full concentration-versus-time profiles, as well as trough levels, (i.e., drawn immediately pre-dose), from each subject.

Duration of dosing and the longest time post-dose at which plasma levels were assessed, for each study are presented in Table 37.

Table 37. Duration of dosing and sampling.

Study	Weeks of Dosing[a]	Longest Time Post-Dose[b]
H1	2	335 hr = 2 wk
H2	2	1488 hr = 8.9 wk
H3	4	1440 hr = 8.6 wk

a. The duration of the once-a-day dosing period.

b. The longest duration of the post – dosing sampling period (*washout*) following the last dose of the multiple-dose regimen.

Study H1 compared fed and fasted conditions in a crossover design in both elderly and young subjects. Subjects were dosed with 250 mg of the drug once a day in the morning for 15 consecutive days. Doses 1-5 and 10-12 were taken at home. It was

assumed that these were all taken at 8 am with breakfast, i.e., under fed conditions as prescribed in the protocol. Doses 6, 7, 9, 13, and 14 were taken at the study centre at 8 am with breakfast, i.e., under fed conditions. There were two sequences for a crossover assessment of the effect of food. In sequence 1, dose 8 was taken at the study centre at 8 am after a 7:30 am breakfast, i.e., fed; and dose 15 was taken at the study centre at 8 am fasting until noon. In sequence 2, the fed/fasted conditions of doses 8 and 15 were reversed: day 8, fasted; day 15, fed. Twenty-four-hour trough levels were drawn following doses 5-7 and 12-14. A 24-hour profile was taken following dose 8, and a 335-hour profile was taken after dose 15. Each subject thus provided 33 blood samples in all: 6 troughs, 11-samples on day 8, and 16 samples on days 15 through 29. (Here pre-dose samples are counted as troughs, not as parts of the profiles.) Fifteen elderly (ages 61-75 years) and fifteen young (ages 19-33 years) subjects were separately randomised to each of the two sequences.

Study H2 investigated the pharmacokinetic interactions with other drugs in a crossover design. No pharmacokinetic interaction was observed, so dosing with the other drug was equivalent to placebo for our analysis. Subjects were dosed with 250 mg of the drug once a day in the morning for 18 consecutive days. Doses 1-10 were taken at home under fed conditions. Doses 13-17 were taken under fed conditions at the study centre. Doses 11, 12, and 18 were taken in the fasted state at the study centre. There were two sequences of ten subjects each, and the study was a two-period crossover study. In the first sequence, subjects received 18 doses of the drug in the first period, and they received placebo in the second period. In the second sequence, subjects received 18 doses of the drug in the second period, having received placebo in the first period. Since the study was blinded, subjects in the first sequence had plasma samples taken after the last active dose through the washout period and through the second treatment period (drug-placebo). Therefore, subjects in the first sequence had samples drawn for as long as 1488 hours post dose.

Study H3 compared 125 mg and 250 mg doses in a parallel design. Subjects were dosed with either 125 mg or 250 mg of the drug, in a parallel design, once a day in the morning for 30 consecutive days (except for the second day). On days 1, 16, and 30, doses were taken at 8 am at the study center with fasting until 11 am. Each of these doses was followed by a PK profile: 48 hours on day 1 (with no dose given on day 2); 24 hours on day 16; and up to 1440 hours on day 30 (through day 58). Doses on days

4, 5, 7, 8, 10, 11, 13-15, 18-20, 22, 23, 25, 26, 28, and 29 were taken at home at 8 am. It was assumed that these doses were taken with breakfast, i.e., under fed conditions (the protocol did not specify). Doses on days 3, 6, 9, 12, 17, 21, 24, and 27 were taken at 8 am at the study centre. It was assumed that these doses were also taken with breakfast, i.e., under fed conditions (the protocol did not specify). Twenty four hours after the doses on days 5, 8, 11, 15, 20, 23, 26, and 29, trough blood samples were taken at the study centre. Each subject provided up to 73 blood samples: 8 troughs, 18 samples on day 1 (through day 2, not counting the pre-dose sample on day 1), 14 samples on day 16, and up to 33 samples on day 30 (through day 58). There were 10 young (18-45 years old), healthy, male volunteers in each dose group.

3.1.2.2 Methods

3.1.2.2.1 Model description

Pharmacokinetic models were investigated within the family of linear, *mammillary* models with *first-order output* from the central compartment.

Mammillary models is a subset of compartmental models in which compartments exchange the drug only though the central one, there is no direct flow between peripheral compartments, like in Figure 2. These are the most often used compartmental models. They correspond to the physiological notion that drug is distributed and eliminated through the blood, and that blood carries drug to all other tissues and eliminating organs. The other consideration is a mathematical one: if measurements are taken only from one compartment (blood, for example), a non-mammillary multi-compartmental system poses an *identifiability* problem (the solution is not unique).

First-order output from the central compartment assumes that the rate of elimination of the drug from the system is proportional to the amount of the drug in the central compartment, as in Eq. 1.

It was assumed that the observed plasma concentration represented the concentration in the central compartment. Based on the previous knowledge of the pharmacokinetics of the drug [Kovarik, *et al.*,1992, 1995], input was modelled as *zero-order* into the central compartment (i.e. rate of input is constant, it does not depend on the amount of

drug outside or in the compartment). It was also known, that the pharmacokinetics of the drug could not be described by a one compartment model [Jensen, 1989, 1990; Kovarik, *et al.*,1992]. Therefore, two- and three-compartment models were tried and compared.

To describe the three-compartment model, let, as before, X_1, X_2 and X_3 represent drug amounts in the central and two peripheral compartments, respectively. Then these quantities are assumed to obey the following system of differential equations in response to a single dose, D:

$$dX_1/dt = I(t) - (k_{12} + k_{13} + k_{el}) X_1 + k_{21} X_2 + k_{31} X_3 , \qquad \text{Eq. 22}$$

$$dX_2/dt = k_{12}X_1 - k_{21}X_2 , \qquad \text{Eq. 23}$$

$$dX_3/dt = k_{13}X_1 - k_{31}X_3 , \qquad \text{Eq. 24}$$

with the following initial conditions

$$X_1(0) = X_2(0) = X_3(0) = 0 . \qquad \text{Eq. 25}$$

Here I(t) is the rate of drug input into the central compartment and is assumed to be given by

$$I(t) = \begin{cases} 0, & t \le t_{lag} \\ FD/t_{abs}, & t_{lag} < t \le t_{abs} + t_{lag} \\ 0, & t_{abs} + t_{lag} < t \end{cases} \qquad \text{Eq. 26}$$

where t_{lag} is the delay between the time of dose until drug first appears in the central compartment, t_{abs} is the duration of zero-order input into the central compartment, and F is the fraction of dose absorbed.

Let V_c be the volume of distribution of the central compartment. The concentration in the central compartment,

$$C(t) = X_1(t)/V_c, \qquad \text{Eq. 27}$$

can be expressed in closed form as follows. Let $b_2>b_3>b_4$ be the three exponential decay constants that are the eigenvalues of the system (Eq. 22 - Eq. 25) with I(t)=0.

Let us define the following quantities:

$$s = V_c t_{abs}/F, \qquad \text{Eq. 28}$$

$$\begin{aligned} c_2(t) &= D(k_{21} - b_i)(k_{31} - b_i)[\exp(b_i(t - t_{lag})) - 1] /[s(b_3-b_2)(b_4-b_2)b_2] \\ c_3(t) &= D(k_{21} - b_i)(k_{31} - b_i)[\exp(b_i(t - t_{lag})) - 1]/[s(b_2-b_3)(b_4-b_3)b_3] \\ c_4(t) &= D(k_{21} - b_i)(k_{31} - b_i)[\exp(b_i(t - t_{lag})) - 1]/[s(b_2-b_4)(b_3-b_4)b_4] \end{aligned} \qquad \text{Eq. 29}$$

$$c_i = c_i(t_{abs} + t_{lag}), \quad i=2,3,4. \qquad \text{Eq. 30}$$

Then the concentration C(t) can be expressed as

$$C(t) = \begin{cases} 0, & t \le t_{lag} \\ \sum_{i=2}^{4} c_i(t) \exp(-b_i(t - t_{lag})), & t_{lag} < t \le t_{abs} + t_{lag} \\ \sum_{i=2}^{4} c_i \exp(-b_i(t - t_{lag})), & t_{abs} + t_{lag} < t. \end{cases} \qquad \text{Eq. 31}$$

C(t) depends on 8 parameters: t_{lag}, t_{abs}, s, b_2, b_3, b_4, k_{21}, and k_{31}.

Parameters t_{lag}, t_{abs} and s are known to be responsible for absorption and bioavailability, and may be influenced by food. The other five parameters describe the distribution of the drug in the body and its elimination, and they usually do not depend

on food intake. To account for the possible effects of food, three variations of the above model were compared for studies H1 and H3:

Reduced model: no differences between fasted and fed

Mid model: s differs between fasted and fed; let s_a denote the parameter under the fasted condition; one additional parameter is thereby introduced

Full model: t_{lag}, t_{abs}, and s differ between fasted and fed; let $t_{lag,a}$, $t_{abs,a}$, and s_a denote the three parameters under the fasted conditions; three additional parameters are thereby introduced relative to the reduced model, or two additional parameters relative to the mid model.

For either the mid or the full model, let $c_{i,a}(t)$ and $c_{i,a}$ represent the coefficients $c_i(t)$ and c_i modified by the substitution of the fasted for the fed parameters, where appropriate.

The model was applied to multiple dosing by superposition. An assumption of dose linearity, necessary for justification of superposition, was based on dose proportionality in a single-dose study [Kovarik *et al.*, 1992].

Let $C(t,d)$ represent the concentration at time t after the d^{th} daily dose, assuming that for a given subject all doses were administered at exactly the same time of day. $C(t,d)$ can be expressed as the sum of two parts: $C_{d-1}(t,d)$, the sum of contributions through the (d - 1)'st dose; and $C_d(t,d)$, the contribution from the d^{th} dose. $C_d(t,d)$ is given by (Eq. 31).

$C_{d-1}(t,d)$ can be found from superposition, by adding contributions from doses through the $(d-1)^{st}$. Assume that most doses are administered in the fed state, but that some are administered in the fasted state, say $d_1, \ldots, d_f$. Then

$$C_{d-1}(t,d) = \sum_{j=1}^{d-1} \sum_{i=2}^{4} c_i e^{-b_i(24j+t-t_{lag})} + C_f(d_1,\ldots,d_f) =$$

$$= \sum_{i=2}^{4} \frac{c_i e^{-b_i(t-t_{lag})} e^{-24b_i}[1-e^{-(d-1)24b_i}]}{1-e^{-24b_i}} + C_f(d_1,...,d_f), \qquad \text{Eq. 32}$$

where $C_f(d_1,...,d_f)$ is a correction for the fasting doses:

$$C_f(d_1,...,d_f) = \sum_{k=1}^{f}\left(\sum_{i=2}^{4} c_{i,a} e^{-b_i[24(d-d_k)+t-t_{lag,a}]} - \sum_{i=2}^{4} c_i e^{-b_i[24(d-d_k)+t-t_{lag}]}\right). \qquad \text{Eq. 33}$$

For the population pharmacokinetic analyses, an additional correction was made when the recorded time of the second-to-last dose was not 24 hours before the time of the last dose. (In the volunteer studies, nominal dosing intervals of exactly 24 hours were assumed.)

Let t_{inter} be the time between the last two doses. Then, to $C_{d-1}(t,d)$ and $C_d(t,d)$ was added the correction term $C_{d-1,c}(t,d)$ where:

$$C_{d-1,c}(t,d) = \sum_{i=2}^{4} c_i e^{-b_i(t-t_{lag})}(e^{-b_i t_{inter}} - e^{-b_i 24}) \qquad \text{Eq. 34}$$

3.1.2.2.2 Model Fitting

Models were fitted by unweighted nonlinear least squares method (OLS) for each subject separately.

This method is valid and is known to deliver the best result [Amisaki & Eguchi, 1995] if the residual error model is additive, and errors are independently normally distributed with mean zero, i.e.

$$C_{j,obs} = C_{j,pred} + \varepsilon_j, \qquad \varepsilon_j \sim N(0, \sigma^2),$$

where $C_{j,obs}$ and $C_{j,pred}$ are the observed and predicted plasma concentrations of a subject. If both assumptions hold, then the least squares estimates are in fact maximum likelihood estimates.

However, most often concentrations are distributed log-normally, not normally. To use OLS method in this situation, the concentrations should be log-transformed. The following model is then appropriate to fit by OLS method:

$$\ln(C_{j,obs}) = \ln(C_{j,pred}) + \varepsilon_{j.}$$

Therefore, the log-transformation of concentrations was used in fitting process. Values below quantification limit (BQL), which on a concentration scale might have been assigned a value of zero, were treated as missing, and were excluded from the analysis.

Fitting was done using SAS. A number of routines were developed and various existing SAS routines were used. Several steps of fitting were employed:

1. Parameters were first estimated by *stripping* the last profile [Gibiansky, 1994]. The stripping technique assumes that by later times all exponential terms except the slowest one have already vanished. Therefore the parameters for the slowest term can be estimated by log-linear regression on several latest data points. The contribution of this term can then be subtracted from the values of all the previously observed data points. The procedure can then be repeated for the next slowest exponential term, and so on till the first [Dunne, 1986].

2. These estimates were then used as initial guesses for nonlinear least squares fitting to the log concentrations. A Nelder-Mead simplex algorithm within PROC IML of SAS was used [Gibiansky, 1995; SAS, 1990]. These are denoted as the second-level estimates.

3 Then, because of evidence that the objective function had multiple local minima, the data for each subject were fitted using 100 sets of initial guesses that were selected by allowing each parameter to vary randomly within 100% of its second-level estimate. The best fits from this process are denoted as the third-level estimates. In several cases, the sum-of-squared-errors (SSE) for the mid model was smaller than the SSE of the full model, or the SSE of the reduced model was smaller than either the mid or the full. In these cases, the

third-level estimates for the better model were used as initial guesses to generate revised third-level estimates for the fuller model.

4. Final estimates were obtained by using the third-level estimates as initial guesses for the secant method of PROC NLIN in SAS [SAS, 1989]. In a couple of cases it further improved the fit. Attempts to shorten the analysis and use a gradient method with the initial guess given by the stripping procedure (step 1), usually resulted in a failure due to non-convergence.

For Study H1, the final fits for the full, mid, and reduced models were compared by means of approximate F statistics [Seber & Wild, 1989]. Estimates of bioavailability t_{lag} and F/V_c ($F/V_c = t_{abs}/s$) from the full model under fed and fasted conditions were compared by signed-rank tests. Estimates of apparent oral clearance CL/F and terminal half-life $t_{1/2}$ ($CL/F = D/AUC_{0-\infty}$, $t_{1/2} = \ln(2)/b_4$) [Gibaldi & Perrier,1982] were compared for elderly and young subjects by Wilcoxon rank-sum tests. A full two-compartment model, that is, one with t_{lag}, t_{abs}, and s differing between fed and fasted conditions, was also fitted to the data from each subject in H1. The two- and three-compartment models were compared by approximate F statistics. Diagnostic plots of standardised residuals assessed the goodness-of-fit of the finally selected model.

For Study H3, third-stage fits were compared by approximate F statistics, and then only the mid model was fitted at the fourth stage.

For Study H2, only the mid model was fitted, and only through the third stage.

The availability of two dose levels in Study H3 permitted an assessment of dose - linearity of the drug, which must be true if superposition is to be valid. As described above, the mid model was fitted to each subject separately. Under dose-linearity, the parameters of the model should not depend on the dose level. To test this independence, parameter estimates from the two dose groups were compared by Wilcoxon rank-sum tests. Dose proportionality was further investigated by similar tests applied to AUC_{0-48} and C_{max} for the concentration versus time profile on Day 30.

Additionally, a linear mixed model (repeated measures) was fitted to all dose - normalised log concentrations through Day 30, 48 hours post dose. DOSE, DAY, and TIME were fixed effects and SUBJECT was a random effect in the model.

3.1.2.3 Results

The effect of food on bioavailability was investigated by the comparison of the full, mid, and reduced models fitted to the data. All three models were fitted separately to data from each individual in Studies H1 and H3. Minimum Sum of Squared Errors (SSE) for the full, mid, and reduced models for each subject from these two studies are shown in Table 38 and Table 39. The p-values from approximate F-tests comparing mid with the full model and reduced with the mid model are also presented.

In Study H1, where full concentration-time profiles were obtained under both fed and fasted conditions, for investigation of a food interaction, the full model was superior to the mid model with $p \leq 0.05$ for 24 of 30 subjects. Absorption and bioavailability parameters were different under fed and fasted conditions as described later, providing the evidence to support the importance of accounting for differences between fed and fasted conditions of dosing on bioavailability when modelling a drug's pharmacokinetics.

In Study H3, a food interaction was not investigated in the design, with all plasma concentration-time samples collected following a dose under fasted conditions. However, the majority of doses, which were taken at home, were taken under fed conditions. For five of the ten subjects at the 250-mg dose, the mid model, which accounts for food effect on bioavailability, was judged superior to the reduced model with $p \leq 0.05$. For all ten of the subjects at the 125-mg dose, where there were more BLQ values, the reduced model adequately described the data.

Table 38. Comparison of individual models: Study H1

Subject	SSE				p-value [a]		
	2 comp	3 comp			Full: 2 comp vs 3 comp	3 comp	
	Full	Full	Mid	Reduced		Full vs Mid	Mid vs Red
1	0.94	0.25	0.27	0.96	***	-	***
2	1.03	0.23	1.40	1.60	***	***	-
3	0.62	0.25	2.46	2.73	***	***	-
4	2.28	0.54	1.93	2.01	***	***	-
5	1.20	0.21	2.43	2.44	***	***	-
6	3.08	0.53	1.59	1.65	***	***	-
7	0.54	0.37	0.78	0.98	*	***	*
8	2.90	0.68	0.79	0.82	***	-	-
9	0.73	0.61	0.67	1.38	-	-	***
10	0.83	0.64	3.83	4.07	-	***	-
11	0.68	0.32	2.31	2.41	***	***	-
12	0.72	0.49	2.13	2.17	*	***	-
13	0.61	0.35	1.71	1.71	**	***	-
14	0.36	0.32	1.02	1.83	-	***	***
15	1.26	0.40	4.16	4.92	***	***	*
17	1.27	0.31	3.51	3.55	***	***	-
18	1.06	0.76	3.29	3.31	*	***	-
20	1.16	0.72	1.61	1.61	**	***	-
21	2.14	1.03	2.51	2.95	***	***	*
22	0.91	0.42	0.57	0.61	***	*	-
23	0.95	0.35	0.50	0.54	***	*	-
24	0.66	0.46	0.96	1.83	*	***	***
25	7.15	5.56	7.10	7.13	-	-	-
26	0.27	0.19	3.44	3.44	*	***	-
27	0.85	0.31	0.82	0.89	***	***	-
28	0.42	0.14	6.89	7.04	***	***	-
29	1.70	1.23	4.92	5.06	*	***	-
30	0.90	0.41	0.49	0.50	***	-	-
31	0.65	0.27	0.34	0.64	***	-	***
32	0.47	0.43	0.84	1.53	-	**	***

a. Indicators of significance: - $p \geq 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table 39. Comparison of individual 3 compartment models: Study H3

Subject	SSE			p-value [a]	
	Full	Mid	Reduced	Full vs Mid	Mid vs Red
125 mg dose group					
2	0.98	0.99	1.15	-	**
3	2.35	2.35	2.36	-	-
11	2.12	2.12	2.12	-	-
12	2.66	2.66	3.53	-	***
15	0.79	0.79	0.87	-	*
17	1.62	1.63	1.72	-	-
19	2.03	2.04	2.35	-	**
105	1.96	1.96	2.08	-	-
108	3.13	3.14	3.43	-	*
404	3.29	3.29	3.30	-	-
250 mg dose group					
1	6.11	6.11	6.11	-	-
6	2.56	2.57	2.62	-	-
7	1.5	1.50	1.50	-	-
13	1.68	1.69	1.87	-	*
14	4.10	4.10	4.13	-	-
16	0.72	0.72	0.74	-	-
18	1.35	1.35	1.36	-	-
109	2.13	2.13	2.18	-	-
110	1.61	1.61	1.75	-	-
120	3.49	3.49	3.49	-	-

a. Indicators of significance: - $p \geq 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

The complexity of the drug pharmacokinetics was investigated by comparing the results of the two- and three-compartment models for each subject in Study H1. Table 38 shows minimum SSEs for full two- and three-compartment models fitted to each subject from H1, together with p-values for the approximate F-tests comparing those two models. For 25 of 30 subjects, the p-value was ≤ 0.05, providing strong evidence that the plasma concentrations of the drug were more consistent with three phases of distribution and elimination than with two.

The three-compartment model fitted the data adequately. For studies H1, H2, and H3, Figure 21, Figure 22, and Figure 23, respectively, trace the average of all fitted curves through the average observed concentrations versus time since the start of the study. The match between the fitted and observed values is good. Figure 24, Figure 25, and Figure 26 plot standardised residuals versus fitted values. The homogeneity of the scatter in the plots justifies the choice of the log scale for concentrations.

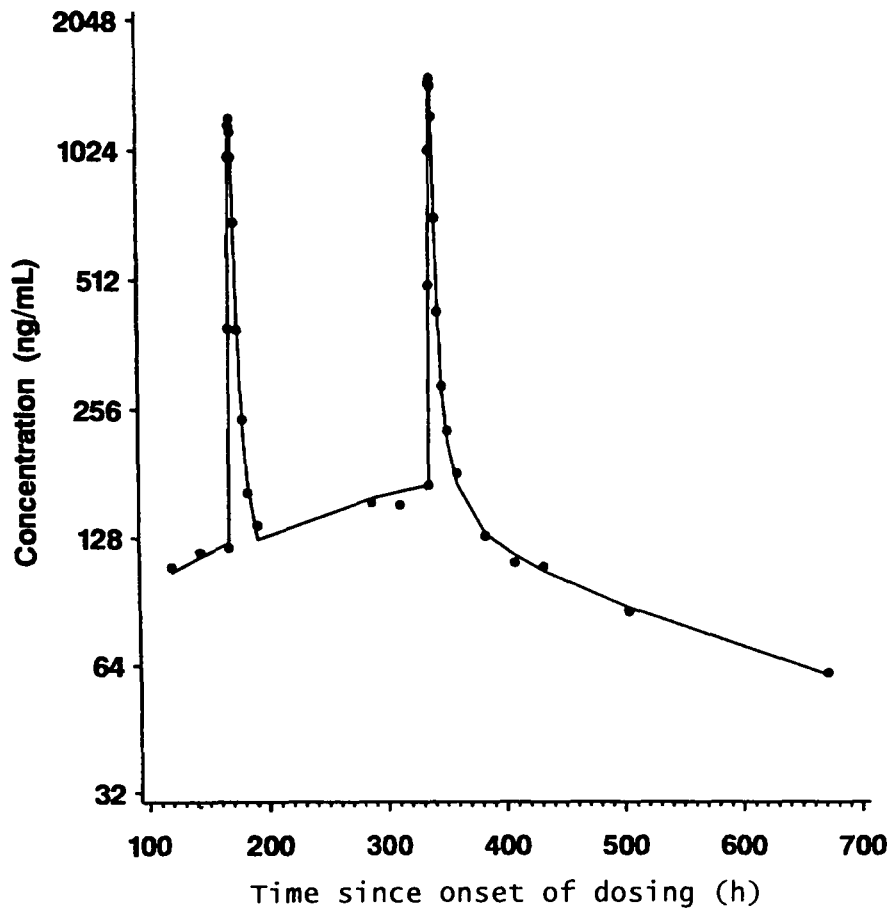

Figure 21. Average fitted values and average observed values versus time since start of dosing for Study H1.

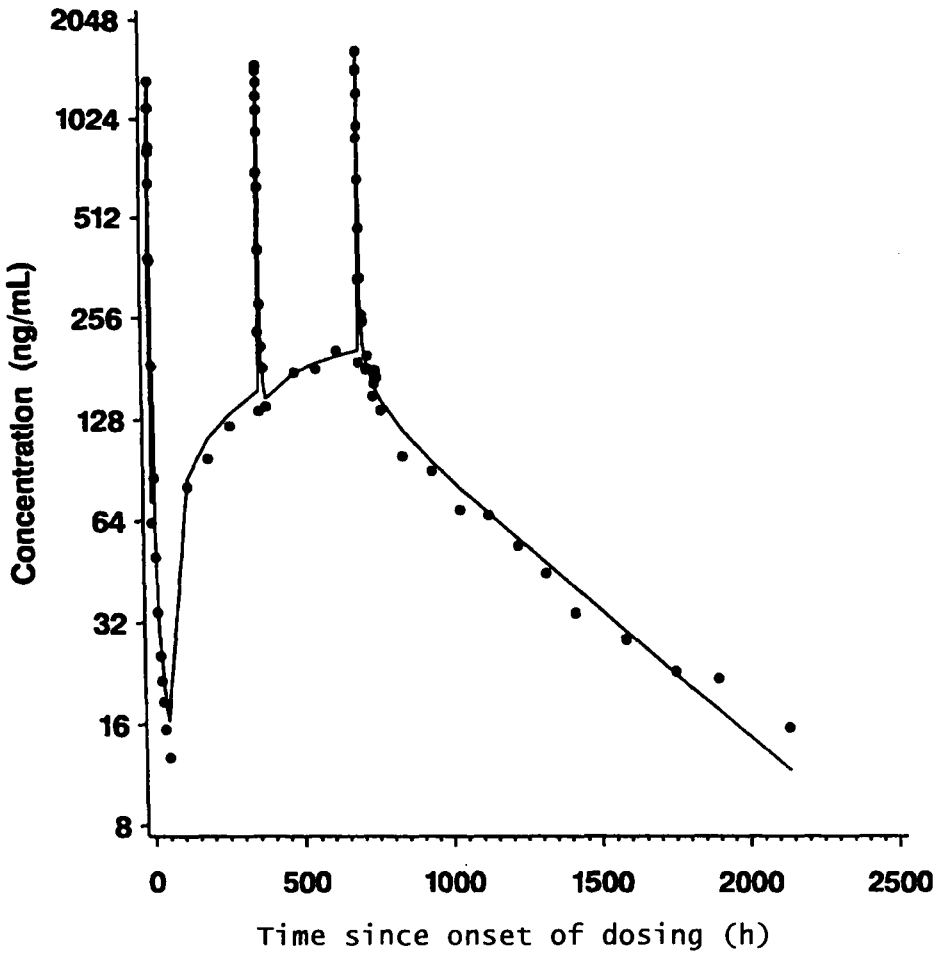

Figure 22. Average fitted values and average observed values versus time since start of dosing for Study H2.

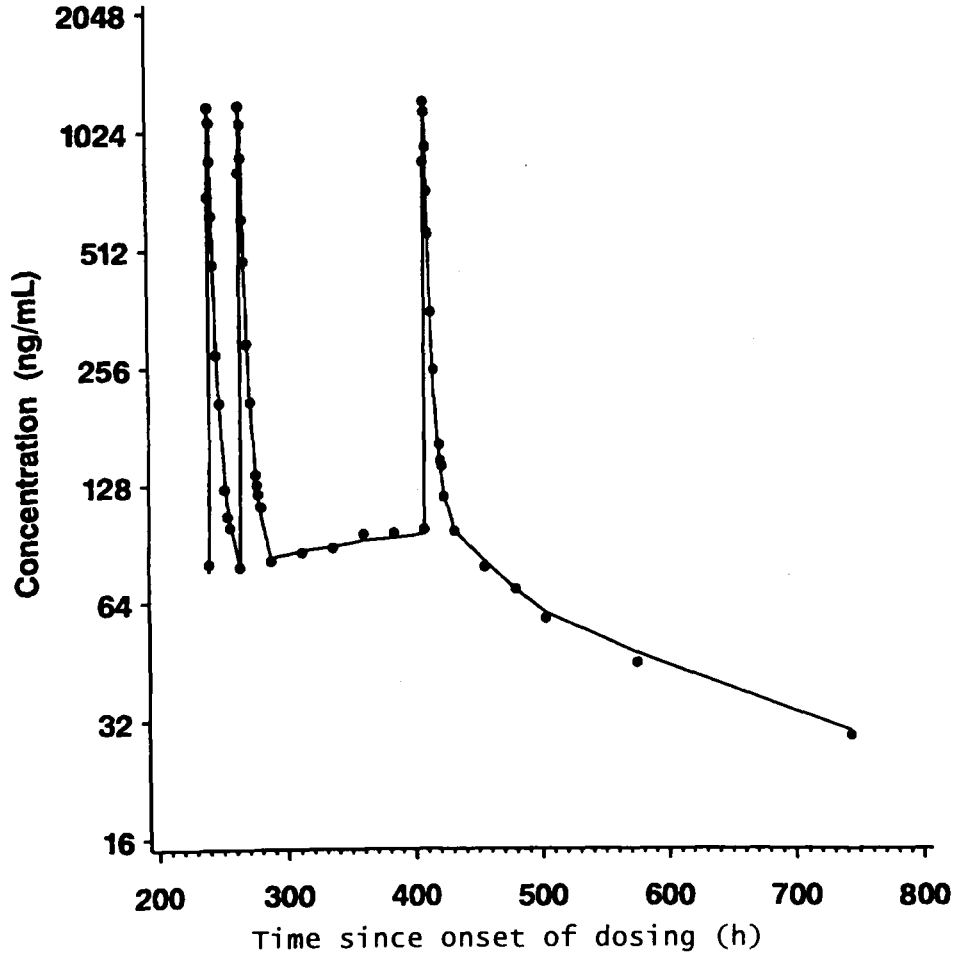

Figure 23. Average fitted values and average observed values versus time since start of dosing for Study H3.

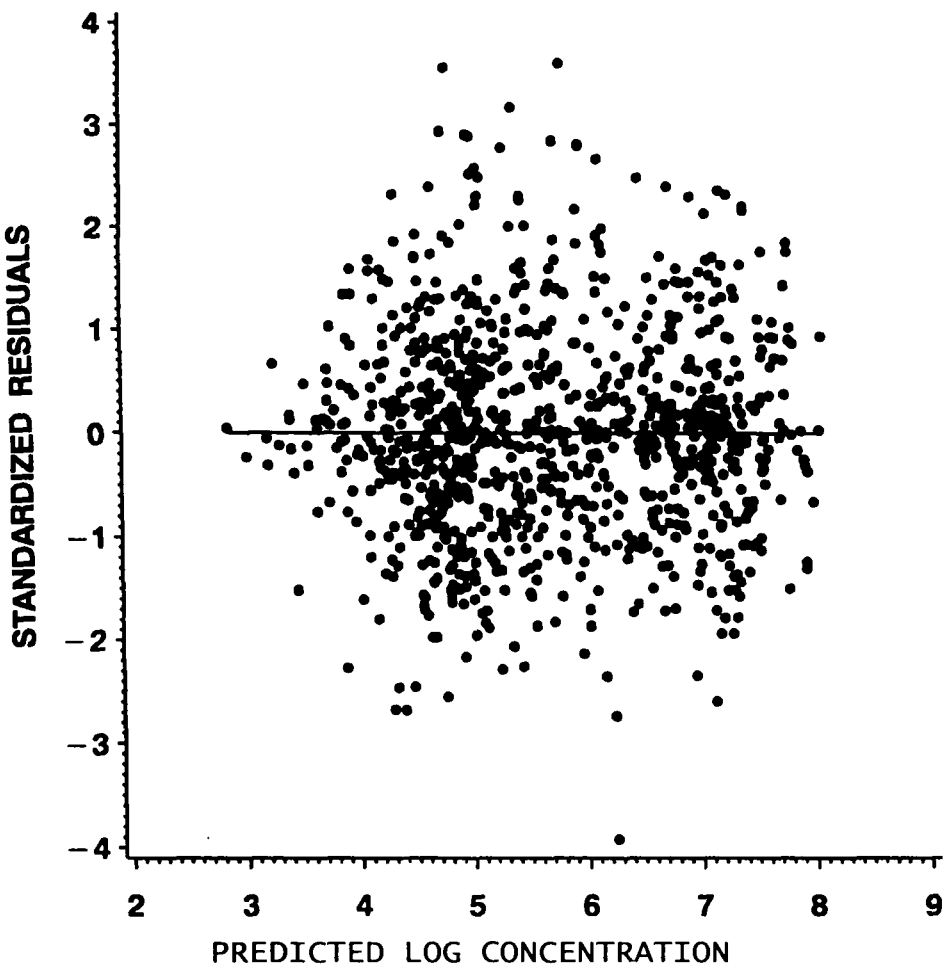

Figure 24. Standardised residuals versus fitted values for Study H1.

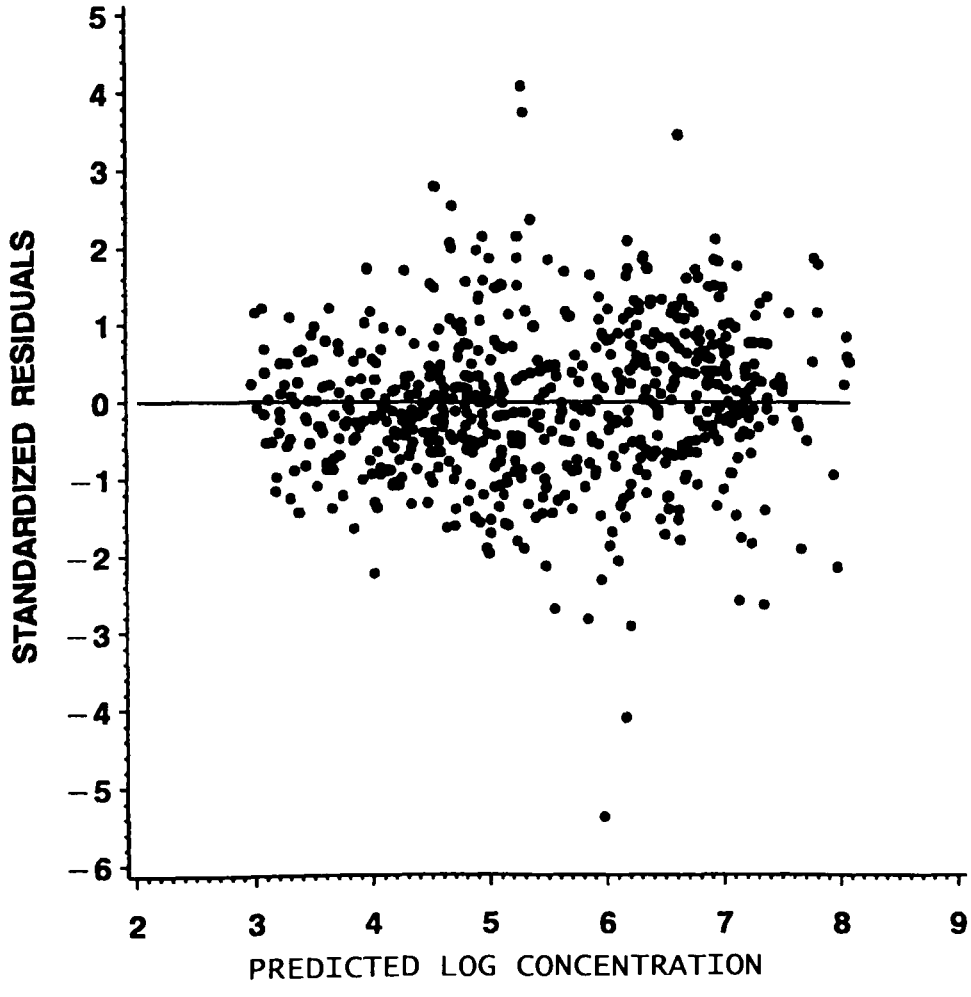

Figure 25. Standardised residuals versus fitted values for Study H2.

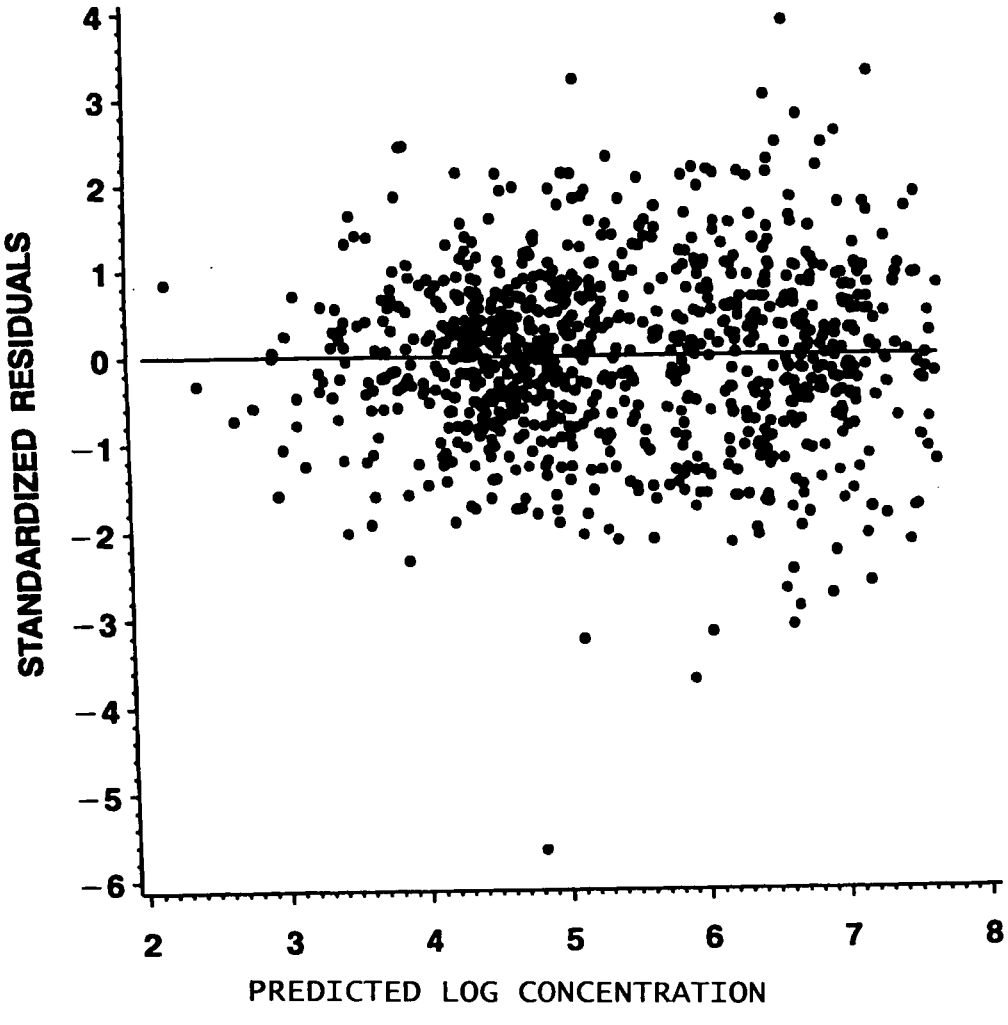

Figure 26. Standardised residuals versus fitted values for Study H3.

Pharmacokinetic parameter estimates from the full model fitted to each subject in study H1 and the mid model fitted to each subject in studies H2 and H3, are summarised in Table 40. The parameters are: t_{lag}, the delay until drug appears in the central compartment, under fed and fasted conditions; t_{abs}, the duration of zero-order absorption, under fed and fasted conditions; $F/V_c = t_{abs}/s$, the bioavailability divided by the volume of the central compartment, under fed and fasted conditions; $t_{1/2}$, the terminal elimination half-life, which, according to the model is independent of food; and Cl/F, the apparent oral clearance, under fed conditions only. Summaries for H1 are provided by age group. Parameters from study H3 are summarised by dose. (Except for the 125-mg dose group in H3, all other doses in all studies were 250 mg).

The effect of food was evident in the food-interaction Study H1: t_{lag} and F/V_c increased; i.e., absorption was delayed and bioavailability increased (assuming V_c, remained constant) with food. These effects were statistically significant ($p < 0.05$) overall and for both elderly and young separately as judged by signed-rank tests (see Table 41). There also appeared to be an effect of age although it was not statistically significant. Older subjects exhibited a longer half-life and reduced clearance.

Table 40. Summaries of Parameter Estimates: Median (Inter-quartile Range)[a]

Study	t_{lag} fed (hr)	t_{lag} fasted (hr)	t_{abs} fed (hr)	t_{abs} fasted (hr)	F/V_c fed (hr)	F/V_c fasted (hr)	$t_{1/2}$ (wks)	Cl/F (L/h)
H1, Elderly	0.70 (0.58)	0.36 (0.27)	2.86 (2.07)	1.78 (0.72)	13.2 (8.5)	10.2 (7.8)	2.13 (2.08)	17.2 (11.5)
H1, Young	0.69 (0.64)	0.41 (0.40)	1.51 (1.18)	1.61 (1.17)	9.4 (5.0)	7.0 (3.9)	1.57 (3.15)	24.0 (13.4)
H3, 250 mg[b]	-[c]	0.22 (0.03)	-	0.98 (0.12)	11.0 (5.2)	7.2 (2.1)	2.23 (0.80)	18.6 (23.8)
H2	-	0.10 (0.30)	-	1.43 (0.43)	6.5 (6.0)	7.6 (41)	1.59 (1.90)	30.8 (19.4)
H3, 125 mg	-	0.20 (0.02)	-	0.92 (0.34)	5.3 (4.2)	5.9 (3.4)	2.14 (6.68)	35.8 (37.5)

a) Obtained from the individual estimates of the pharmacokinetic parameter in the respective study or a group (dose or age group) within a study;

b) Excludes one subject for whom a two-compartment model fit adequately but the three-compartment model yielded $k_{31}=0$ with no improvement in overall fit;

c) The mid model did not distinguish between fed and fasted for these parameters. Since the concentration profiles comprising the bulk of a subjects data were taken in the fasted state, the parameter estimates were associated with the fasted state.

Table 41. P-values of fed versus fasted comparisons in Study H1 by Wilcoxon signed-rank test.

Subjects	t_{lag}	t_{abs}	F/V_{ss}
All	***	-	***[a]
Young	***	-	***
Elderly	***	-	*

a. Indicators of significance: - $p \geq 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

A fundamental assumption for all of the models used here was linearity of pharmacokinetic response. This assumption implies that:

a. The same model should fit both the 125-mg and 250-mg doses, i.e. the fitted parameters of the model as well as derived pharmacokinetic responses (dose-normalised AUC, Cmax, etc.) should be the same for individuals from the 125 mg and 250 mg groups;
b. Observed pharmacokinetic response (dose-normalised concentrations) across those two doses should be dose proportional.

To test this assumption:

a. All fitted parameters and derived dose-normalised AUC_{0-48} and C_{max} of the final profile (Day 30) were compared for the 125 mg and 250 groups by the Wilcoxon rank-sum test. Amongst all of the parameters, no differences were significant at α=0.05 level except for k_{21} ($p = 0.009$) and b_3 ($p = 0.01$). Across subjects, k_{21} and b_3 were highly correlated, so it was not surprising that either both were significant or not. Since k_{21} and b_3 were parameters related to the tail-behaviour of the concentration curve, their differences across dose groups may have been due to the greater number of BLQs at the 125-mg dose. Neither dose-normalised AUC_{0-48} nor C_{max} differed significantly between the groups.
b. A linear mixed model was fitted to all dose-normalised log concentrations through Day 30, 48 hours post dose. DOSE, DAY, and TIME were fixed effects and SUBJECT was a random effect in the model. DOSE was not significant (but all the other effects were).

Thus, given the limitations of the data, the evidence is consistent with the assumption of dose proportionality.

3.1.3 POPULATION MODEL

3.1.3.1 Data

A nonlinear mixed-effects version of the three-compartment model was applied to the data from Studies P1 and P2 used for the model-free analysis, as described in Section 2.2.2. Since blood sampling was sparse in both studies, data from Study H1 (described in Section 3.1.2 above) was also included in the analysis. Blood samples from all three studies had been analysed at the same laboratory. Moreover, all doses in Studies P1 and P2 had been taken under fed conditions (according to the protocols), and full

pharmacokinetic profiles following dosing under fed conditions were available from Study H1 for appropriate supplementation of the sparse patient data. Figure 27 and Figure 28 show the plots of concentrations versus time post dose for Studies H1, P1, and P2. From Study H1, only the plasma levels from the full profile after doses in the fed state on day 15 were used in the plot. The overlap of the different symbol types in these figures justified the pooling of the studies.

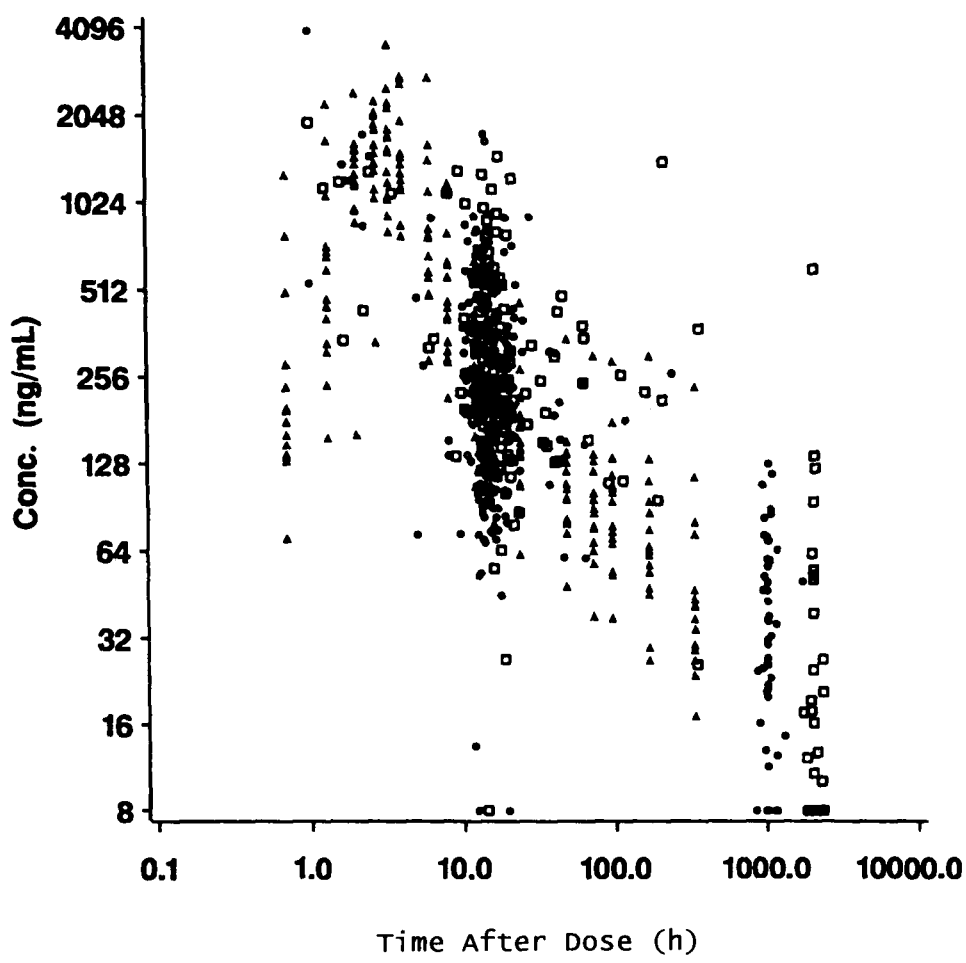

Figure 27. Concentration versus time post dose for Studies: H1 ▲▲▲, P1 □□□, and P2 ooo.

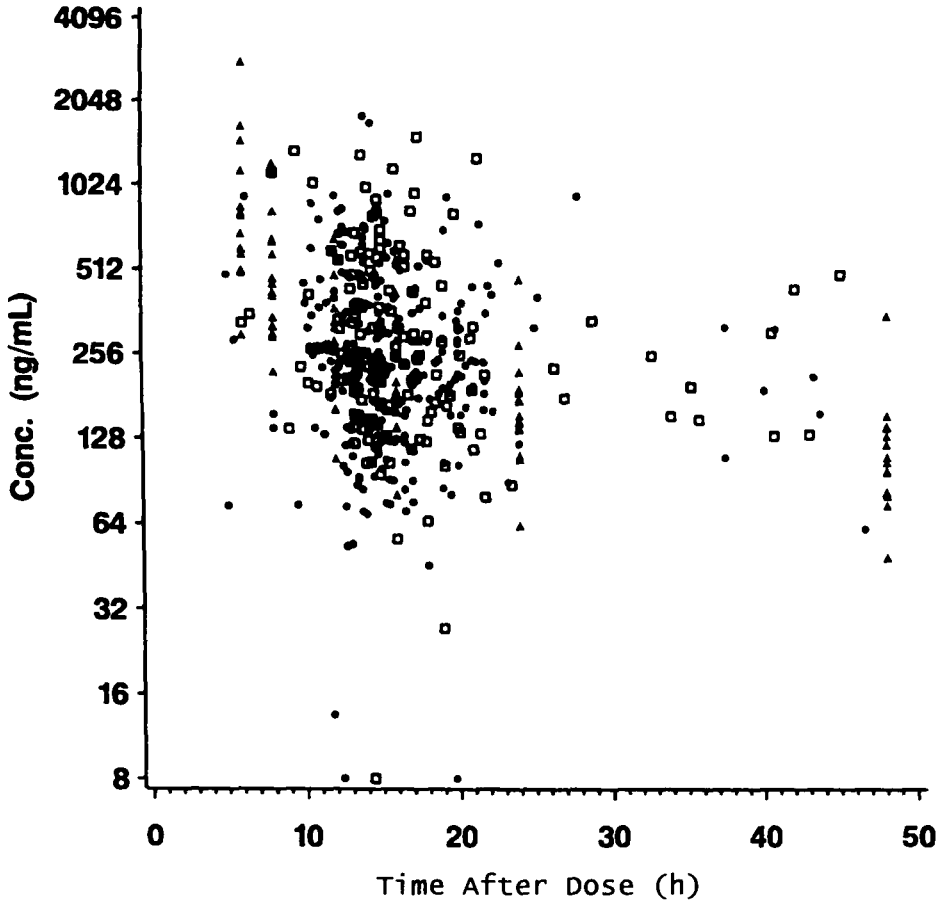

Figure 28. Concentration versus time post dose with times in the range 5-48 hours for Studies: H1 ▲▲▲, P1 □□□, and P2 o o o.

Based on the observed difference in pharmacokinetic parameters between fed and fasted conditions, only those blood levels following doses in the fed state in H1 were used for the model-based analysis. The consequence of this restriction was that for subjects in Sequence 1, the 16-sample profile following the fasted dose on day 15 was excluded; and for subjects in Sequence 2, the 11 -sample profile following the fasted dose on day 8 was excluded.

Plasma levels below the limit of quantification (BLQ) were retained for the model-based analysis. This was feasible because the added uncertainty due to lack of knowledge of the exact level could be incorporated into the model.

3.1.3.2 Methods

3.1.3.2.1 Model description

The general framework of a mixed-effects model (Eq. 2 - Eq. 6) may be represented for our purposes here as:

$$y_{ij} = f(D_{ij}, t_{ij}; \varphi_i) + \sigma(z_{ij}, \underline{\theta})\varepsilon \qquad \text{Eq. 35}$$

$$\varphi_i = \underline{h}(\underline{x}_i; \eta_i, \underline{\theta}) \qquad \text{Eq. 36}$$

where:

y_{ij} is the log concentration for the j^{th} observation of subject i which occurred at time t_{ij} after the d_{ij}^{th} dose;

φ_i is a vector of the i^{th} subject's pharmacokinetic parameters (t_{lag}, t_{abs}, s, b_2, b_3, b_4, k_{21}, k_{31}).

f is the predicted log concentration from the three-compartment model described above.

z_{ij} is a vector of subject-and-time specific covariates that determine the intra-subject variance at time t_{ij} for subject i. It permits the variance to accommodate extra variability from two sources. First, observations from patient studies were expected to be less reliable than those from the pharmacokinetic study because of less control over the timing of dosing in the former two studies. Second, some plasma levels in patient studies were below the detection limit of the assay that was 10 ng/mL. These observations were modelled by assuming that they had a value of 5 ng/mL (half the limit) but a greater variance. The extra parameter for the variance associated with these observations permitted them to be retained while downweighting their influence on the fit.

Let PATI be an indicator variable for patient versus healthy volunteer:

$$PATI = \begin{cases} 1, & \text{for patients in P1 and P2;} \\ 0, & \text{for volunteers in H1;} \end{cases} \qquad \text{Eq. 37}$$

and let IBLQ be an indicator variable for an observation being below the quantification limit:

$$\text{IBLQ} = \begin{cases} 1, & \text{if the observation is BLQ;} \\ 0, & \text{otherwise.} \end{cases} \qquad \text{Eq. 38}$$

Then $PATI_i$ and $IBLQ_{ij}$ are the two components of z_{ij}.

$\underline{\theta}$ is a vector of population mean parameters.

$\sigma(z_{ij}, \underline{\theta})$ is the standard deviation of i^{th} subject's intra-subject error at time t_{ij}. It was given the form:

$$\sigma(\text{PATI}, \text{IBLQ}, \underline{\theta}) = \theta_{0\sigma} + \theta_{1\sigma} * \text{PATI} + \theta_{2\sigma} * \text{IBLQ}. \qquad \text{Eq. 39}$$

ε is an intra-subject random error, assumed to be normally distributed with mean zero.

$\underline{x}_i$ is a vector of the i^{th} subject's covariates, as determined by the model-free screening (age, smoker or not, hypertension or not).

η_i is a vector of inter-subject random effects that is assumed to have a normal distribution with mean zero and covariance matrix Ω.

$\underline{h}$ is the mapping that determines a subject's pharmacokinetic parameters from $\underline{\theta}$, x_i, and η_i.

The mapping $\underline{h}$ was one of the two forms:

$$\log(\text{Par}_k) = \theta_{0k} + \theta_{1k} * \text{age} + \theta_{2k} * \text{smoker} + \theta_{3k} * \text{hypertension} + \eta_k \qquad \text{Eq. 40}$$

$$\text{sqrt}(\text{Par}_k) = \theta_{0k} + \theta_{1k} * \text{age} + \theta_{2k} * \text{smoker} + \theta_{3k} * \text{hypertension} + \eta_k \qquad \text{Eq. 41}$$

where Par_k is one of $(t_{lag}, t_{abs}, s, b_2, b_3, b_4, k_{21}, k_{31})$.

Thus, each parameter was assumed to have a log-normal or square-root-normal distribution with the median determined by a linear function of the components of $\underline{\theta}$.

The choice of distribution for each parameter was based on comparing with normal the distributions of different transformations of individual values of the parameter obtained from individual fitting of healthy volunteers' data. Not all of the components of $\underline{\theta}$ were nonzero. All θ_{0k}'s were nonzero. But θ_{1k}, θ_{2k}, and θ_{3k} were assumed to be zero to start, and then they were allowed be nonzero sometimes for some parameters as part of the exploratory process of determining what covariates were important.

3.1.3.2.2 Model fitting

The base model without covariates was firstly fit to the data. Different structures of variance-covariance matrix of random inter-individual effects were tried based on correlation between the estimates, limitations imposed by NONMEM (blocks of dimension ≤ 5), and of feasibility of convergence. The likelihood ratio test at the significance level $\alpha=0.05$ was used to discriminate between alternative hierarchical models and the Akaike Information Criterion (AIC) was used to distinguish between non-hierarchical models [Beal & Sheiner, 1992; Judge *et al.*, 1980]. The alpha level of 0.05 corresponds to a reduction of 3.8 (χ^2, $p<0.05$; 1 degree of freedom (df)) in the minimum objective function when one parameter is added to the model. When more than one parameter was added, the critical reduction in the objective function that corresponded to $\alpha=0.05$ was used. In addition to the minimum objective function value, diagnostic goodness-of-fit plots were used for model building and selection.

The full model was built by adding one covariate at a time to each of the parameters s, b_2, b_3, and b_4 (i.e. allowing non-zero θ_{1k}, θ_{2k}, or θ_{3k} for the respective parameters). Absorption parameters t_{lag}, t_{abs}, as well as inter-compartmental rate constants k_{21} and k_{31} were not explored for inclusion of covariates. After the full model was built, the backward elimination procedure at $\alpha=0.05$ significance level was used to arrive at the final model.

The software NONMEM was used to fit the model to the data. The resulting model was highly parameterised, and difficulties were encountered with the conditional estimation method of NONMEM. Consequently, only the first-order method was used.

A Monte-Carlo simulation study was conducted to assess the bias and precision of the NONMEM parameter estimates. It was done to justify the use of the first-order method as well as validate the code. Ten data sets of y_{ij} 's were generated with the same numbers of subjects and observations, and the same values of day and time-post-dose, as in the real data set. All ten had had the same values for $\underline{\theta}$ and Ω, which were similar to the final estimates from the real data. SAS was used to generate pseudo-random values of η_i and ε and with them to compute y_{ij} 's.

To validate the modelling approach further, the fit of the finally selected model was assessed with diagnostic plots of residuals.

3.1.3.3 Results

Parameterisation of the final model had the following form:

$$
\begin{aligned}
\text{sqrt}(t_{lag}) &= \theta_1 \exp(\eta_1) \quad , \\
\log(t_{abs}) &= \theta_2 + \eta_2 \quad , \\
\log(s) &= \theta_3 + \theta_{17}\,\text{HPRT} + \eta_3 \quad , \\
\log(b_4) &= \theta_4 + \theta_{13}\,\text{AGE1} + \eta_4 \quad , \\
\log(k_{31}) &= \theta_5 + \eta_5 \quad , \\
\log(k_{21}) &= \theta_5 + \theta_6 + \eta_6 \quad , \\
\log(b_3) &= \theta_4 + \theta_7 + \eta_7 \quad , \\
\log(b_2) &= \theta_4 + \theta_7 + \theta_8 + \theta_{14}\,\text{SMOKE} + \eta_8 \quad , \\
\\
\sigma &= \theta_9 + \theta_{10}\,\text{PATI} + \theta_{20}\,\text{IBLQ} \quad ,
\end{aligned}
$$

where

the covariates HPRT and SMOKE are the indicators for hypertension and smoking with the values 0 – No, and 1 – Yes;

age covariate AGE1 = Age/10; and

indicators PATI and IBLQ are described in (Eq. 37, Eq. 38).

The variance- covariance matrix of random effects was the following:

$$\Omega = \mathrm{cov}(\eta) = \begin{bmatrix} \omega_{11} & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & \omega_{22} & \omega_{23} & 0 & 0 & 0 & 0 & 0 \\ 0 & \omega_{23} & \omega_{33} & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & \omega_{44} & \omega_{45} & 0 & 0 & 0 \\ 0 & 0 & 0 & \omega_{45} & \omega_{55} & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & \omega_{66} & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & \omega_{77} & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & \omega_{88} \end{bmatrix}.$$

The final parameter estimates and estimated standard errors of the final model are presented in Table 42.

Table 42. Parameter estimates of the final population model.

Fixed parameter	Estimate	Standard Error	Random parameter	Estimate	Standard Error
θ_1	0.731	0.047	ω_{11}	0.0326	0.038
θ_2	0.839	0.055	ω_{22}	0.192	0.077
θ_3	-1.28	0.072	ω_{33}	0.269	0.13
θ_4	-6.42	0.13	ω_{44}	0.0871	0.063
θ_5	-6.24	0.14	ω_{55}	0.0695	0.079
θ_6	2.49	0.21	ω_{66}	0.00735	0.0039
θ_7	2.35	0.24	ω_{77}	$8.89*10^{-9}$	$1.6*10^{-6}$
θ_8	3.00	0.29	ω_{88}	0.0667	0.019
θ_9	0.222	0.026	ω_{23}	0.200	0.091
θ_{10}	0.191	0.042	ω_{45}	0.0667	0.073
θ_{13}	-0.0604	0.013			
θ_{14}	0.347	0.087			
θ_{17}	-0.263	0.12			
θ_{20}	1.35	0.28			

According to this model, hypertension decreased s, smoking increased b_2, and age decreased b_4. The pharmacokinetic parameters were pair-wise independent except for correlation of t_{abs} with s, and b_4 with k_{31}.

The parameter $s = V_c t_{abs}/F$ serves as a multiplicative factor that adjusts the level of the concentration curve; decrease of s with hypertension increases plasma levels. The parameter b_4 is reciprocal to the terminal elimination half-life ($t_{1/2} = \log(2)/b_4$); so terminal elimination half life increased with age. The parameter b_2, the largest of the three exponential rate constants is primarily associated with the absorption and early disposition portion of the concentration curve. The effect of its increase with smoking was decrease of plasma levels for smokers.

The value of the NONMEM objective function, which, apart from an additive constant, is approximately -2log(maximum likelihood), was -789.5 for this model. To establish the adequacy of this model, several extensions and simplifications were fitted.

Firstly, in order to assess the adequacy of the covariance structure, a model was fitted with eight η's, one for each of the parameters: t_{lag}, t_{abs}, s, b_4, k_{31}, k_{21}, b_3, and b_2; and with a full 3x3 covariance block for the first three "bioavailability" parameters and a full 5x5 covariance block for the final five "distribution/elimination" parameters, with independence between the two subsets of parameters. This model increased the number of covariance parameters from 10 to 20, but the NONMEM objective function decreased only to -801.1, i.e. the decrease of only 10.6 Thus, the covariance structure of the final model appeared to be sufficiently rich.

To assess the importance of the off-diagonal terms, models were fitted that constrained each of the two covariances to zero. When b_4 and k_{31} were forced to be independent, the objective function rose to -785.7. When t_{abs} and s were forced to be independent, the objective function rose to -741.4. Thus, the data strongly indicated the importance of the latter correlation, and mildly supported the former.

To assess the appropriateness of the dependencies of the pharmacokinetic parameters on the covariates, reduced models with one covariate constrained to zero were fitted (Table 43). The importance of age in b_4 and smoking in b_2 were strongly supported by the data; the effect of hypertension in s was marginally significant.

Table 43. Summary of reduced covariate models

Covariates	Criteria	
	Objective Function	AIC[a]
b_2(Smoking), b_4(Age), s(Hypertension)	-789.5	-741.5
b_2(Smoking), s(Hypertension)	-765.6	-719.6
b_4(Age), s(Hypertension)	-771.4	-725.4
b_2(Smoking), b_4(Age)	-785.1	-739.1

a. Akaike Information Criterion = Objective function + 2(number of parameters)

To assess further the adequacy of the finally selected model, diagnostic plots of residuals (observed plasma concentrations minus subject-specific predicted values) versus subject-specific predicted values, time, each of the three covariates, and Study were plotted in Figure 29 - Figure 34. In Figure 29, residuals corresponding to the BLQ values that were set at 5 ng/mL, stand out as the downward trending line at the low end of the range of predicted values. Several outliers also are evident. Otherwise the scatter is fairly homogeneous, justifying the log scale. Those residuals from BLQ values cluster just below zero at 2000 hours in Figure 30, rendering the distribution of residuals at 2000 hours skewed toward the positive. A similar skewness is evident around the one-hour time. Otherwise, residuals versus time show no obvious trends that would indicate deficiencies in the model. In Figure 31 - Figure 33, residuals show homogeneous scatter when plotted versus covariates. In Figure 34, the similar distributions of residuals across the three studies demonstrate that the model describes the pharmacokinetics of both patients and healthy volunteers equally well.

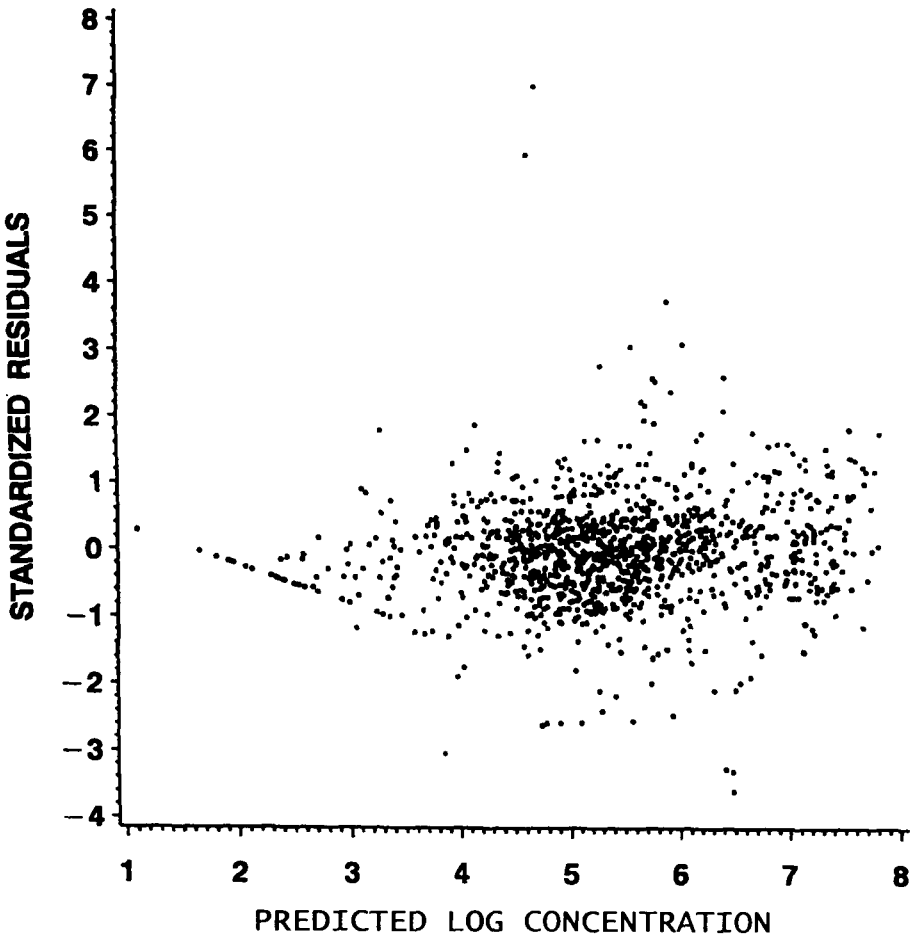

Figure 29. Standardised residuals versus predicted values from NONMEM fit.

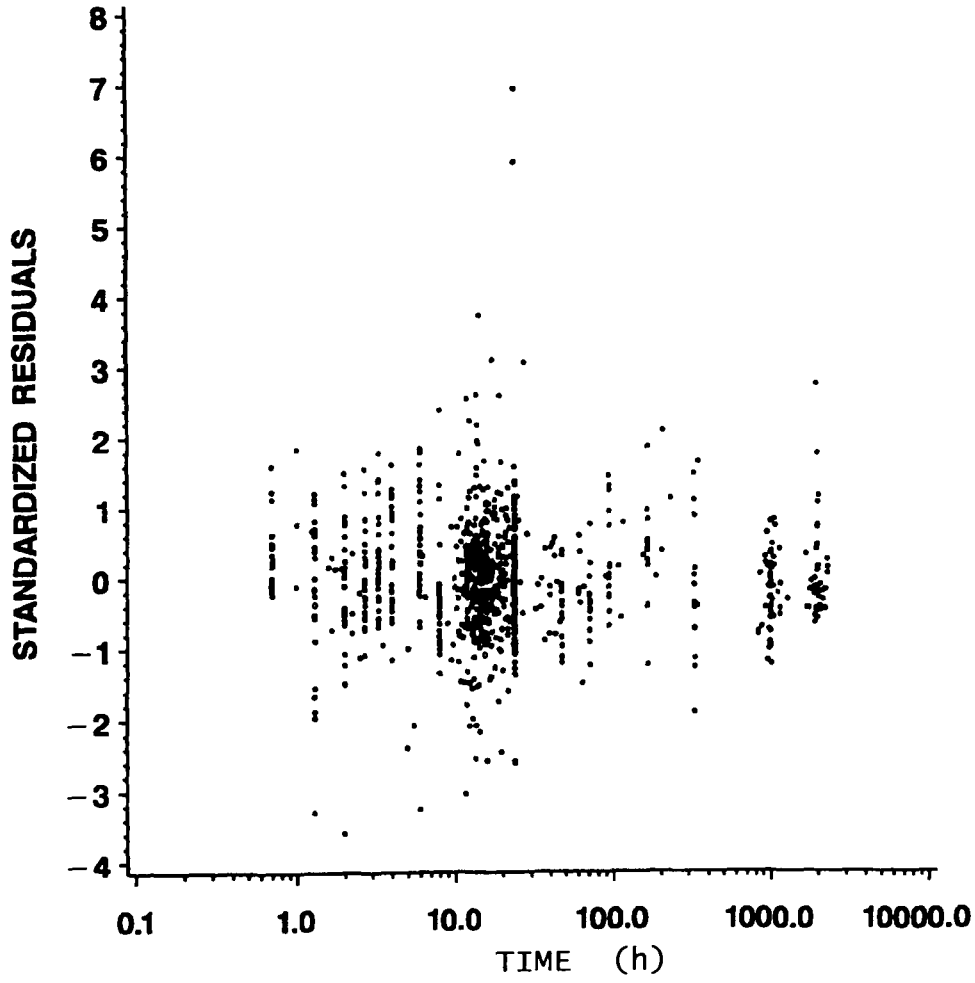

Figure 30. Standardised residuals versus time from NONMEM fit.

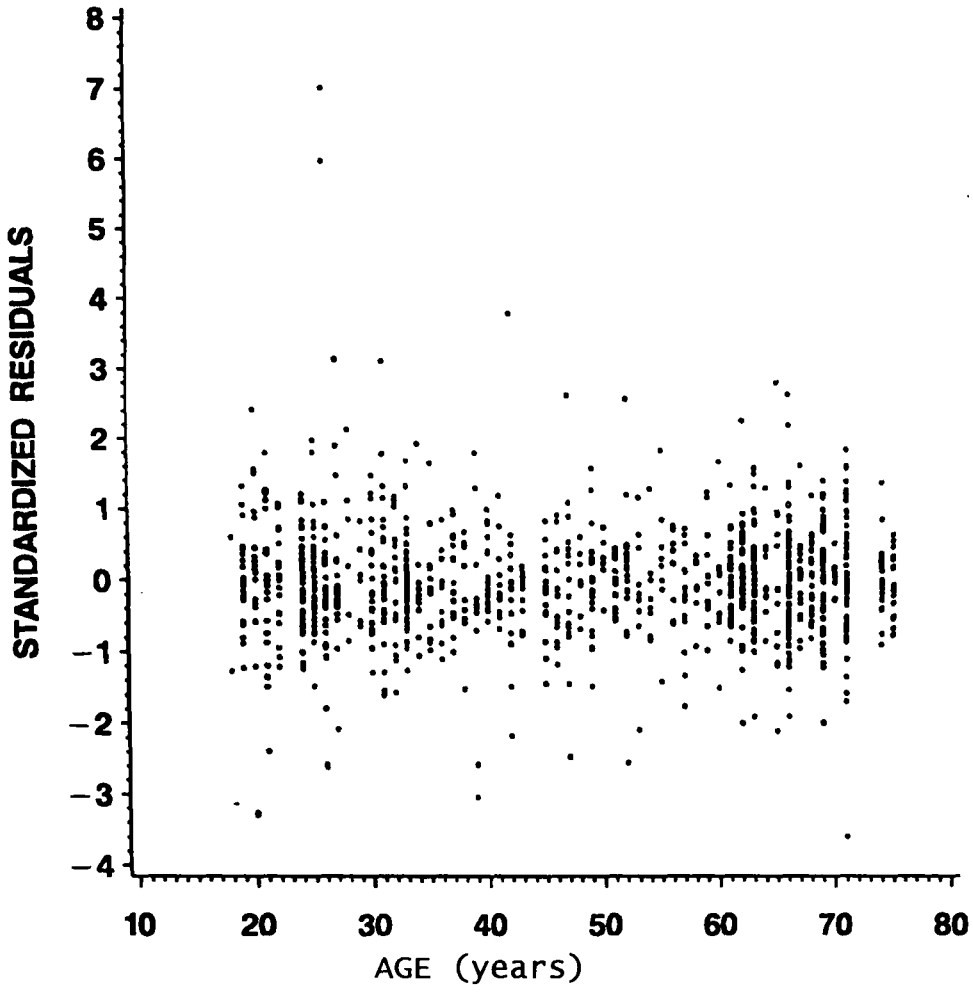

Figure 31. Standardised residuals versus age from NONMEM fit.

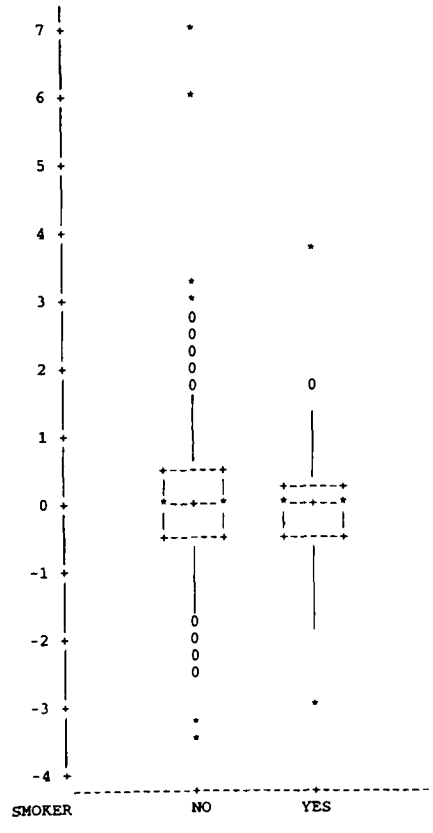

Figure 32. Standardised residuals versus smoking status from NONMEM fit.

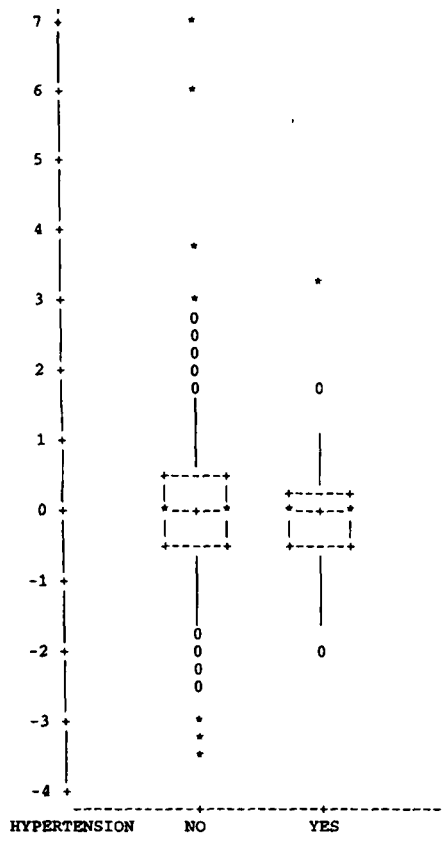

Figure 33. Standardised residuals versus hypertension from NONMEM fit.

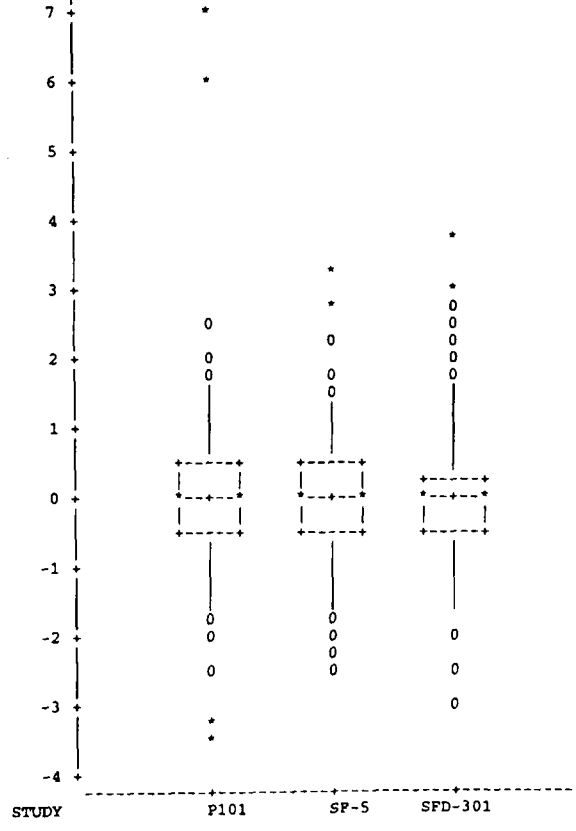

Figure 34. Standardised residuals versus Study from NONMEM fit.

To assess the ability of the first-order estimation method of NONMEM to estimate parameters from such a model, a simulation study was performed. Results of the simulation are summarised in Table 44. Although only 10 simulations were performed, some trends were evident. θ_4, the parameter that is the intercept of the linear relationship between $\log(b_4)$ and age, was slightly biased downward by about 2%. θ_{13}, the slope of that relationship, did not exhibit any bias. When the resultant bias in $\log(b_4)$ was translated into an effect on half-life, $\log(2)/b_4$, it became a 10% upward bias in the $t_{1/2}$. Estimates of the bioavailability parameters θ_1 ($\sqrt{t_{lag}}$), θ_2 ($\log(t_{abs})$), and θ_3 ($\log(s)$) all exhibited biases on the order of 10%. Other pharmacokinetic structural parameters were estimated without significant bias. ω_{22} and ω_{33}, random-effect variances associated with the bioavailability parameters θ_2 and θ_3, respectively, exhibited slight biases. ω_{44}, the variance of $\log(b_4)$, was underestimated by about 50%. This translated into an approximate 25% underestimation of the standard deviation of $\log(b_4)$, which is approximately the coefficient of variation of b_4 or of the terminal half-life. The variance of $\log(b_2)$, that is ω_{88}, was slightly upward biased.

Table 44. Simulation study: true parameters, means and standard deviations of 10 NONMEM runs.

Parameter	True	Mean	STD	Parameter	True	Mean	STD
θ_1	0.731	0.661	0.081	$t_{1/2}$	3.325	3.678	19.52
θ_2	0.839	0.941	0.127	Cl/F	19.05	0.266	0.799
θ_3	-1.28	-1.01	0.127	ω_{11}	0.10	0.30	0.57
θ_4	-6.42	-6.54	0.134	ω_{22}	0.20	0.15	0.064
θ_5	-6.24	-6.37	0.083	ω_{32}	0.20	0.21	0.091
θ_6	2.49	2.5	0.112	ω_{33}	0.30	0.40	0.13
θ_7	2.35	2.38	0.113	ω_{44}	0.090	0.047	0.026
θ_8	3.00	2.994	0.137	ω_{54}	0.070	0.053	0.043
θ_9	0.222	0.254	0.016	ω_{55}	0.070	0.073	0.062
θ_{10}	0.191	0.17	0.027	ω_{66}	0.010	0.0074	0.0064
θ_{13}	-0.06	-0.06	0.019	ω_{77}	0.00010	0.0020	0.0027
θ_{14}	0.347	0.336	0.093	ω_{88}	0.070	0.073	0.020
θ_{17}	-0.26	-0.29	0.157				

In conclusion, the first-order estimation method of NONMEM did an adequate job at capturing the average disposition/elimination behaviour. It did less well at identifying the bioavailability parameters t_{lag}, t_{abs} and s, although it accurately estimated average apparent oral clearance. It exhibited a downward bias in the estimation of the variability of the terminal half-life.

3.1.4 APPLICATIONS OF THE POPULATION MODEL

3.1.4.1 Pharmacokinetics Of The Drug

3.1.4.1.1 Methods

In order to summarise the important features of the drug pharmacokinetics, the following pharmacokinetic parameters and other summary measures were computed from the final model: apparent oral clearance (Cl/F), apparent volume of distribution at steady state (Vss/F), terminal elimination half life ($t_{1/2}$), area accumulation ratio, trough accumulation ratio, and tail fraction after n weeks of dosing.

Apparent oral clearance, apparent volume of distribution at steady state and terminal elimination half life were computed as [Gibaldi & Perrier, 1982]:

$$AUC_{0-\infty} = \int_0^{\infty} C(t)dt\,, \qquad \text{Eq. 42}$$

$$AUMC = \int_0^{\infty} tC(t)dt\,, \qquad \text{Eq. 43}$$

$$CL/F = D/AUC_{0-\infty}\,, \qquad \text{Eq. 44}$$

$$V_{ss}/F = D * AUMC/ [AUC_{0-\infty}]^2\,, \qquad \text{Eq. 45}$$

$$t_{1/2} = \ln(2)/b_4 \qquad \text{Eq. 46}$$

Area accumulation ratio, trough accumulation ratio, and tail fraction after n weeks of dosing were defined as:

Area accumulation ratio ($AUC_{0-24,ss}$ / $AUC_{0-24,single\ dose}$)	the ratio of the AUC over 24 hours at steady state to the AUC over the 24 hours after a single dose;

Trough accumulation ratio ($C_{24,ss} / C_{24,single\ dose}$)	the ratio of the concentration at 24 hours post-dose at steady state to the concentration at 24 hours after a single dose;
tail fraction after n weeks of dosing ($AUC_{24-\infty,n} / AUC_{0-\infty}$)	the ratio of the AUC from 24 hours to ∞ following the final dose after n weeks of dosing to the cumulative AUC from the very first dose to ∞ following the final dose after n weeks of dosing; i.e., the fraction of total systemic exposure that occurs after an n-week dosing period ceases.

Distributions of these summary measures were explored for a population of patients described by the final model. To do this, 1000 random $\underline{\eta}$ vectors had to be generated with a mean 0 and covariance matrix Ω, as estimated by the final model. To generate the $\underline{\eta}$'s, 1000 sets of eight normal random numbers r_k, k=1,...,8 were generated, with a mean 0 and variance 1. From $\underline{r}$, individual random parameters were computed as

$$\eta = L * \underline{r}, \qquad \text{Eq. 47}$$

where L is a matrix such that

$$\Omega = L * L^T. \qquad \text{Eq. 48}$$

These η's, were then used to compute pharmacokinetic parameters t_{lag}, t_{abs}, s, b_2, b_3, b_4, k_{21}, k_{31} according to Eq. 40 and Eq. 41. From these parameters the above summary measures were computed. From the 1000 resulting values of each summary measure, percentiles (5th, 25th, 50th, 75th, and 95th) were computed. This was done for each of several combinations of covariates found significant during the analysis. Finally, to summarise the effects of the covariates, the medians of each summary measure were fitted to multiplicative models with the covariates as predictors.

3.1.4.1.2 Results

The components of Ω determine the distribution of pharmacokinetic parameters amongst individuals. The importance of subpopulations determined by age, smoking

status, and history of hypertension has been identified. Within each subpopulation, there is a distribution of the pharmacokinetic parameters.

Figure 35 - Figure 40 summarise the distributions of the parameters for the eight subpopulations determined by the eight combinations of age (30 or 60), smoking (yes or no) and hypertension (yes or no). Each vertical line in these plots extends from the first to the third quartile of the distribution for the parameter in the subpopulation of all individuals with the combination of demographic covariates specified by the horizontal axis. The central tick is the median. Table 45 summarises the medians.

Table 45. Medians of pharmacokinetic parameters by demographic subgroup.

Age	30 years				60 years			
Smoker?	No		Yes		No		Yes	
Hypertension?	No	Yes	No	Yes	No	Yes	No	Yes
Cl/F (L/h)	21.0	16.2	29.8	22.9	17. 6	13.5	24.8	19.1
V_{ss} /F (L)	5028	3866	7081	5443	6781	5213	9566	7354
$t_{1/2}$ (wks)	3.07	3.07	3.07	3.07	3.68	3.68	3.68	3.68
Area Accumulation Ratio	1.78	1.78	1.77	1.77	2.13	2.13	2.11	2.11
Trough Accumulation Ratio	7.01	7.01	7.34	7.34	9.50	9.50	9.99	9.99
Tail Fraction 12 Weeks	0.11	0.11	0.11	0.11	0.17	0.17	0.17	0.17
Tail Fraction 24 Weeks	0.059	0.059	0.059	0.059	0.094	0.094	0.094	0.094

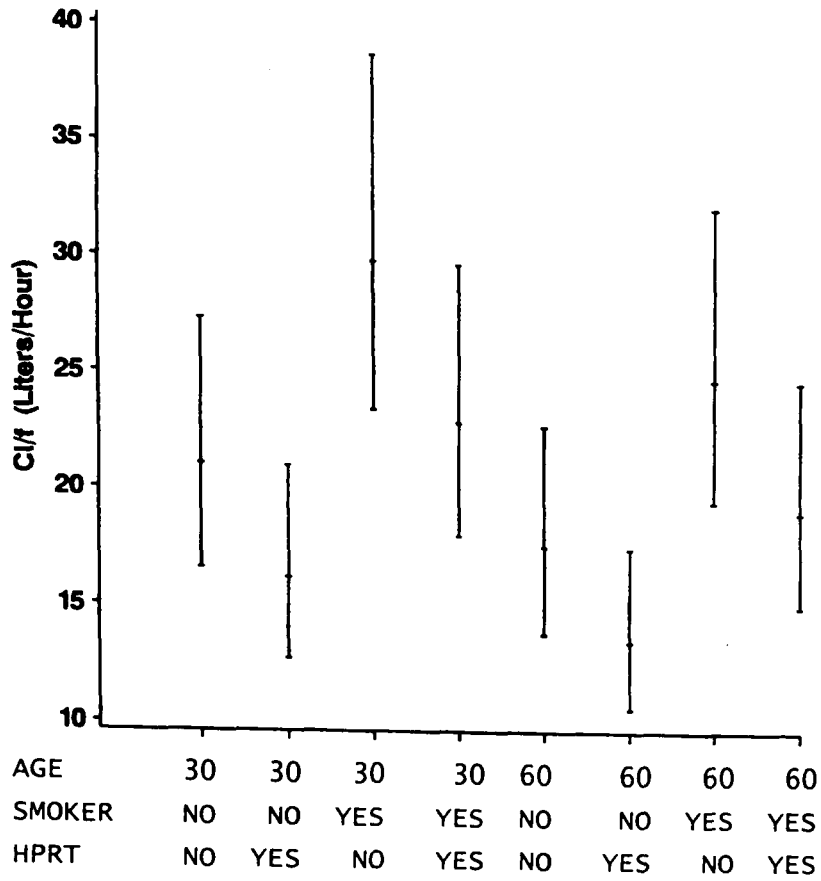

Figure 35. Lower, middle, and upper quartiles of apparent oral clearance in subpopulations determined by demographic covariates.

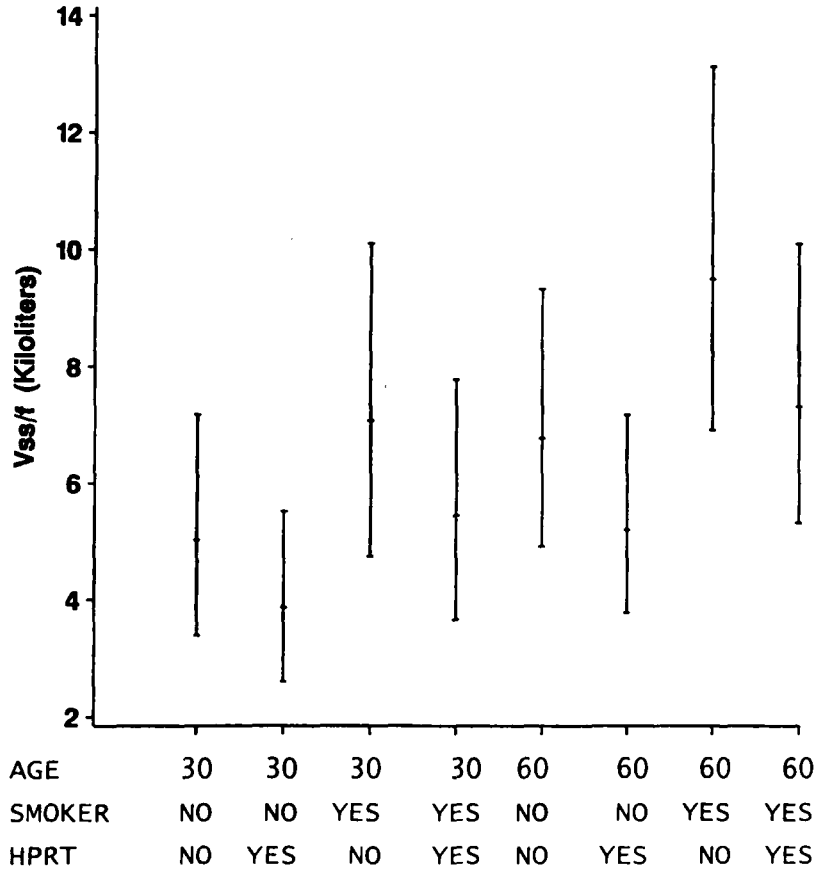

Figure 36. Lower, middle, and upper quartiles of apparent volume of distribution at steady state in subpopulations determined by demographic covariates.

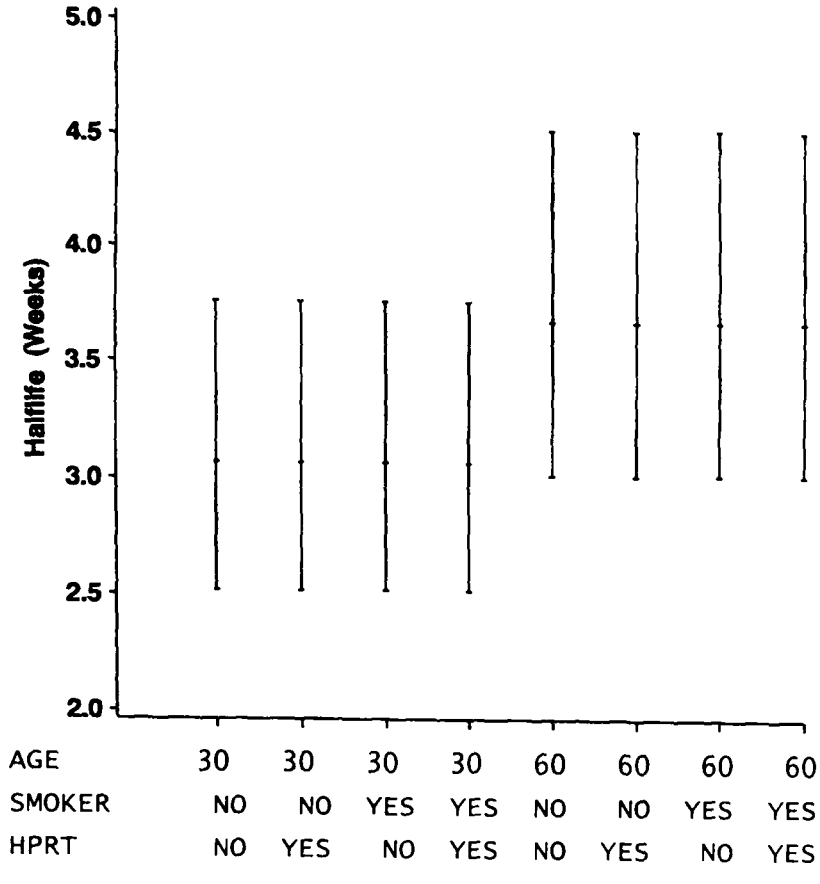

Figure 37. Lower, middle, and upper quartiles of half-life in subpopulations determined by demographic covariates.

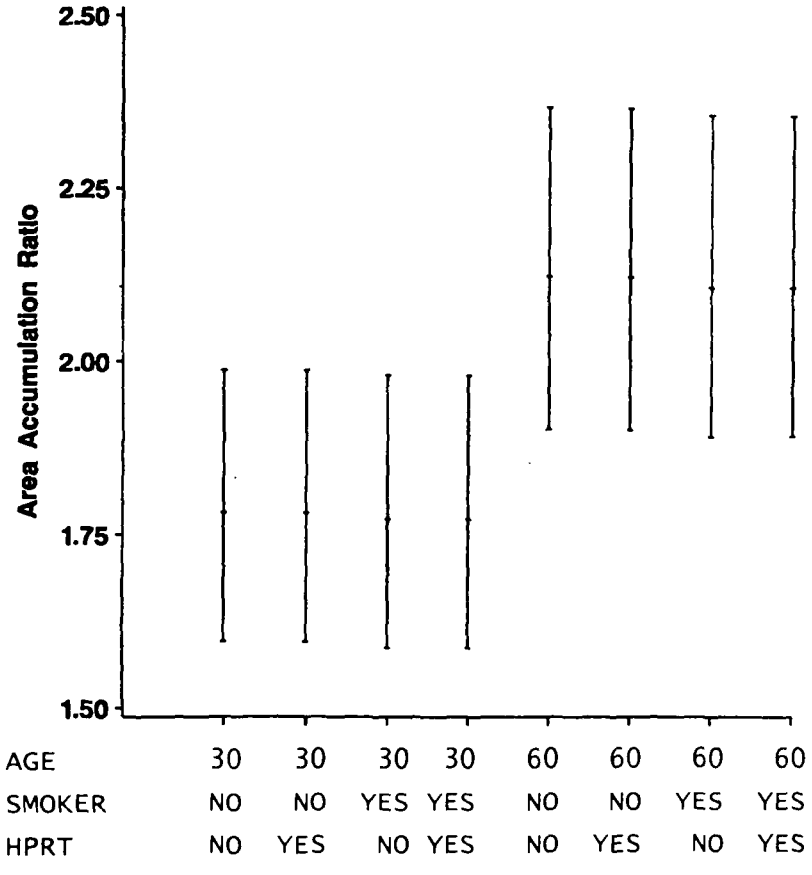

Figure 38. Lower, middle, and upper quartiles of Area Accumulation Ratio in subpopulations determined by demographic covariates.

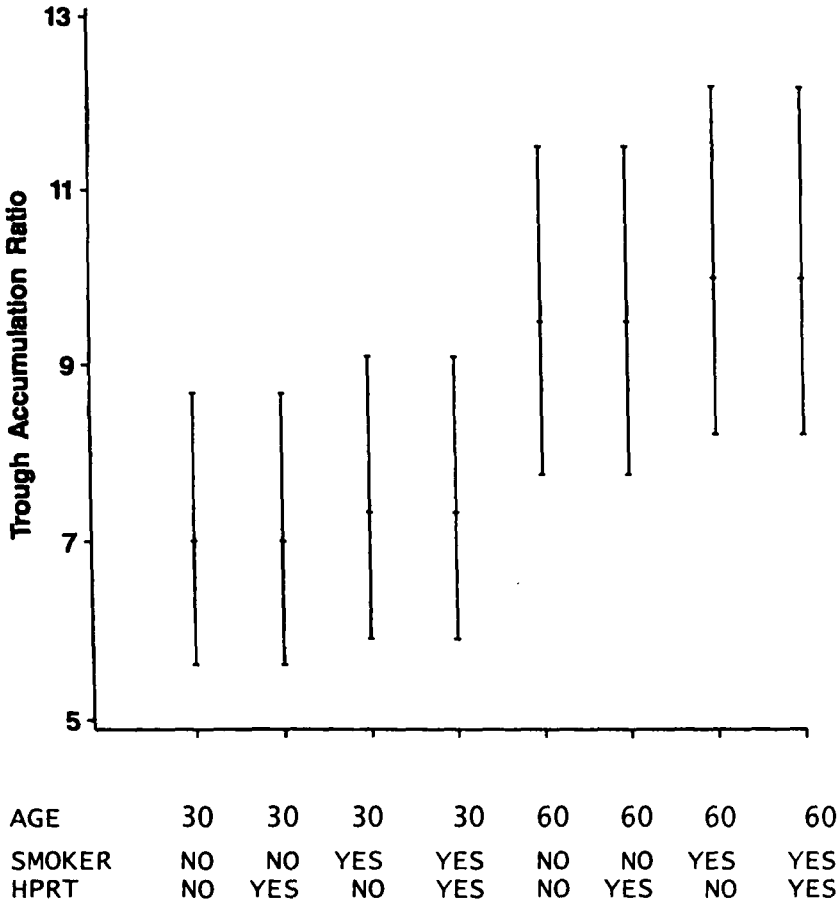

Figure 39. Lower, middle, and upper quartiles of Trough Accumulation Ratio in subpopulations determined by demographic covariates.

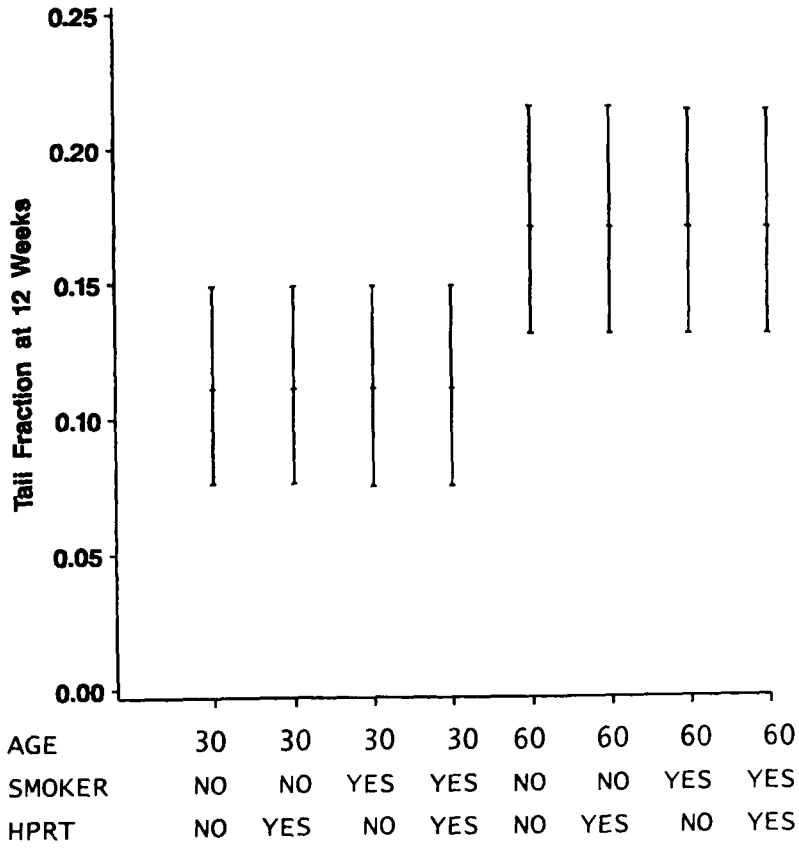

Figure 40. Lower, middle, and upper quartiles of Tail Fraction at 12 weeks in subpopulations determined by demographic covariates.

As one can see, median half-lives and tail fractions do not depend on smoking status and hypertension, they stay constant within the age groups. This behaviour is expected from the model: only AGE covariate influences b_4, the parameter that defines the half-life and the tail fractions.

For each of the seven pharmacokinetic parameters, which are presented in Table 45, the eight estimated median values in the corresponding row were fitted to a multiplicative main-effects model. For example, for the parameter Cl/F the model had the following form

$$Cl/F = (\mu)(\alpha_{age})(\beta_{smoker})(\gamma_{hypertension})(1+\varepsilon), \qquad \text{Eq. 49}$$

where age $\in \{30, 60\}$, smoker $\in$ {no, yes}, hypertension $\in$ {no, yes}, and ε is a residual error. For none of the seven parameters ε ever exceeded 0.002. The small residuals from the fits indicated the adequacy of the multiplicative structure for each parameter. Such a structure implies that each parameter changes proportionally across levels of each demographic variable independently of the other two demographic variables. Using the estimated multiplicative effects, Table 46 displays percent differences in each of the seven parameters across each of the three demographic dichotomies.

Median half-lives ranged from 3 to 3.7 weeks across subpopulations, with age driving the variation in the medians. Correcting for possible bias in the NONMEM estimate, these may reduce to 2.7 - 3.3 weeks. Within subpopulations, the inter-quartile range in half-life was about 40% of the median, with the 75th percentile being 1.5 times the 25th percentile. Correcting for possible bias in the NONMEM estimate of the inter-subject variance, the inter-quartile range of half-life may increase to approximately 50% of the median. Steady-state levels were about twice the single-dose levels on average, although troughs increased as much as 10-fold at steady state, relative to the first dose. By the 12th week, and certainly the 24th week, of a therapeutic regimen, steady state would be achieved. Following cessation of such a regimen, systemic exposure continued and contributed 11-17% of the total exposure from a 12-week regimen on average, or 6-9% of the total exposure from a 24-week regimen. Again, age drove the variation in medians.

Table 46. Percent differences in medians of pharmacokinetic parameters across demographic subgroups.

Parameter	Demographic subgroups		
	Age	Smoker	Hypertension
	60 relative to 30	Yes relative to No	Yes relative to No
Cl/F	-16.6	41.4	-23.1
V_{ss}/F	35.0	40.9	-23.1
$t_{1/2}$	19.9	0.0	0.0
Area Accumulation Ratio	19.4	-0.8	0.0
Trough Accumulation Ratio	35.8	4.9	0.0
Tail Fraction 12 Weeks	54.5	0.0	0.0
Tail Fraction 24 Weeks	59.3	0.0	0.0

Age increased the *extent* of exposure. Clearance decreased with age, which increased the steady-state plasma level. The steady-state volume of distribution also increased with age, so drug was distributing more widely. The relative level of steady-state concentrations compared with single-dose concentrations also increased with age, as expressed by both accumulation ratios. Increasing age also increased *duration* of exposure. With age, the half-life increased, and the fraction of total exposure that occurred after the cessation of a therapeutic regimen of 12-24 weeks also increased.

Smoking decreased steady-state plasma levels by increasing clearance, but it also increased apparent steady-state volume of distribution. Smoking had a negligible effect on duration of exposure.

Hypertension increased steady-state plasma levels by decreasing clearance, and it also decreased steady-state volume of distribution. It had a negligible effect on duration of exposure.

3.1.4.1.3 Discussion

The duration and extent of exposure were the two major foci of this investigation. Duration of exposure was determined largely by half-life, and steady-state levels were determined by clearance. In Table 47 the estimates of terminal half-life and clearance by duration of dosing in the studies from which the estimates were derived are summarised. For the three healthy-volunteer studies, the listed values are medians of individual estimates. For the combined studies H1, P1, and P2, the values are the medians of the distributions, as predicted by the nonlinear-mixed-effects model for 30-year-old (young) or 60-year-old (elderly), non-smoking, non-hypertensive subjects.

As can be seen, the estimates of half-life increased with duration of dosing. On the first glance it contradicts the assumption of linear time-independent kinetics. However, the observed trend can be explained within the model of time-independent, linear, three-compartment kinetics.

Table 47. Median values of terminal half-life and apparent clearance by duration of dosing.

Study	Duration of Dosing	$t_{1/2}$ (weeks)		Cl/F (L/h)	
		Young	Elderly	Young	Elderly
H1	15 days	1.57 [a]	2.13 [b]	23.96 [a]	17.16 [b]
H2	18 days	1.59 [c]		30.81 [c]	
H3	30 days	2.23 [d]		18.59 [d]	
H1+P1+ P2	15 days -24 weeks	3.07 [e]	3.68 [f]	21.05 [e]	17.56 [f]

a. Median value of parameter estimates from fits to 15 young (19-33 years) healthy subjects.

b. Median value of parameter estimates from fits to 15 elderly (61-75 years), healthy subjects.

c. Median value of parameter estimates from fits to 20 young (19-40 years), healthy subjects.

d. Median value of parameter estimates from fits to 10 young (21-45 years) healthy subjects at the 250-mg dose.

e. Median value of estimated distribution of parameter for 30-year-old, non-smoking, non-hypertensive subjects, according to nonlinear mixed-effects model.

f. Median value of estimated distribution of parameter for 60-year-old, non-smoking, non-hypertensive subjects, according to nonlinear mixed-effects model.

The notion of terminal half-life is related to the visual appearance of the concentration versus time curve when plotted on semi-logarithmic axis (i.e. the log-concentration versus time curve). Commonly, at sufficiently long times post dose, the curve appears linear; that is, the instantaneous slope, or derivative, of the curve eventually becomes constant. The slope of the terminal, linear portion of log-concentration curve is inversely proportional to the terminal half-life (Eq. 46).

Figure 41 displays log-concentration curves following various duration of dosing for an average 30-year-old, non-smoking, non- hypertensive, according to the nonlinear mixed-effects model. All five curves eventually become linear and parallel, but curvature persists longer for the shorter duration of dosing. The estimate of half-life provided by fitting a compartmental model is highly dependent on whether the last several observations are in the linear portion of the curve or still in the curved portion. To the extent that these observations are still in the curvilinear region, the estimated half-life is actually estimating *instantaneous half-lives* ($t_{1/2,\ inst}$), which are defined as inversely proportional to the instantaneous slopes or derivatives of the curve.

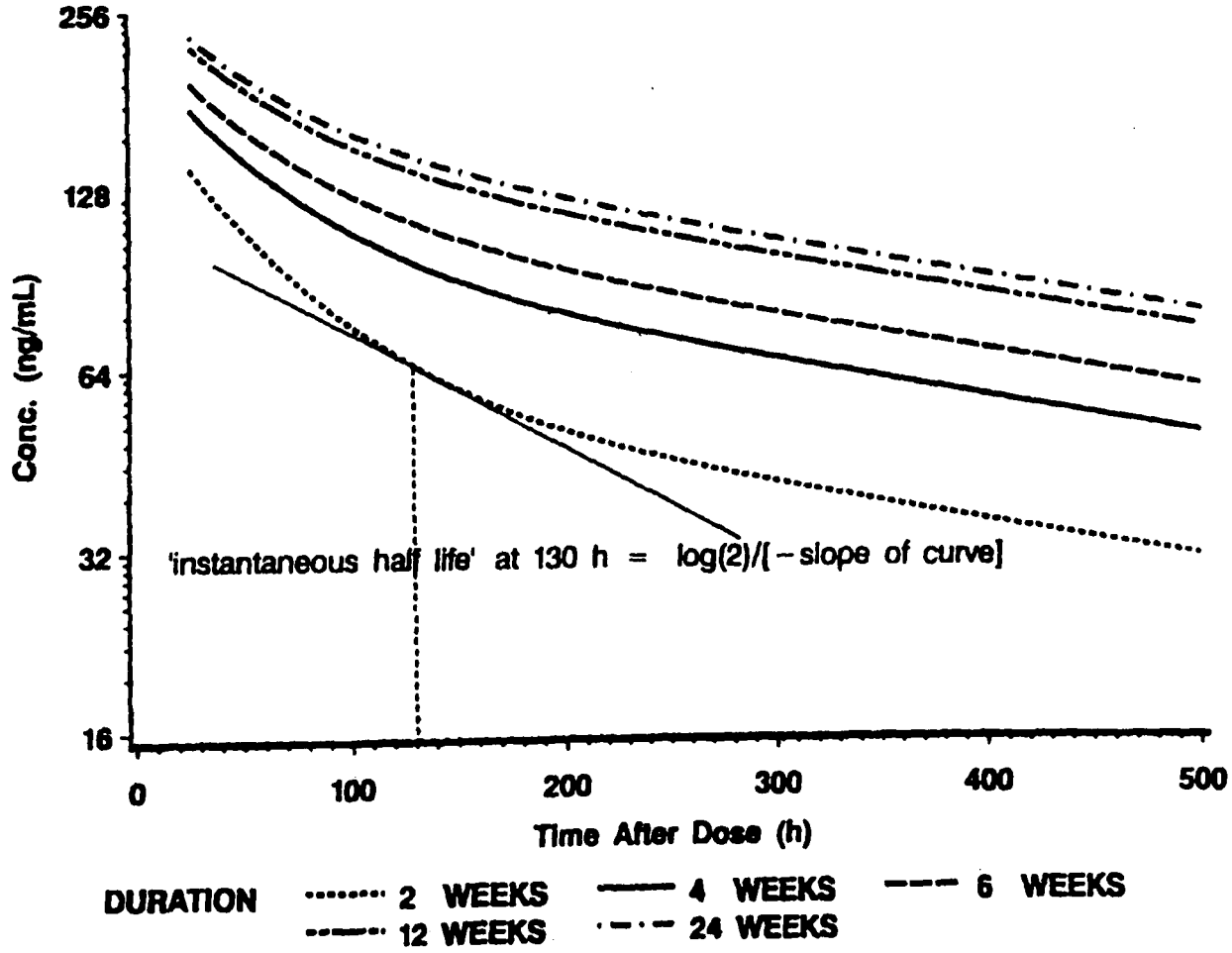

Figure 41. Predicted concentration versus time curves for an average 30-year-old, non-smoking, non-hypertensive subject, after different dosing duration as predicted by the nonlinear mixed-effects model. Also displayed is the definition of instantaneous half-life.

Most of the observations in all five of the studies were indeed in the curvilinear region, and the proportion was greater for the studies of shortest duration.

Table 48 displays values of instantaneous half-life as a function of duration of dosing and hours post dose, as they would be for an average 30-year-old, non-smoking, non-hypertensive subject, with a fixed terminal half-life of 3.1 weeks, according to the nonlinear mixed-effects model. The combined effects of dosing duration and times of observation, evident in Table 48, suggest that the pattern of estimated half-lives in Table 47 may reflect an underestimation of $t_{1/2}$ due to short dosing duration and short follow-up times, not a dependence of the true value of $t_{1/2}$ on duration of dosing.

Table 48. Instantaneous half-life (weeks) versus day of dosing and time post dose.

Time Post Dose (hours)	Day of Dosing					
	15	18	30	42	84	168
100	0.70	0.75	0.91	1.00	1.13	1.17
200	1.59	1.69	1.92	2.04	2.18	2.22
300	2.53	2.58	2.70	2.75	2.80	2.81
400	2.91	2.93	2.96	2.97	2.98	2.99
500	3.01	3.01	3.02	3.02	3.02	3.03
600	3.03	3.03	3.03	3.03	3.03	3.03

Table 48 helps to explain the observed dependence of half-life on the duration of dosing (see Table 47). For example, in Study H1, following 15 days of consecutive dosing, the last three times of blood sampling after the final dose were 96, 168, and 335 hours. The estimate of half-life, based on these three time points, should be close to the instantaneous half-life in the middle of the time interval of 96 – 335 hours. According to Table 48, this would yield an estimated half-life that is close to the observed value of 1.6 weeks.

There were two major concerns with regards to a drug that has a long terminal half-life. The first concern was that a long half-life would generate marked

accumulation of the drug. Basic pharmacokinetic theory, commonly used to estimate the accumulation, suggests [Rowland & Tozer, 1995] that with a half-life of $t_{1/2}$ hours, dosing every 24 hours would produce an accumulation ratio R_{acc} of

$$R_{acc} = 1/[1 - \exp(-24*\log(2)/t_{1/2})] = 1/[1 - \exp(-16.6/t_{1/2})]. \quad \text{Eq. 50}$$

A half-life of 3.03 weeks (509 hours) would thus yield an accumulation ratio of 31. However, the above formula applies only to the one-compartment model with a mono-exponential concentration curve, whereas the drug exhibits poly-exponential behaviour. The estimate based on the three-compartment model resulted in the area accumulation ratio of around 2, and the trough accumulation ratio of around 8. Complete verification of the model-based estimates of accumulation was not possible in principle because only one of the five Studies, H3, had single-dose plasma levels; and 30 days of dosing in that study were not sufficient to attain steady state according to a half-life of 3 weeks. Nonetheless, some partial verification was possible by considering observed versus predicted accumulation at 30 days. The median *model-independent* (computed by trapezoidal rule: a simple summation of observed trapezoidal areas*) area* estimates of AUC_{0-24} on days 1 and 30 from the 250-mg dose in Study H3 were 4760 and 8841 ng/mL, respectively, for a ratio of 1.866. For young (age=30 years), non-hypertensive non-smokers, the nonlinear mixed-effects model fitted to the combined data from Studies H1, P1, and P2 estimated median AUC_{0-24} on days 1 and 30 to be 6778 and 10580 ng/mL, respectively, for a ratio of 1.56. The two ratios are in general agreement.

The second concern about a long terminal half-life was prolonged exposure following extended treatment. If one measures total exposure by cumulative AUC from the start of dosing until plasma levels decrease to zero after the very last dose, then one can quantify the effective duration of exposure to the drug after cessation of dosing by computing what fraction of that cumulative AUC is contributed by the time period after cessation of dosing. That fraction was estimated for 12 and 24 weeks of dosing and for various combinations of age, smoking, and hypertension. That fraction decreased with duration of dosing, and it increased with age. Smoking and hypertension had little effect on the tail fraction. This is because age entered the model through the terminal exponential rate constant, whereas smoking and

hypertension affected bioavailability and early disposition parameters. Estimated median values were 11-17% for 12 weeks of dosing and 6-9% for 24 weeks of dosing for ages 30-60 years.

3.1.4.2 Exposure And Predictors Of Exposure Versus Safety

After exposure and demographic predictors of exposure were quantitatively characterised, possible effects of varying exposure on safety were explored. Data from two patient studies, P1 and P2, was used for this analysis.

3.1.4.2.1 Data

In order to perform the pharmacokinetic/safety analysis, data regarding study visits, safety indicators, and pharmacokinetics were merged together. Patients from placebo arms were included in the analysis. Only data from those centres participating in blood sampling could be utilised. Adverse events were associated with visits by matching the date of onset of the event to the inter-visit interval preceding the visit with which it was associated. The resulting data set had 2288 visit records corresponding to 282 subjects, seven of whom were excluded for having no pharmacokinetic assessments. Those patients contributed 28 visit records to the 2288. So, data from 2260 visits of 275 patients were used.

In order to examine relationships between safety indicators and demographics, these two types of data were merged together. Only patients on active drug were included. For the analyses involving age and hypertension, there were 434 patients from all centres in the final data set. For the analysis involving smoking, some of the centres in Study P2 had to be excluded because smoking histories were collected from patients only when pharmacokinetic blood sampling was done. The resulting data set had 228 patients.

3.1.4.2.2 Methods

Two types of safety indicators were investigated: scores summarising frequency and severity of adverse events often recorded as drug related by investigators, and scores summarising frequency and severity of abnormal laboratory tests of liver function. Associations between the safety indicator scores and pharmacokinetic response were

investigated, as well as associations between the safety indicator scores and demographic covariates that were found to be significantly related to pharmacokinetic response. Only data from the administration periods, not from baseline or follow-up periods, were considered.

The following adverse events were investigated: headache, dizziness, diarrhoea, dyspepsia, flatulence, nausea, abdominal pain, rash, pruritus, erythema, and taste disturbance. Adverse events occurred infrequently, therefore they were grouped for analysis into the following four categories:

CNS = {headache, dizziness}
GI = {diarrhoea, dyspepsia, flatulence, nausea, abdominal pain}
SKIN = {rash, pruritus, erythema}
TASTE = {taste disturbance}.

Patients visited their physicians for clinical assessments at weeks 2, 4, 6, 8, and 12 in the Study P1 and at weeks 2, 4, 8, 12, 18, and 24 in the Study P2. Adverse events were associated with a visit number if the onset of the event occurred prior to that visit but after the preceding visit. An adverse-event occurrence was scored as 1 if mild, 2 if moderate, and 3 if severe. If the event did not occur, it was given a score equal to 0. Thus, although frequency of drug-related attribution determined which adverse events were selected for investigation, drug-relatedness was not considered for scoring of specific occurrences of those adverse events. If more than one occurrence of the event was associated with a visit, the maximum score for that event was retained. Group scores for CNS, GI, SKIN, and TASTE were then computed for each visit by summing the scores of events associated with that visit from the components of the group.

Liver function tests were performed at weeks 4, 8, and 12 in the Study P1, and at weeks 4, 8, 12, and 24 in the Study P2. Parameters tested for this investigation included gamma-glutamyl transferase (GGT), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and total bilirubin. These were scored as follows, where "test" is a test result and "U" is the upper limit of the normal range for that test:

$0 < \text{test} \leq U$, score=0;

$U < \text{test} \leq 1.5U$, score=0.5;

$1.5U < \text{test} \leq 2U$, score=1;

$2U < \text{test} \leq 3U$, score=2;

$3U < \text{test}$, score=3.

A group liver score, called LIVER, was computed by summing the scores from the four separate tests. Liver test scores were associated with the visit at which the tests were conducted.

The above-described safety scores for adverse events and liver function test results were explored for correlation with systemic exposure. The NONMEM analysis provided Bayes post hoc estimates of pharmacokinetic parameters for each patient. Using those estimates in the model, the patient's average plasma concentration during the inter-visit interval preceding a visit was computed. Correlation was examined between average concentrations and the visit-specific safety scores. Patients in placebo/placebo arms were assigned an inter-visit average concentration of zero for each visit. But patients in the placebo half of the active/placebo arms were assigned average concentration values appropriate to their residual plasma levels. That is, even after dosing with active drug ceased, continued systemic exposure due to the drug's long half-life was accounted for by calculation of the area under the appropriate portion of the terminal tail of the concentration-versus-time curve.

Observations were partitioned into five exposure groups: those where inter-visit average concentration fell into one of four quartiles for the nonzero (i.e., non-placebo /placebo-arm) values, and those where inter-visit average concentration was zero (i.e. placebo/ placebo-arm values). The frequencies of patient visits with nonzero scores, i.e., when any liver function abnormalities or adverse event in their groups occurred, were tested for association with exposure groups by means of the Mantel-Haenszel chi-square tests, which accounted for the ordering of the exposure categories [SAS, 1989b]. To assess the severity of adverse events when they occurred, means and standard deviations of inter-visit safety scores were tabulated by inter-visit average concentration group for patient visits with nonzero scores. Side-by-side box plots were also constructed.

In addition, *patient-summary score*, the maximum over all visit-specific scores for each patient was constructed. A nonzero patient summary score for a safety indicator means that the patient at some time during the study experienced one of the adverse events or abnormal tests. As a patient summary measure of exposure, the average concentration at steady state for each patient was computed from the NONMEM post hoc parameter estimates. Patients in placebo/placebo arms were assigned values of zero. Patients in active/placebo arms were treated just like patients in active/active arms. Observations were partitioned into five exposure groups based on average steady state concentration in the same manner as for inter-visit average concentrations, and were similarly analysed. Note, however, that from the patient-summary perspective the patient was the observational unit, whereas from the visit specific perspective the patient visit was the observational unit.

To investigate associations between safety outcomes and demographic predictors of pharmacokinetics, frequencies of nonzero patient-summary safety scores were tested for association with levels of the demographic covariates by means of Fisher's exact test or Mantel-Haenszel chi-square test. Means and standard deviations of safety indicator scores were also tabulated by level of each covariate. For categorical analysis, age was partitioned into three groups: ≤ 40 years, 41 to 55 years, and > 55 years. Hypertension and smoking were both dichotomous.

3.1.4.2.3 Results

Table 49 and Table 50 summarise means and standard deviations of safety indicator scores associated with specific visits, by groups based on inter-visit average concentration as estimated by the nonlinear mixed-effects model. Table 51 and Table 52 summarise patient-summary scores, where the patient groups were determined by estimated steady-state average concentrations.

For adverse events, a score of 1 indicated a mild occurrence whereas a score of 0 indicated no occurrence. A similar interpretation may be applied to scores for liver - function tests, where a score of 0 meant the test was within the normal range and scores of 0.5 or 1 meant the test was at most 1.5 or 2 times the upper limit of the normal range, respectively. No average safety indicator score in Table 49 - Table 52

exceeded 0.5. Thus, on average, frequency and severity of the studied adverse events and liver function abnormalities were low, across all patient groups.

Table 49. Means and standard deviations of adverse-event safety indicator scores by inter-visit average concentration

Adverse event group	Metrics	Inter-visit average concentration group				
		0	1	2	3	4
CNS	N	448	453	453	453	453
	Mean	0.04	0.01	0.02	0.06	0.06
	Std	0.20	0.10	0.15	0.25	0.29
GI	N	448	453	453	453	453
	Mean	0.02	0.00	0.02	0.03	0.06
	Std	0.20	0.05	0.16	0.19	0.33
SKIN	N	448	453	453	453	453
	Mean	0.01	0.00	0.00	0.02	0.02
	Std	0.11	0.05	0.05	0.18	0.18
TASTE	N	448	453	453	453	453
	Mean	0.00	0.00	0.00	0.01	0.01
	Std	0.05	0.00	0.00	0.11	0.10

Table 50. Means and standard deviations of liver-function safety indicator scores by inter-visit average concentration

Liver function test	Metrics	Inter-visit average concentration group				
		0	1	2	3	4
Bilirubin	N	175	175	174	175	175
	Mean	0.03	0.00	0.00	0.01	0.01
	Std	0.19	0.00	0.04	0.05	0.07
GGT	N	175	175	175	175	175
	Mean	0.05	0.15	0.14	0.08	0.06
	Std	0.33	0.51	0.46	0.34	0.20
SGOT	N	174	175	175	174	175
	Mean	0.03	0.05	0.05	0.01	0.02
	Std	0.24	0.32	0.25	0.10	0.16
SGPT	N	175	175	175	175	175
	Mean	0.04	0.08	0.12	0.05	0.05
	Std	0.16	0.39	0.42	0.20	0.15
LIVER	N	174	175	174	174	175
	Mean	0.15	0.29	0.31	0.15	0.14
	Std	0.55	0.94	0.88	0.50	0.33

Exposure to the drug increases frequency and severity of adverse events and liver function abnormalities if means of the safety scores are increasing with increasing exposure (groups from 1 to 4), and if safety scores of active treatment groups are higher than those of placebo/placebo group (group 0). Only adverse-event safety scores from Table 49 showed an increasing trend, with the scores of groups 3 and 4, higher than placebo. But the increase was small, standard deviations of mean scores were much greater than the increase, and the scores of groups 1 and 2 were lower than placebo. Thus, it was concluded that there were no clinically significant relationships between drug exposure and adverse event scores or liver function scores.

Table 51. Means and standard deviations of adverse-event safety indicator scores by steady-state concentration group.

Adverse event group	Metrics	Steady-state concentration group				
		0	1	2	3	4
CNS	N	56	54	55	55	55
	Mean	0.23	0.28	0.35	0.20	0.22
	Std	0.43	0.56	0.58	0.45	0.46
GI	N	56	54	55	55	55
	Mean	0.14	0.09	0.20	0.27	0.24
	Std	0.55	0.35	0.56	0.59	0.58
SKIN	N	56	54	55	55	55
	Mean	0.05	0.00	0.13	0.09	0.07
	Std	0.30	0.00	0.47	0.40	0.26
TASTE	N	56	54	55	55	55
	Mean	0.02	0.07	0.04	0.00	0.02
	Std	0.13	0.33	0.27	0.00	0.13

Table 52. Means and standard deviations of liver-function safety indicator scores by steady-state concentration group

Liver function test	Metrics	Steady-state concentration group				
		0	1	2	3	4
Bilirubin	N	53	54	54	55	55
	Mean	0.05	0.01	0.02	0.01	0.01
	Std	0.28	0.07	0.10	0.07	0.07
GGT	N	53	54	54	55	55
	Mean	0.08	0.07	0.19	0.24	0.15
	Std	0.42	0.31	0.57	0.62	0.46
SGOT	N	53	54	54	55	55
	Mean	0.09	0.17	0.09	0.05	0.05
	Std	0.43	0.57	0.39	0.18	0.28
SGPT	N	53	54	54	55	55
	Mean	0.09	0.20	0.11	0.17	0.08
	Std	0.24	0.68	0.25	0.41	0.19
LIVER	N	53	54	54	55	55
	Mean	0.30	0.44	0.41	0.47	0.29
	Std	0.79	1.43	0.82	0.91	0.56

Table 53 summarises patient-summary safety scores in subgroups of patients according to hypertension status, age, or smoking status.

Hypertension increased plasma levels, but safety scores were not consistently higher among patients with hypertension. Plasma levels increased with age, but average safety scores generally did not. Smokers had lower plasma levels than non-smokers, but non-smokers did not have consistently higher safety scores than smokers.

Table 53. Means and standard deviations of adverse-event safety indicator scores and liver-function safety indicator scores by hypertension, smoking and age group.

Group	Metrics	Hypertension		Smoking		Age group		
		No	Yes	No	Yes	Elderly	Middle	Young
CNS	N	396	36	185	43	118	167	147
	Mean	0.19	0.25	0.21	0.44	0.24	0.19	0.17
	Std	0.50	0.55	0.47	0.63	0.55	0.51	0.46
GI	N	396	36	185	43	118	167	147
	Mean	0.25	0.28	0.18	0.23	0.18	0.24	0.21
	Std	0.68	0.70	0.51	0.57	0.53	0.82	0.61
SKIN	N	396	36	185	43	118	167	147
	Mean	0.09	0.11	0.08	0.05	0.11	0.11	0.07
	Std	0.42	0.40	0.34	0.30	0.43	0.40	0.43
TASTE	N	396	36	185	43	118	167	147
	Mean	0.05	0.00	0.04	0.00	0.01	0.06	0.05
	Std	0.28	0.00	0.24	0.00	0.09	0.32	0.28
Bilirubin	N	390	36	184	43	116	165	145
	Mean	0.02	0.03	0.01	0.00	0.01	0.02	0.03
	Std	0.10	0.12	0.08	0.00	0.07	0.09	0.13
GGT	N	390	36	184	43	116	165	145
	Mean	0.13	0.10	0.15	0.16	0.13	0.14	0.12
	Std	0.48	0.26	0.50	0.46	0.41	0.44	0.41
SGOT	N	390	36	184	43	116	165	145
	Mean	0.15	0.14	0.08	0.12	0.04	0.04	0.12
	Std	0.44	0.39	0.36	0.43	0.22	0.17	0.45
SGPT	N	390	36	184	43	116	165	145
	Mean	0.36	0.31	0.12	0.22	0.05	0.17	0.20
	Std	0.92	0.56	0.31	0.71	0.17	0.44	0.54
LIVER	N	390	36	184	43	116	165	145
	Mean	0.36	0.31	0.36	0.50	0.22	0.36	0.46
	Std	0.92	0.56	0.80	1.48	0.52	0.91	1.10

3.1.4.3 Exposure And Predictors Of Exposure Versus Efficacy

3.1.4.3.1 Data

Data from two patient studies, P1 and P2, was used for this analysis. Only data from patients on active drug was used, since there were no improvements for patients in the placebo/placebo group.

3.1.4.3.2 *Methods*

To relate exposure to efficacy, three efficacy outcomes were identified: Complete Cure, Effective Treatment but Not Complete Cure, and Ineffective Treatment. Associations were explored between these efficacy outcomes and exposure levels among patients on active drug. Exposure was quantified by average steady-state concentrations estimated from the Bayes post hoc parameter estimates of the nonlinear mixed-effects model.

Each of the four treatment groups (6 or 12 weeks of active drug in Study P1, and 12 or 24 weeks of active drug in Study P2) was analysed separately. Within each treatment group, patients taking active drug were partitioned into quartiles according to their average concentrations at steady state. Frequencies of the three efficacy outcomes among the four exposure quartiles were computed, and associations were assessed by the Fisher's exact test and the Mantel-Haenszel test. Fisher's exact test does not account for the ordering of the categories as the Mantel-Haenszel test does, but the expected frequencies may have been too small for validity with the Mantel-Haenszel test.

Relationships between efficacy and demographic predictors of pharmacokinetics were explored in a similar way to the safety analysis. For each of the four treatment groups, frequency tables of efficacy outcomes by demographic predictors for patients who received some active drug were constructed by study. Frequencies were based on all patients, not only patients who had plasma samples drawn, and efficacy assignments were determined by the last observation for which such assessments were not missing. Fisher's exact test was applied to test the hypotheses of no differences among subgroups. Tests were applied separately for each treatment group.

3.1.4.3.3 Results

Table 54 summarises frequencies of efficacy outcomes for the overall patient population, for young and elderly patients, for smokers, and for hypertensive patients. There are no evident trends in the results. None of the tests conducted to examine the association between efficacy outcomes and exposure quartiles, age, smoking, or hypertension were significant.

Table 54. Efficacy Outcomes for demographic groups: frequency and percent within treatment group.

Study Treatment	Demographic subgroup	Cure Category					
		Complete Cure		Effective		Ineffective	
		N	%	N	%	N	%
P1 6-Week	All	36	50%	12	17%	24	33%
	Age ≤ 40	11	58%	3	16%	5	26%
	Age > 55	16	55%	2	7%	11	38%
	Smokers	8	38%	6	29%	7	33%
	Hypertensives	5	50%	1	10%	14	40%
P1 12- Week	All	36	50%	15	21%	21	29%
	Age ≤ 40	12	57%	3	14%	6	29%
	Age > 55	15	54%	5	18%	8	29%
	Smokers	8	50%	4	25%	4	25%
	Hypertensives	4	44%	1	11%	4	44%
P2 12- Week	All	45	31%	26	18%	72	50%
	Age ≤ 40	19	37%	9	17%	24	46%
	Age > 55	7	22%	9	28%	16	50%
	Smokers	2	40%	3	60%	0	0%
	Hypertensives	5	50%	1	10%	4	40%
P2 24- Week	All	62	42%	34	34%	51	35%
	Age ≤ 40	28	51%	9	16%	18	33%
	Age > 55	10	34%	8	27%	11	38%
	Smokers	1	100%	0	0%	0	0%
	Hypertensives	2	29%	1	14%	4	57%

3.1.4.4 Design Of A New Study

3.1.4.4.1 Objectives

Subsequent to the development of the population PK model, a new clinical study in healthy volunteers was planned with the following objectives:

1) To determine the pharmacokinetics of higher doses (500, 750, and 1000 mg), administered once or twice a day;

2) To evaluate the safety of these drug regimens.

In order to evaluate the safety, it was necessary for steady state to be attained during the study; it was not enough to model steady state, using non-steady state data. The first question to be answered was: for how long should the drug be dosed to attain 80 to 90% of steady state for each treatment regimen? With the considerable inter-patient variability of pharmacokinetic parameters established by the model, the question was transferred into the following one: for how long should one dose in order that the concentration of the drug (i.e. AUC, C_{max}, C_{trough}) would reach 90% of that for steady state for half of individuals and 80% for 75% of individuals?

The second question arose because of the need to follow up until the complete elimination of the drug from the body. For how long should subjects be monitored after the cessation of dosing?

For the purpose of the study it was important that subjects be kept in a clinic for the duration of the treatment. That made the study very expensive and led to the third question: were there alternative, acceptable ways to dose the drug to reach steady state earlier? Specifically, would a loading dose at the beginning of the study help? Or, would one or two weeks of 'pre-treatment' (that is, administering lower doses of the drug before the start of the monitored study) help?

3.1.4.4.2 Methods

To answer the above questions, concentration-time profiles were simulated using SAS [SAS, 1990] for different regimens of the drug. The following assumptions were made for the simulations:

1) Pharmacokinetics is linear in these higher doses, i.e. concentration at a given time increases proportionally with dose. This also implies that pharmacokinetic parameters at twice-a-day dosing are the same as for once-a-day administration;

2) Doses are administered regularly every 24 hours for once-a-day and every 12 hours for twice-a-day dosing.

The basis for the first assumption was the dose linearity for doses 125 and 250 mg a day that was shown earlier. Also, one study with 250, 500, and 750 mg single doses had already been conducted, its results suggested dose-linearity [Kovarik, *et al.*, 1992].

The second point was not actually an assumption about the drug, but rather a statement of the way the experiment would run, allowing the model to be written.

According to the model, the plasma concentration C of the drug at t hours after the last d^{th} dose and before the $(d+1)^{st}$ dose, when the dose D is administered every T hours is

$$C(t,d) = [\, C(d-1, T, t) + C(t) \,] \exp(\varepsilon) . \qquad \text{Eq. 51}$$

Here C(d-1,T, t) is the portion of the concentration due to all the doses except the last, C(t) is the contribution of the last dose, and ε is a random error due to the intra-subject variability and the measurement error. It is normally independently distributed with mean zero and variance σ^2. According to (Eq. 32) concentrations C(d-1,T) can be written as

$$C(d-1,T,t) = D\sum_{i=2}^{4} \frac{c_i e^{-b_i(T+t-t_{lag})}[1-e^{-b_i T(d-1)}]}{1-e^{-b_i T}}, \qquad \text{Eq. 52}$$

C(t) is given by (Eq. 31), coefficients $c_i(t)$ and c_i are given by (Eq. 29),(Eq. 30) and parameters t_{lag}, t_{abs}, s, b_2, b_3, b_4, k_{21}, and k_{31} are described by (Eq. 40), (Eq. 41).

The new study was to be conducted in young, healthy, non-smoking subjects, therefore estimates of θ_k appropriate for such subjects were used. The estimates of Ω and σ^2 were taken directly from the population model.

Concentrations of 1000 individuals were simulated according to (Eq. 51), (Eq. 52). Individual pharmacokinetic parameters were generated as described in the earlier simulation.

To study the approach to the steady state, dose-normalised concentrations at 11 time points between zero and 12 hours post dose for twice a-day regimens, and at 12 time points between zero and 24 hours for once-a-day regimens were computed for 1 through 31 days of dosing for each of the individuals. The same was done for the steady state (1000 days of dosing). From each of the concentration-time profiles, Area Under the dose-normalised concentration Curve (AUC), maximum concentration (C_{max}), and trough concentration (C_{trof}) were computed. Ratios of them to the respective steady state values show how far the individual is from the steady state on a particular day of dosing. Medians and 25% percentiles of the ratios for all 1000 subjects characterise approach to steady state by 50% and 75% of the subjects, respectively.

To answer the question about the duration of monitoring (time necessary to follow the subjects), the time after cessation of dosing till their plasma concentration drop to the quantification limit of the assay (5 ng/ml in this case) was estimated.

The concentration on the p^{th} day after the stop of dosing (24*p-12 hours post last dose for twice-a-day regimens) was computed as

$$C(p,d,T) = D\sum_{i=2}^{4} \frac{c_i e^{-b_i(T+24(p-1)-t_{lag})}\left[1-e^{-b_i Td}\right]}{1-e^{-b_i T}} \quad . \qquad \text{Eq. 53}$$

As before, *d* denotes the number of doses taken, which is equal to number of days on the drug for once-a-day dosing, and is twice that for twice-a-day regimens.

The concentrations for a number of different monitoring times p after three weeks of dosing were computed for 1000 simulated subjects for three different doses of interest 500, 750 and 1000 mg/day, administered twice-a-day. For each of the regimens the monitoring times p were sought for which medians (50% of subjects) or 75% percentiles (75% of subjects) of concentration drop to 5 ng/ml.

To explore ways to shorten the time to steady state, formulas for concentration were modified to accommodate one or two loading doses of dose D_0 and specified number of days of 'pre-treatment doses D_{pr} given once a day before the start of monitored period.

The following regimens were investigated: twice-a-day dosing with one or two loading doses of 750 mg, and twice-a-day dosing with one or two weeks of 250 mg once-a-day, taken at home prior to start of the clinic period of the study ('pre-treatment').

Again, 1000 concentration profiles were simulated for each combination of dosing and 'pre-treatment' regimens. Ratios of AUC, C_{max}, and C_{trof} to their steady state values were summarised and compared with the respective values without loading doses or 'pre-treatment'.

3.1.4.4.3 Results

Simulations showed that plasma concentrations built up very gradually (Table 55). Three weeks of dosing were necessary for median AUC (i.e. half of the subjects) to reach 90% of steady state, and for the 25% percentile (i.e. 75% of the subjects) to exceed 80% of steady state in both once-a-day and twice-a-day regimens. By this time peak concentrations reach 95% and 81% of steady state, respectively. Trough concentrations build up even slower than AUC. After three weeks of dosing trough medians and the 25% percentiles exceed only 75%, and 60% of steady state. Even after four weeks of dosing they do not reach required 90% and 80% of steady state.

Table 55. Percent of steady state reached by 50% and 75% of the simulated subjects on the 16th, 21st and 28th day of dosing for twice-a-day dosing.

	Day 16		Day 21		Day 28	
	50% of subjects	75% of subjects	50% of subjects	75% of subjects	50% of subjects	75% of subjects
AUC/AUC_{ss}	87	79	90	83	93	86
$C_{max}/C_{max,\ ss}$	93	79	95	81	96	82
$C_{trof}/C_{trof,ss}$	72	57	79	63	86	69

Elimination was very slow. To monitor the washout period until the concentrations of 75% of the subjects drop to 5 ng/ml, the limit of quantification of the assay, the monitoring duration should be 70, 85, and 95 days for twice-a-day regimens of 500, 750 and1000 mg/day, respectively. Half of the subjects reach the limit after 60, 70, and 75 monitor days, respectively.

Surprisingly, alternative schedules aimed to reach steady state faster did not produce a significant improvement (Table 56). One or two loading doses of 750 mg as well as one week of 'pre-treatment' with 250 mg once a day do not shorten the time to steady state. Two weeks of 'pre-treatment' decrease the time to steady state by five days, but only for the 500 mg/day twice-a-day regimen. For higher doses improvement is negligible.

Table 56. Percent of AUC at steady state reached by 50% and 75% of the simulated subjects on the 16th and 21st days of dosing after different 'pre-treatment' regimens.

Regimen		Day 16		Day 21	
Loading or 'pre-treatment'	Dose, BID mg	50% of subjects	75% of subjects	50% of subjects	75% of subjects
First loading dose of 750 mg	500	87	80	91	83
	750	86	79	91	83
First and third loading doses of 750 mg	500	88	80	92	84
	750	87	79	91	83
1 week pre-treatment with 250 mg a day	500	88	81	92	84
	750	87	78	91	83
2 week pre-treatment with 250 mg a day	500	90	83	93	85
	750	88	81	92	84

3.1.5 CONCLUSIONS

The developed pharmacokinetic model of the drug was able to describe diverse pharmacokinetic data. It identified demographic predictors of the drug exposure and quantified their influence on pharmacokinetic parameters. In particular:

1. The linear mammillary three compartment model adequately described individual's data from pharmacokinetic studies in healthy volunteers;
2. Results from Study H1 demonstrated the differences in bioavailability between fed and fasted conditions of dosing, and these results were supported by the findings in H3;
3. Results from Study H1, and from Studies H1, P1, and P2, combined, demonstrated the decreased clearance of the drug in elderly subjects;

4. Fitting nonlinear mixed-effects models to combined data from 30 healthy volunteers and 219 patients corroborated the assumptions of the linear, mammillary, three-compartment model and demonstrated that healthy volunteers and patients have similar multiple-dose pharmacokinetics;
5. Nonlinear mixed effects population pharmacokinetic model identified age, smoking, and hypertension as covariates with statistically significant effects on observed plasma levels of the drug, thus confirming the results of the model-free approach;
6. The model allowed one to quantify the dependencies of pharmacokinetic parameters on age, smoking, and hypertension that were consistent with the observed data. Age and hypertension decreased apparent clearance (Cl/F); smoking increased it. Age and smoking increased apparent steady-state volume of distribution (V/F); hypertension decreased it. Elimination half-life ($t_{1/2}$) increased with age.

The model proved to be a useful tool in further exploration of pharmacokinetics of the drug. It answered the clinical concerns about the accumulation of the drug, about long half-life and the residual exposure after the cessation of dosing. It established:

7. A median half-life of 3.1 and 3.7 weeks for young and elderly, respectively;
8. An area accumulation ratio of 1.8 to 2.1;
9. A tail Fraction to be 11% -17% after 12 weeks of dosing and 6%-9% after 24 weeks of dosing, for young and elderly, respectively.

The pharmacokinetic summaries of the drug exposure derived from the model allowed one to explore relationships of exposure versus safety and efficacy of the drug:

10. Generally, adverse events and liver function abnormalities were rare and mild. There were no clinically significant relationships between systemic drug exposure and adverse event scores or liver function scores;
11. Despite relationships between pharmacokinetics and age, hypertension, and smoking:
    a. There were no trends in adverse event scores or liver function scores across age groups, hypertension status, or smoking status that were consistent with variation in the drug exposure with age, hypertension, and smoking;

b. There were no statistically significant changes in efficacy across age groups, hypertension status, or smoking status.

Finally, the developed model was used to guide the design of a new study, with different doses and different dosing regimens of the drug. Simulation of the study answered the questions of duration and monitoring of dosing, needed to satisfy the objectives of the study. It also explored the alternative ways to speed up the attainment of steady state.

3.2 Population Dose-Response Of An Anti-Hypertension Agent

This project is devoted to the development of a population dose-response model of an anti-hypertension drug. The drug was given to hypertensive patients to reduce their diastolic blood pressure. The main difference between the clinical trials described earlier and ones analysed here is that the dose and/or regimen of the drug given to a particular patient change during the trial. More importantly, this change depends on how well the drug works in the patient, so called *titration according to response*. With this design, different patients receive different doses of the drug during the trials. Only the patients most resistant to the therapy receive the highest doses.

3.2.1 OBJECTIVES

This investigation had two main objectives:

1. To describe the dose-response relationship of the drug in placebo-controlled dose-escalation studies;
2. To characterise the efficacy of the drug in the Black population versus other races in those studies.

The second objective was important because there were more cardiovascular adverse events among Black patients than among Caucasians during the trials. This may or may not have reflected the lower efficacy of the drug in this population.

3.2.2 DATA

Data came from two clinical dose-escalation studies. Both studies were 6-week, double-blind, parallel-group, randomised, placebo-controlled trials in patients with hypertension. To enter the studies, patients' average sitting (Study 1) or supine (Study 2) diastolic blood pressure (DBP) had to be ≥ 100 mm Hg, and in Study 2 also had to be < 120 mm Hg. Additionally, there should not have been a decreasing trend in blood pressure. Patients were stratified before randomisation according to their DBP (100 to 105, and > 105). Trough measurements of DBP (an average of two measurements three minutes apart) were taken once a week.

3.2.2.1 Study 1.

After stratification patients were randomised into three groups: placebo twice-a-day, the active drug once-a-day and placebo once-a-day, and the active drug twice-a-day. Patients were instructed to take their medications at the specified windows of time, so that the interval between the trough blood pressure measurements and the previous morning dose was usually 22-25 hours, and 10-12 hours following the previous evening dose. Patients on the active drug received 12 mg/day during Weeks 1 and 2. If at the end of Week 2, the DBP was > 90 mm Hg, then the dose was increased to 24 mg/day. A similar decision was made at the end of Week 4. If the above criterion was satisfied, the dose was increased to the next dose level. Patients that had a dose increase at the end of Week 2 received 48 mg/day; patients that did not have a dose increase before the end of Week 4, received 24 mg/day (see Table 57 for schematic dosing schedule).

Table 57. Dosing schedule for Study 1.

Treatment group	Week		
	1-2	3-4	5-6
Placebo (b.i.d)	0	0	0
Active drug (mg): Once-a-day (q.d.)	12	12 - 24	12- 48
Active drug (mg): Twice-a-day (b.i.d.)	6	6 -12	6- 24

3.2.2.2 Study 2.

After stratification, patients were randomised into two groups: placebo and the active drug. All patients were instructed to take their study medication twice-a-day (b.i.d.) at specified windows of hours, so that the interval between measurements of blood pressure and the previous morning dose was usually 22-25 hours, and 11-15 hours following the previous evening dose. All patients randomised to the active drug group received the active drug once-a-day (q.d.) in the morning for Weeks 1,2, and 3 (and

placebo in the evening). If at the end of Week 3 the DBP decreased to < 95 mm Hg with at least a 10 mm Hg decrease from baseline, patients were continued on the q.d. schedule for the duration of the study (Weeks 4, 5, and 6). If however, these criteria were not satisfied by the end of Week 3, the drug was administered twice a day.

The initial dose for all patients on the active drug was 12.5 mg for Days 1-3 of Week 1, followed by 25 mg for Days 4-7 of Week 1. If at the end of Week 1 the above response criteria were not satisfied, then the dose was increased to 50 mg for Week 2. At the end of Week 2, a similar decision was made regarding dose-escalation. If the response criteria were not satisfied, the dose was increased to the highest level of 75 mg. (or 50 mg if 25 mg was administered during Week 2). At the end of Week 3, patients who satisfied the response criteria continued to receive the active drug on a once-a-day schedule for Weeks 4-6. Patients receiving a dose of less than 75 mg who did not satisfy the response criteria had the dose increased to the next level and continued to take the drug once-a-day for Weeks 4-6. Patients who did not satisfy the response criteria while receiving the maximum of 75 mg or who could not tolerate a higher dose of the drug switched to the twice-a-day regimen. They received 25 mg/day during Week 4. If at the end of Week 4 the response criteria were still not satisfied, the dose was increased to 50 mg/day. Similarly, the dose was increased to 75 mg/day at the end of Week 5 for Week 6 if the response criteria were not satisfied at the end of Week 5 (See Table 58 for schematic schedule of dosing).

In both studies, dose may have been reduced in the case of an adverse reaction.

Observations were excluded from the analysis if:

1. Patient did not satisfy entry requirements;
2. Patient took non-study blood pressure medication or other disallowed medication during the week before the visit;
3. Patient was not compliant. That is:
    a) not taking at least 80% of prescribed doses;
    b) not taking the required doses the day prior to the visit, or taking the morning dose on the day of the visit;
    c) the blood pressure measurement did not fall in the allowed range of hours on the day before the visit;

Table 58. Dosing schedule for Study 2.

<table>
<tr><td rowspan="3">Treatment group</td><td colspan="7">Week</td></tr>
<tr><td colspan="2">1</td><td rowspan="2">2</td><td rowspan="2">3</td><td rowspan="2">4</td><td rowspan="2">5</td><td rowspan="2">6</td></tr>
<tr><td>Day 1-3</td><td>Day 4-7</td></tr>
<tr><td>Placebo (b.i.d.)</td><td colspan="7">0</td></tr>
<tr><td rowspan="3">Active drug (mg)</td><td rowspan="3">12.5
q.d.</td><td rowspan="3">25
q.d.</td><td rowspan="3">25-50
q.d.</td><td rowspan="3">25-75
q.d.</td><td colspan="3"><u>Responders:</u>

q.d. dose continued</td></tr>
<tr><td colspan="3"><u>Nonresponders:</u></td></tr>
<tr><td>12.5
b.i.d.</td><td>12.5-25
b.i.d.</td><td>12.5-37.7
b.i.d.</td></tr>
</table>

After exclusion of invalid observations there were 1186 observations from 179 patients (52 African-Americans, 104 Males), and 1256 observations from 200 patients (125 African-Americans, 128 Males) from studies 1 and 2, respectively.

3.2.3 METHODS

Models for Studies 1 and 2 were investigated separately. Data from Weeks -1 (baseline), and 1-6 were used in the models. All analyses were done in a context of linear and nonlinear mixed-effects models. The first order conditional estimation (FOCE) method of NONMEM (version IV) was used to fit the data.

The general form of the population dose-response model for diastolic blood pressure was:

$$\begin{aligned} \ln(DBP) = Baseline - \{ & (Plac + Qd + Bid) * \text{Placebo effect} \\ & + Qd * Drug_{Qd}\ \text{effect} \\ & + Bid * Drug_{Bid}\ \text{effect} \} + \varepsilon, \end{aligned} \qquad \text{Eq. 54}$$

where Plac, Qd, and Bid were the dummy variables that identified observations on placebo, or q.d. and b.i.d. regimen of the active drug, respectively. Thus, the effect of the drug was modelled as a sum of a placebo effect and an appropriate incremental treatment effect.

Each of the terms Baseline, Placebo effect, $Drug_{Qd}$ effect, and $Drug_{Bid}$ effect included structural parameters that describe the basic shape of the dose-response curve (population means), and parameters that account for inter-patient variability (random effects). To address the objective of characterising efficacy in Blacks versus Nonblacks, covariates for race were included in the models when the thereby enlarged model was judged to fit the data better. Structural models considered for the drug effects were no dose dependence (constant or step model), linear dose response, and E_{max} models [Holford & Sheiner, 1981, 1982]. The form of the E_{max} model was

$$Eff = E_{\max} * \frac{Dose}{D_{50} + Dose}. \qquad \text{Eq. 55}$$

In this model *Eff* is the effect (log(DBP)), E_{max} is the maximal drug effect (at ∞ dose), and D_{50} is the dose of the drug that would produce half of the maximal effect.

For the placebo effect, only a step structural model was considered. This corresponds to no effect of time or number of capsules on the placebo effect.

The models were built in a stepwise fashion. Assessment of current parameter estimates and random effect estimates guided the direction of the search, and terms were added to or deleted from models if they decreased or increased, respectively, the Akaike Information Criterion (AIC) [Beal & Sheiner, 1992; Judge *et al.*, 1980].

First only the baseline and placebo data of Study 1 was used to identify a variability model for the baseline and placebo effects. Submodels of the following general model were considered:

$$\ln(DBP_{ij}) = (\theta_{Base} + \sigma_{Base}\eta_{Base,i}) - Plac_{ij}\,(\theta_{Plac} + \sigma_{Plac}\eta_{Plac,i}) + \sigma_{\varepsilon}\varepsilon_{ij}\,, \qquad \text{Eq. 56}$$

where

$Plac_{ij}$ =1 for placebo observations, and =0 otherwise;

θ_{Base} and θ_{Plac} are the population mean baseline and placebo values;

η_{Base} and η_{Plac} are the random inter-subject baseline and placebo effects;

ε_{ij} is the random intra-subject effect. Random variables η_{Base}, η_{Plac}, and ε are assumed to be normally distributed with zero means and unit variances;

σ_{Base}, σ_{Plac}, and σ_{ε} are the standard deviations; and

the index ij stands for the j^{th} observation of the i^{th} subject.

Then the form of the model for the baseline and placebo effects was retained during model building for the drug effect using all the data of Study 1. Submodels of the following general model were considered:

$$\ln(DBP_{ij}) = \theta_{Base} - (Plac_{ij} + Qd_{ij} + Bid_{ij})(\theta_{Plac} + \sigma_{Plac}\eta_{Plac,i})$$
$$- Qd_{ij}\{(1-QdEmax)[\theta_{QdIntcpt} + \sigma_{QdIntcpt}\eta_{QdIntcpt,i} + (\theta_{QdSlope} + \sigma_{QdSlope}\eta_{QdSlope,i})Dose_{ij}]$$
$$+ QdEmax[\theta_{QdEmax} + \sigma_{QdEmax}\eta_{QdEmax,i})Dose_{ij} / (\theta_{QdD50} + \sigma_{QdD50}\eta_{QdD50,i} + Dose_{ij})]\}$$
$$- Bid_{ij}[\theta_{BidIntcpt} + \sigma_{BidIntcpt}\eta_{BidIntcpt,i} + (\theta_{BidSlope} + \sigma_{BidSlope}\eta_{BidSlope,i})Dose_{ij}]$$
$$+ \sigma_{\varepsilon}\varepsilon_{ij} \quad , \qquad \text{Eq. 57}$$

where, for parameters and variables not defined after (Eq. 56):

Qd_{ij} and Bid_{ij} are dummy variables that identify observations on q.d. and b.i.d. dosing of the active drug, respectively. Note that $Plac_{ij} + Qd_{ij} + Bid_{ij} = 0$ for a baseline observation, and = 1 otherwise;

QdEmax = 1, if an Emax model is used for q.d. dosing, and = 0 otherwise;

$\theta_{QdIntcpt}$, $\theta_{QdSlope}$, $\theta_{BidIntcpt}$, and $\theta_{BidSlope}$ are the population mean parameters describing a linear response on q.d. and b.i.d treatment;

$\eta_{QdIntcpt}$, $\eta_{QdSlope}$,$\eta_{BidIntcpt}$, and $\eta_{BidSlope}$ are unit-variance, random, inter-subject q.d. and b.i.d effects for linear responses; and $\sigma_{QdIntcpt}$, $\sigma_{QdSlope}$, $\sigma_{BidIntcpt}$, and $\sigma_{BidSlope}$ are the corresponding standard deviations;

θ_{QdEmax} and θ_{QdD50} are the population mean parameters for the Emax model;

η_{QdEmax} and η_{QdD50} are unit-variance, random, inter-subject effects under the Emax model; and σ_{QdEmax} and σ_{QdD50} are the corresponding standard deviations.

After the structural model and the model for variability were developed for both the placebo and drug effects in Study 1, the covariates for race were explored for inclusion. Submodels of the following general model were considered:

$$\ln(DBP_{ij}) = \theta_{Base} - (Plac_{ij} + Qd_{ij} + Bid_{ij})(B_i\theta_{PlacB} + W_i\theta_{PlacW} + \sigma_{Plac}\eta_{Plac,i})$$
$$- Qd_{ij}\{B_i[\theta_{QdIntcptB} + \sigma_{QdIntcptB}\eta_{QdIntcptB,i} + (\theta_{QdSlopeB} + \sigma_{QdSlopeB}\eta_{QdSlopeB,i})Dose_{ij}]$$
$$+ W_i[\theta_{QdIntcptW} + \sigma_{QdIntcptW}\eta_{QdIntcptW,i} + (\theta_{QdSlopeW} + \sigma_{QdSlopeW}\eta_{QdSlopeW,i})Dose_{ij}]\}$$
$$- Bid_{ij}\{B_i[\theta_{BidIntcptB} + \sigma_{BidIntcptB}\eta_{BidIntcptB,i} + (\theta_{BidSlopeB} + \sigma_{BidSlopeB}\eta_{BidSlopeB,i})Dose_{ij}]$$
$$+ W_i[\theta_{BidIntcptW} + \sigma_{BidIntcptW}\eta_{BidIntcptW,i} + (\theta_{BidSlopeW} + \sigma_{BidSlopeW}\eta_{BidSlopeW,i})Dose_{ij}]\}$$
$$+ \sigma_\varepsilon\varepsilon_{ij} \quad , \qquad \text{Eq. 58}$$

where, for parameters and variables not defined after (Eq. 56) or (Eq. 57):

B and W are dummy variables denoting Blacks and Nonblacks, respectively; B + W=1;

θ_{PlacB} and θ_{PlacW} are the population mean placebo effects for Blacks and Nonblacks, respectively;

Suffixes B and W on subscripts indicate that the variable or parameter is associated with Blacks and Nonblacks, respectively.

A similar stepwise procedure was then followed for Study 2, except that model identification for the baseline and placebo data of Study 2 was not redone. Rather, the same model for the baseline and placebo effects that was determined for Study 1 was used, although parameters were re-estimated.

Bayes posthoc estimates of subject random effects for each individual were computed by NONMEM. They and the estimates of fixed effects were then used to compute fitted values according to the models. Residuals were computed as the difference between observed and fitted values. Standardised random subject effects, i.e. ratios of estimated individual subject effects to their standard deviations were computed.

Residuals and standardised random subject effects of the baseline-placebo model for Study 1 and of the final models for both studies were plotted [S-Plus, 1997] to check the adequacy of assumptions underlying the models and to explore for other candidate variables for inclusion. Plots of observed versus fitted values, residuals versus fitted values, and residuals versus quantiles of the standard normal distribution were constructed to check for bias, heteroscedasticity, and departure from normality. Box and scatter plots of residuals and standardised random subject effects versus week of study, number of capsules (baseline - placebo model only), race, age, duration of hypertension, and sex were constructed to check for the differences in distributions of random effects with respect to those variables.

To compare q.d. and b.i.d. drug effects across the studies, to compare the q.d. effect with the b.i.d. effect within each study, and to compare Blacks with Nonblacks, 95% confidence bands for q.d. and b.i.d. effects were constructed for each race group for each study. These confidence bands consisted of simultaneous confidence intervals for

$$Dose_{lowest} \le Dose \le Dose_{highest},$$

appropriate for maximum likelihood estimation of mixed-effect linear models (Miller 1985):

$$\theta_{Intcpt} + \theta_{Slope} * Dose \pm \sqrt{\chi^2_{2,1-\alpha}[(1, Dose)\Sigma_\theta(1, Dose)^T]} \quad , \qquad \text{Eq. 59}$$

where θ_{Intcpt} is the estimate of the population mean intercept, θ_{Slope} is the estimate of the population mean slope, Σ_θ is the estimated covariance matrix of the mean estimates, and $\chi^2_{2,1-\alpha}$ is a critical value of chi-square distribution with two degrees of freedom at α level of significance.

In cases where θ_{Intcpt} or θ_{Slope} was fixed to zero and, therefore, there was only one degree of freedom, (Eq. 59) reduced respectively to

$$\theta_{Intcpt} \pm z_{1-\alpha}\sigma_{Intcpt} \qquad \text{Eq. 60}$$

$$\text{or} \quad (\theta_{Slope} \pm z_{1-\alpha}\sigma_{Slope}) * Dose\,, \qquad \text{Eq. 61}$$

where σ_{Intcpt} and σ_{Slope} are the estimated standard errors of θ_{Intcpt} and θ_{Slope}, respectively.

3.2.4 RESULTS

3.2.4.1 Study 1

Among different variability models for the baseline and step placebo effect, the selected model was:

$$\ln(DBP_{ij}) = \theta_{Base} - Plac_{ij}\,(\theta_{Plac} + \sigma_{Plac}\eta_{Plac,i}) + \sigma_{\varepsilon}\varepsilon_{ij} \qquad \text{Eq. 62}$$

Note that even though the study design recognized baseline variability in DBP by stratifying on DBP, an inter-subject random effect for baseline DBP was not retained in the model building process.

To assess the adequacy of the model and screen the residuals for the dependence on time (Week) and on 'dose' (number of placebo capsules), diagnostic plots of residuals were plotted in Figure 42. Residuals appeared to be fairly homogeneous and normally distributed, justifying the model. Box plots of the residuals versus Week, and versus number of placebo capsules did not suggest any time or 'dose' dependence.

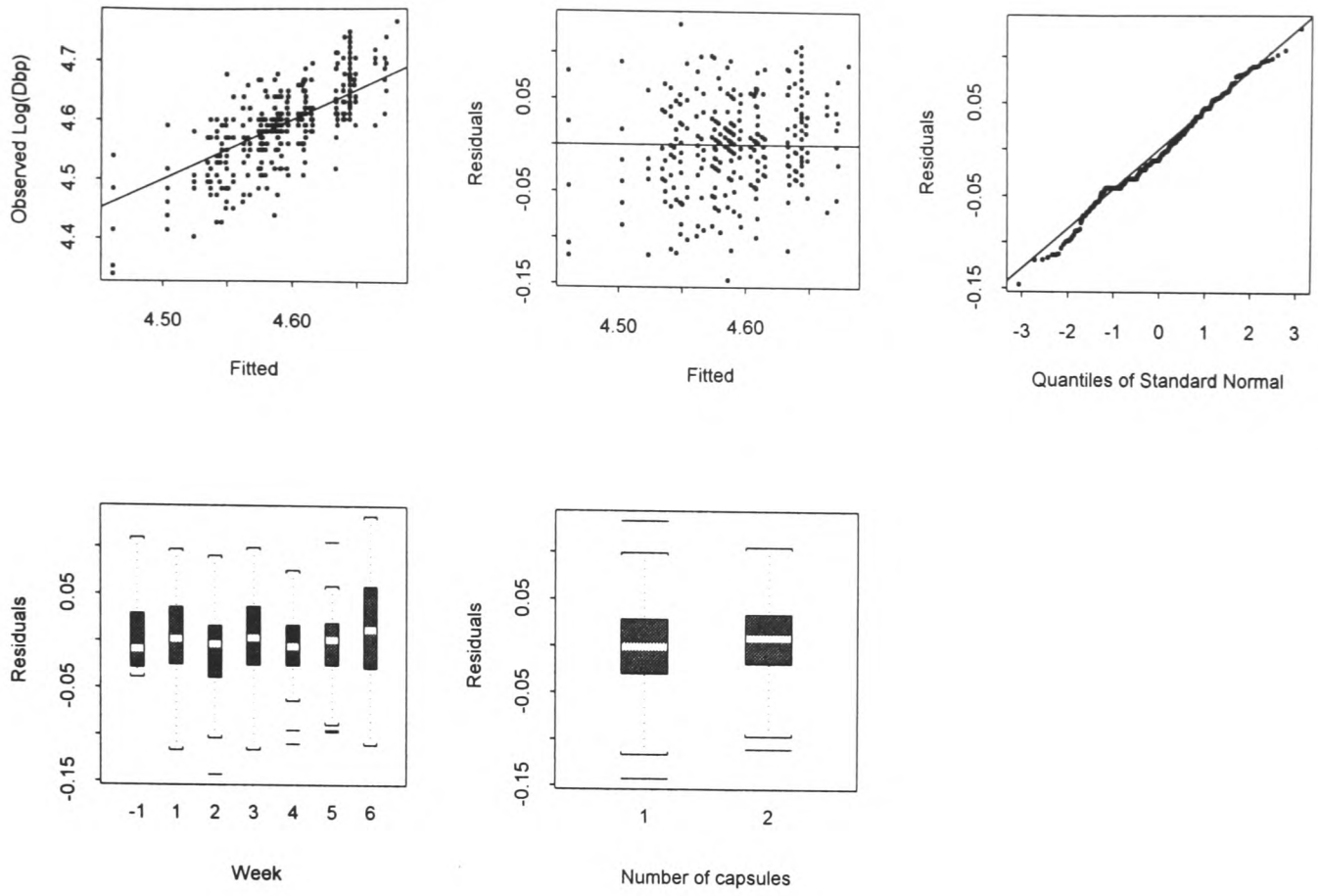

Figure 42. Study 1: Baseline – Placebo model. Diagnostics of residuals.

The form of the model for the placebo effect was used in subsequent modelling of the drug effect. Among all the step, linear and E_{max} models tried, the model with linear dose response for both q.d. and b.i.d. treatments was selected:

$$\begin{aligned}\ln(DBP_{ij}) = \theta_{Base} &- (Plac_{ij} + Qd_{ij} + Bid_{ij})(\theta_{Plac} + \sigma_{Plac}\eta_{Plac,i}) \\ &- Qd_{ij}[\theta_{QdIntcpt} + (\theta_{QdSlope} + \sigma_{QdSlope}\eta_{QdSlope,i})Dose_{ij}] \\ &- Bid_{ij}[\theta_{BidIntcpt} + (\theta_{BidSlope} + \sigma_{BidSlope}\eta_{BidSlope,i})Dose_{ij}] \\ &+ \sigma_{\varepsilon}\varepsilon_{ij}\end{aligned} \qquad \text{Eq. 63}$$

After the race covariates were included in the model, the final selected model was:

$$\begin{aligned}\ln(DBP_{ij}) = \theta_{Base} &- (Plac_{ij} + Qd_{ij} + Bid_{ij})(B_i\theta_{PlacB} + W_i\theta_{PlacW} + \sigma_{Plac}\eta_{Plac,i}) \\ &- Qd_{ij}[W_i(\theta_{QdSlopeW} + \sigma_{QdSlopeW}\eta_{QdSlopeW,i})Dose_{ij} \\ &\quad + B_i(\theta_{QdSlopeB} + \sigma_{QdSlopeB}\eta_{QdSlopeB,i})Dose_{ij}] \\ &- Bid_{ij}[\theta_{Bid\ Intcpt} + W_i(\theta_{BidSlopeW} + \sigma_{BidSlopeW}\eta_{BidSlopeW,i})Dose_{ij}] \\ &+ \sigma_{\varepsilon}\varepsilon_{ij}\end{aligned} \qquad \text{Eq. 64}$$

The parameter estimates with their estimated standard errors are shown in Table 57. Diagnostic plots of the residuals in Figure 43 did not show any heteroscedasticity or considerable departure from normality. Plots of residuals versus week, race, sex, age, and duration of hypertension (Figure 43, Figure 44), as well as plots of the standardised random subject effects versus demographic covariates (Figure 45 - Figure 47) did not suggest any strong dependencies other than already in the model. The possibility of a dependence of the b.i.d. effect on sex, suggested in Figure 47, was not pursued further.

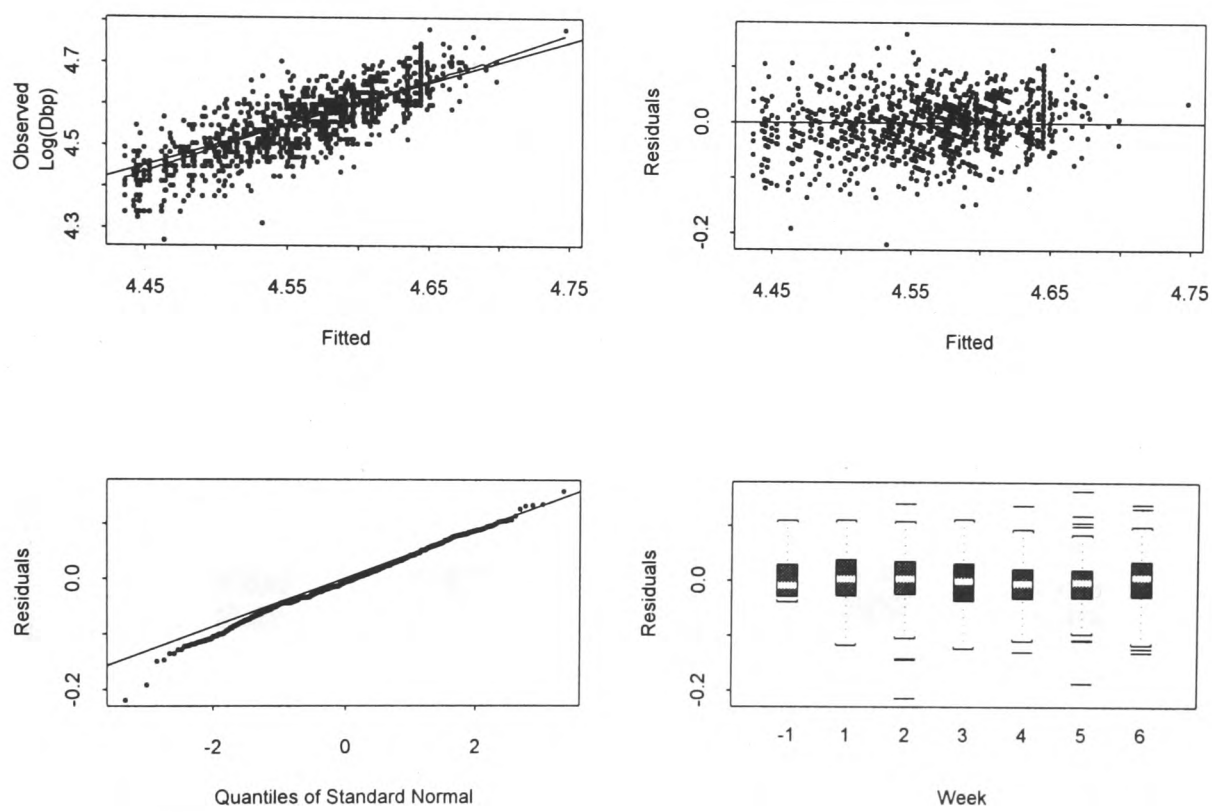

Figure 43. Study 1: Final model. Diagnostics of residuals

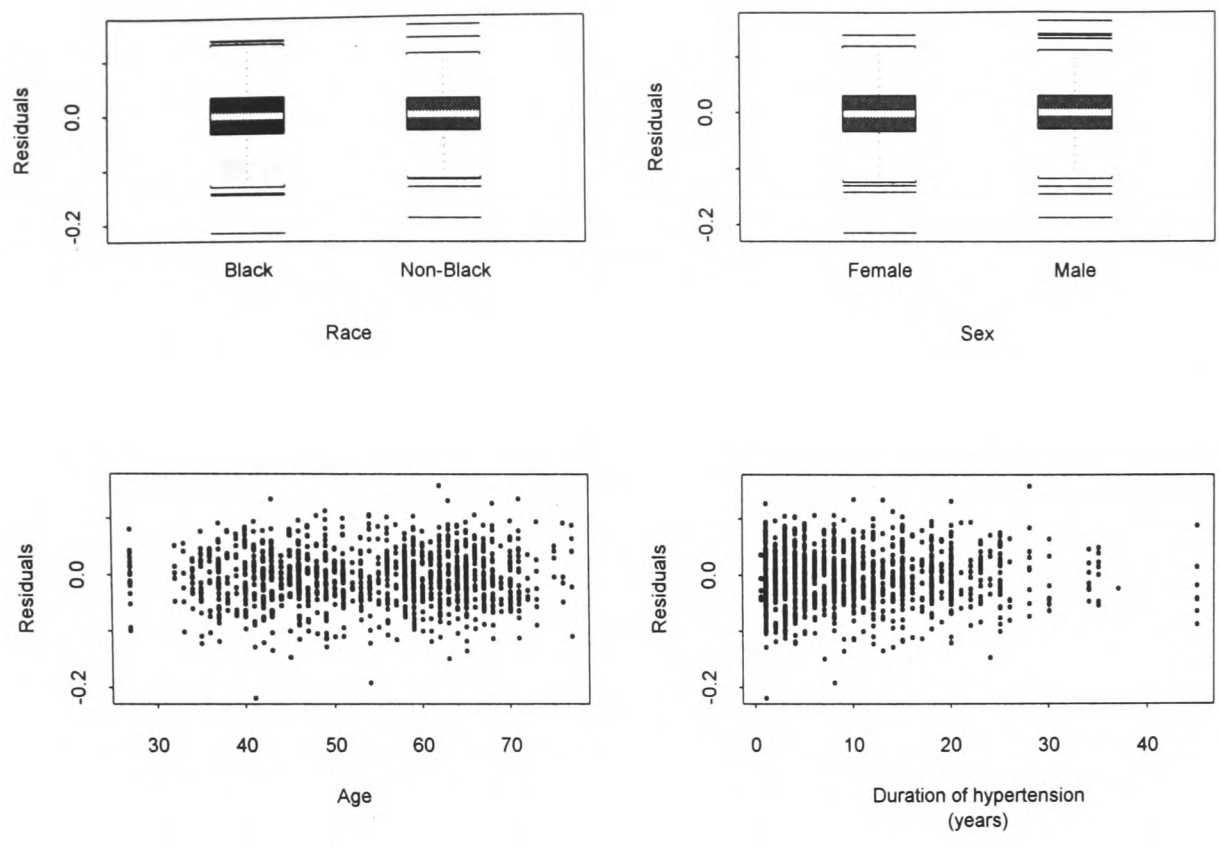

Figure 44. Study 1: Final model. Diagnostics of residuals (Cont.)

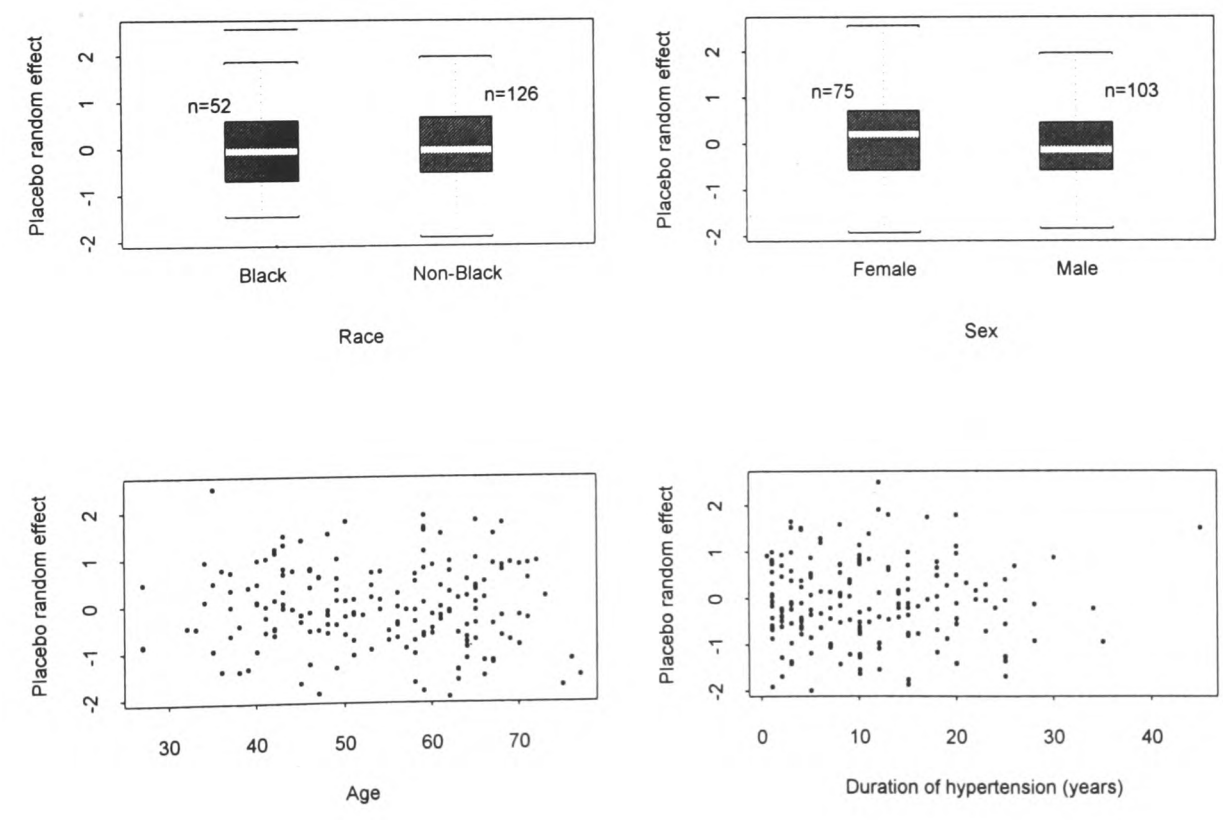

Figure 45. Study 1: Standardised Placebo random effect.

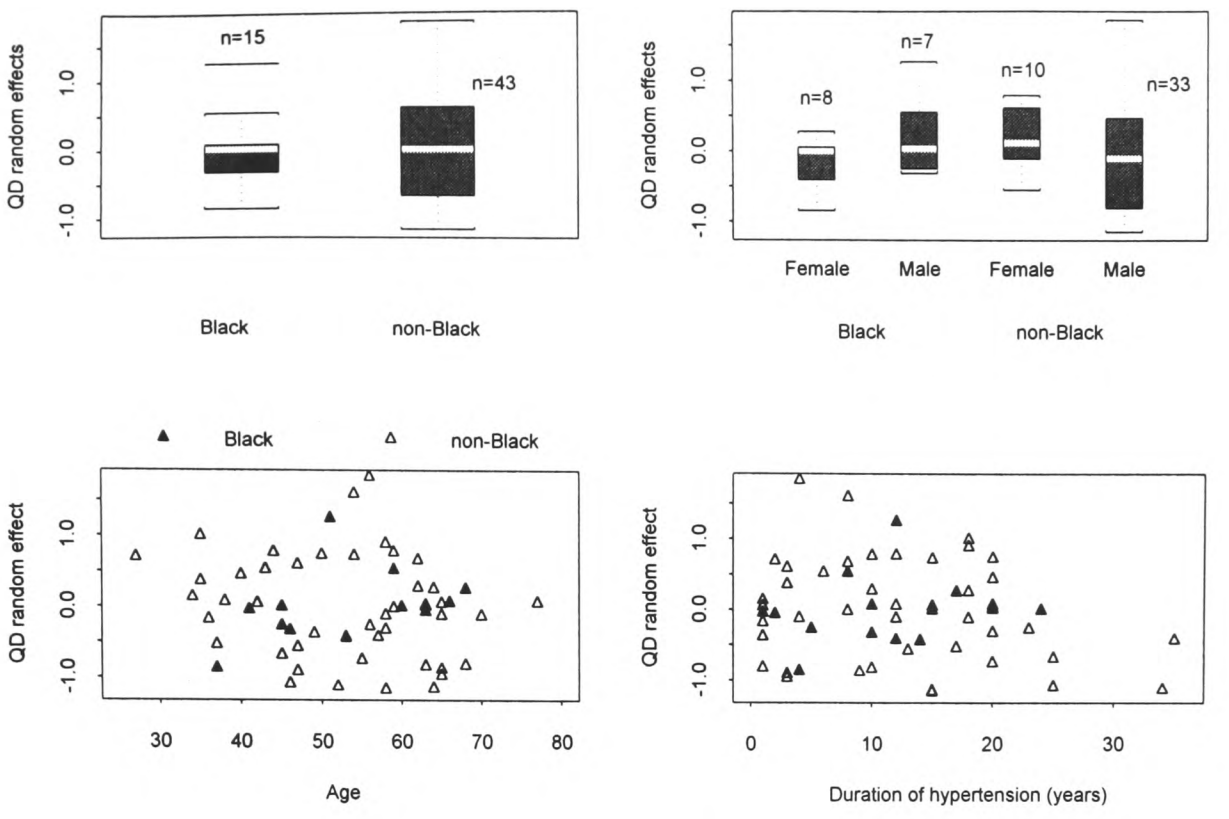

Figure 46. Study 1: Standardised QD random effect.

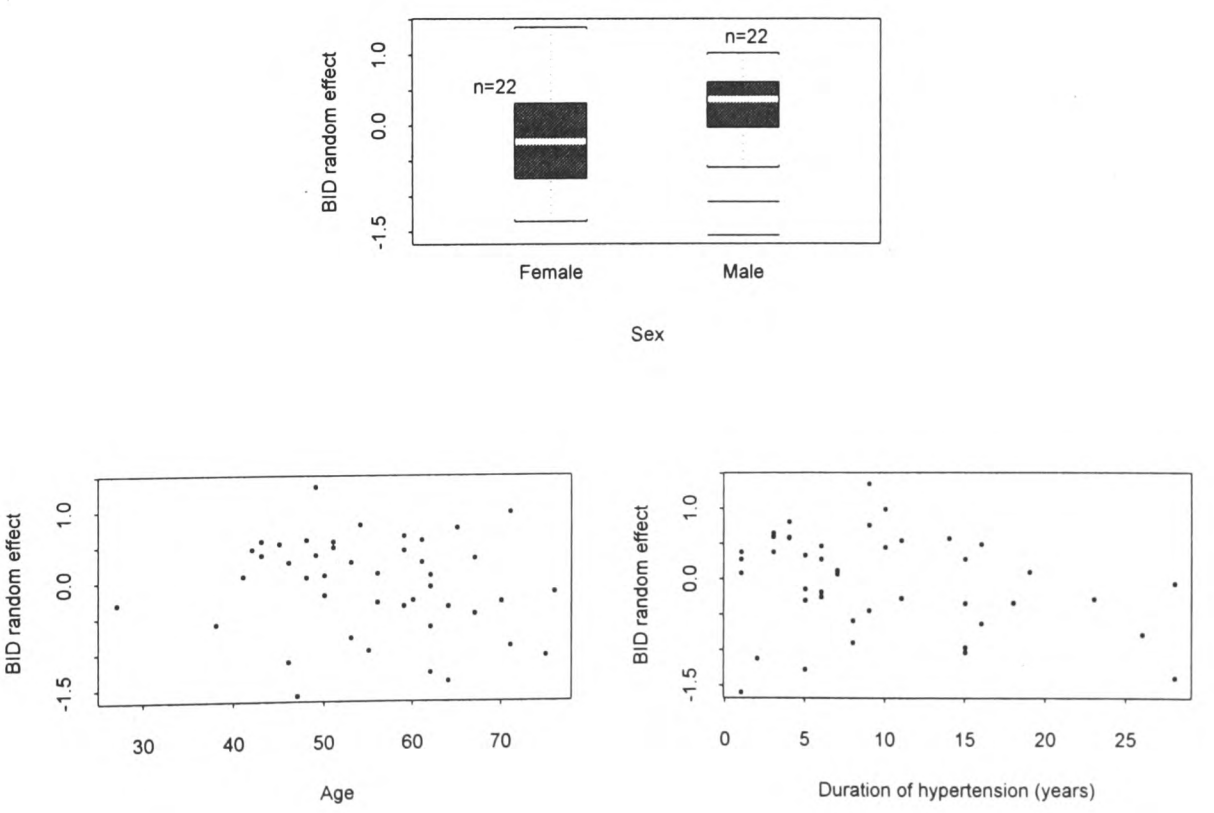

Figure 47. Study 1: Standardised BID random effect for Non-Blacks.

3.2.4.2 Study 2

A model of the form (Eq. 62) from Study 1 was used to describe the placebo effect.

Among the models without the race covariates, the one selected had a linear response for the q.d. regimen and a step response for the b.i.d. regimen. After accounting for the race effects, the linear dependence of the q.d. effect on dose disappeared for Blacks. The final model included a linear q.d. dose-response in Nonblacks, a step q.d. dose-response in Blacks, and no dose dependence for b.i.d. treatment in both race groups.

The form of the final model was:

$$\ln(DBP_{ij}) = \theta_{Base} - (Plac_{ij} + Qd_{ij} + Bid_{ij})(B_i\theta_{PlacB} + W_i\theta_{PlacW} + \sigma_{Plac}\eta_{Plac,i})$$
$$- Qd_{ij}[\theta_{QdIntcpt} + W_i(\theta_{QdSlopeW} + \sigma_{QdSlopeW}\eta_{QdSlopeW,i})Dose_{ij}]$$
$$- Bid_{ij}(\theta_{BidIntcpt} + \sigma_{BidIntcpt}\eta_{BidIntcpt,i})$$
$$+ \sigma_\varepsilon\varepsilon_{ij} \quad . \qquad \text{Eq. 65}$$

The parameter estimates with their estimated standard errors are shown in Table 59.

Diagnostic plots of the residuals are shown in Figure 48. Plots of residuals versus week, race, sex, age, and duration of hypertension are shown in Figure 48, Figure 49. Plots of the standardised random subject effects versus demographic covariates are shown in Figure 50 - Figure 52. Residuals appeared to be fairly homoscedastic. The tails of their distribution were heavier than in a normal distribution, but the distribution was fairly symmetric. Plots of residuals and random effects versus demographic variables did not suggest any dependencies other than already in the model.

Table 59. Parameter estimates of the final dose-response models for both studies.

Population parameters	Study 1	Study 2	Random effects	Study 1	Study 2
θ_{Base}	4.64 (0.003)	4.64 (0.003)	σ_{Plac}	0.053 (0.004)	0.061 (0.003)
θ_{PlacW}	0.059 (0.006)	0.061 (0.008)	$\sigma_{QdSlopeW}$	0.002 (0.0007)	0.001 (0.0003)
θ_{PlacB}	0.047 (0.011)	0.040 (0.008)	$\sigma_{QdSlopeB}$	0.0007 (0.0004)	0
$\theta_{QdIntcpt}$	0	0.021 (0.010)	$\sigma_{BidIntcpt}$	0	0.027 (0.010)
$\theta_{QdSlopeW}$	0.0018 (0.0006)	0.0005 (0.0003)	$\sigma_{BidSlopeW}$	0.002 (0.0007)	0
$\theta_{QdSlopeB}$	0.0007 (0.0004)	0	σ_{ε}	0.051 (0.002)	0.047 (0.002)
$\theta_{BidIntcpt}$	0.035 (0.011)	0.040 (0.012)			
$\theta_{BidSlopeW}$	0.0007 (0.0006)	0			

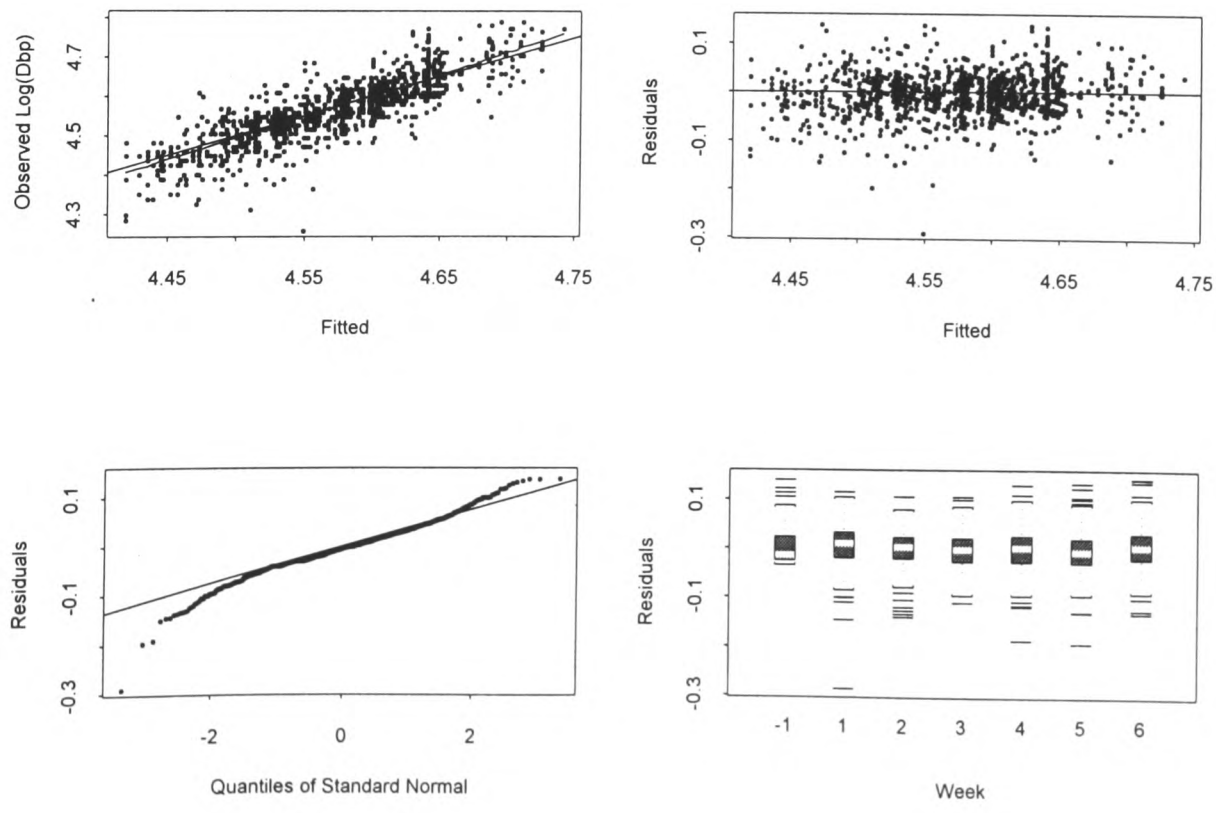

Figure 48. Study 2: Diagnostics of residuals.

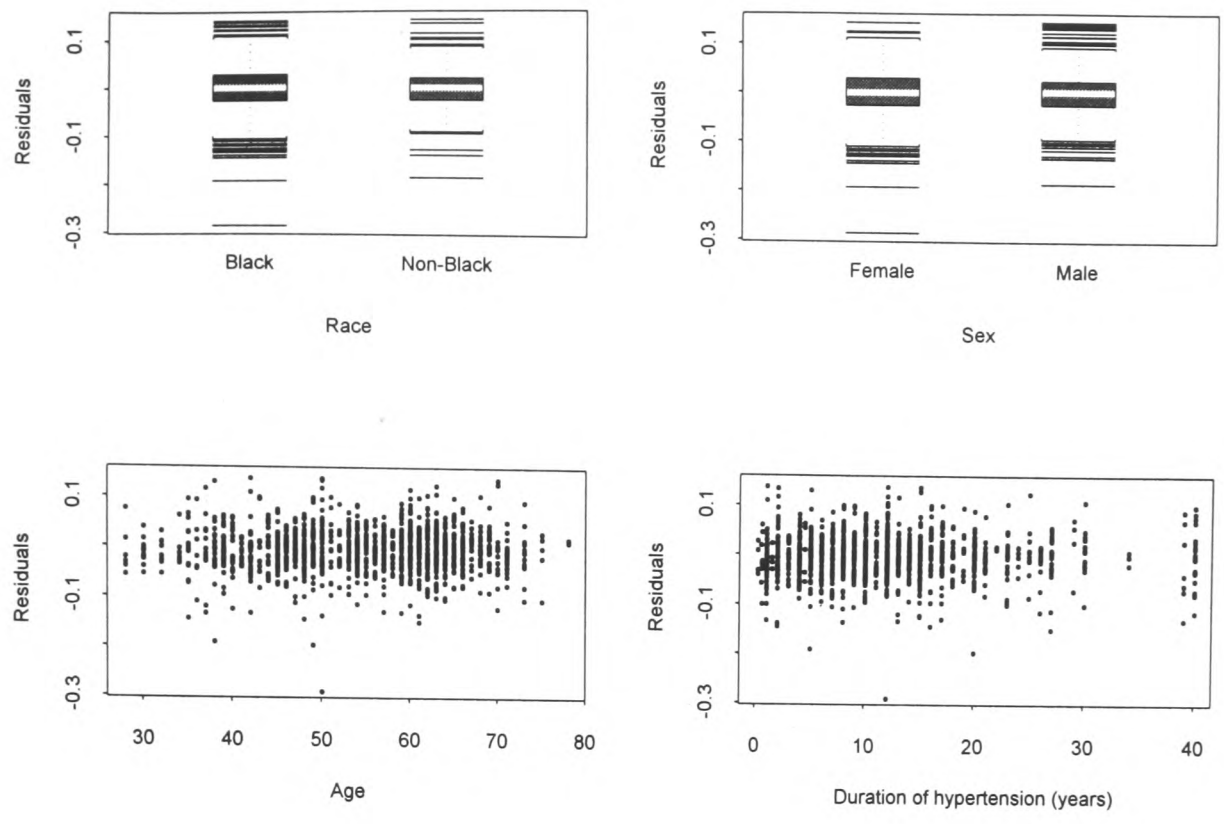

Figure 49. Study 2: Diagnostics of residuals (Cont.)

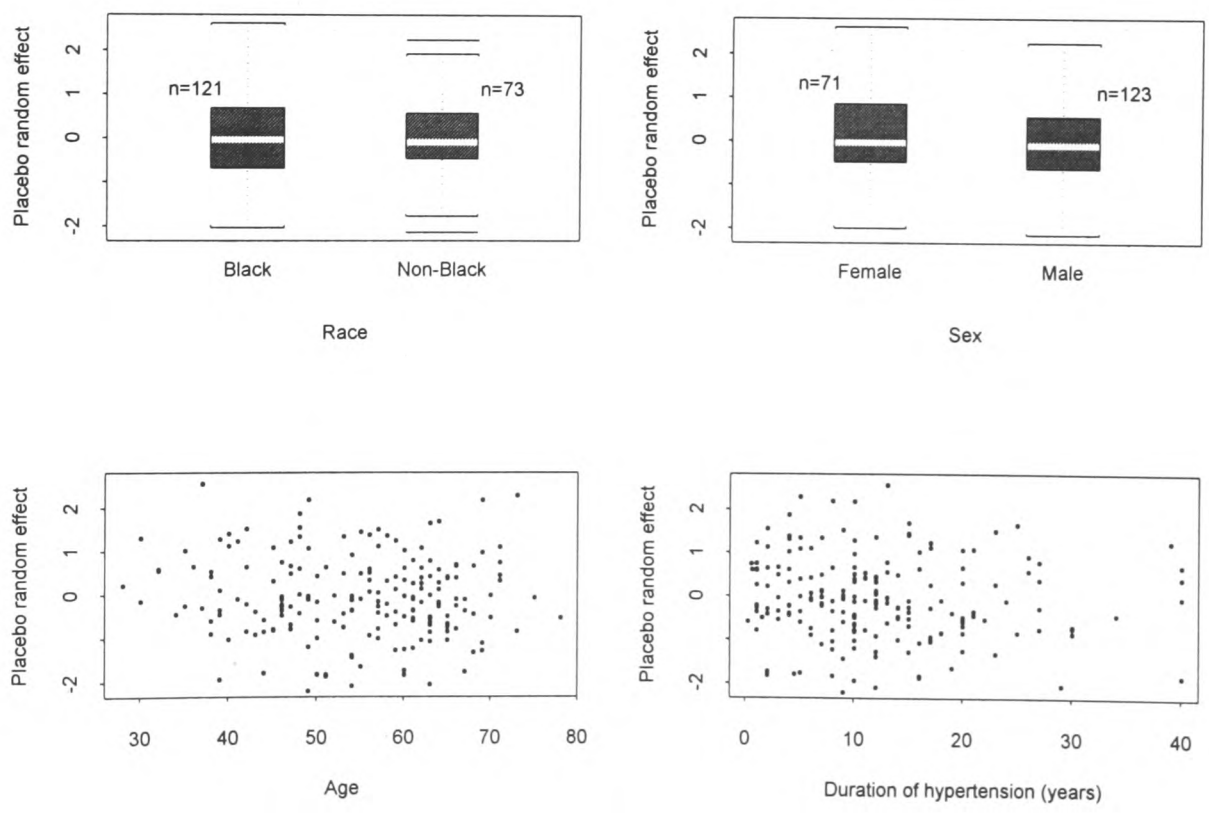

Figure 50. Study 2: Standardised Placebo random effect.

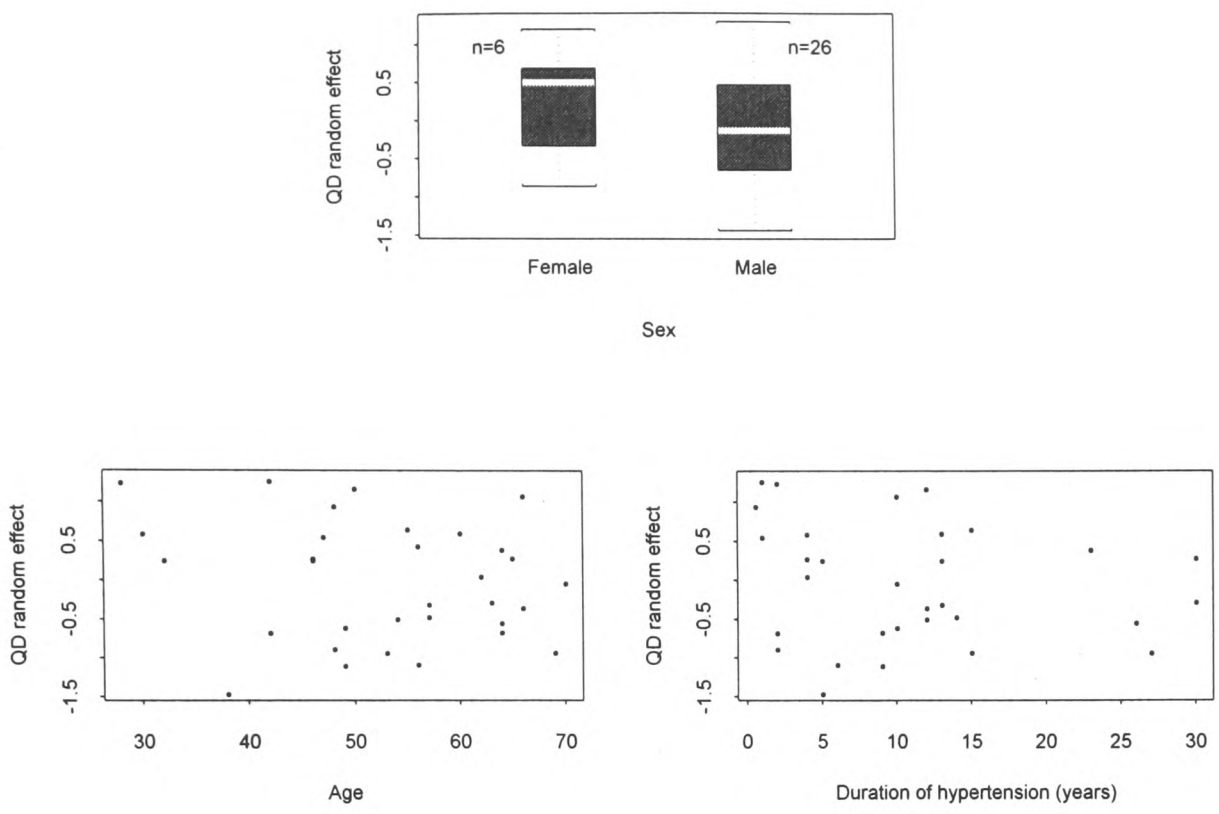

Figure 51. Study 2: Standardised QD random effect for Non-Blacks.

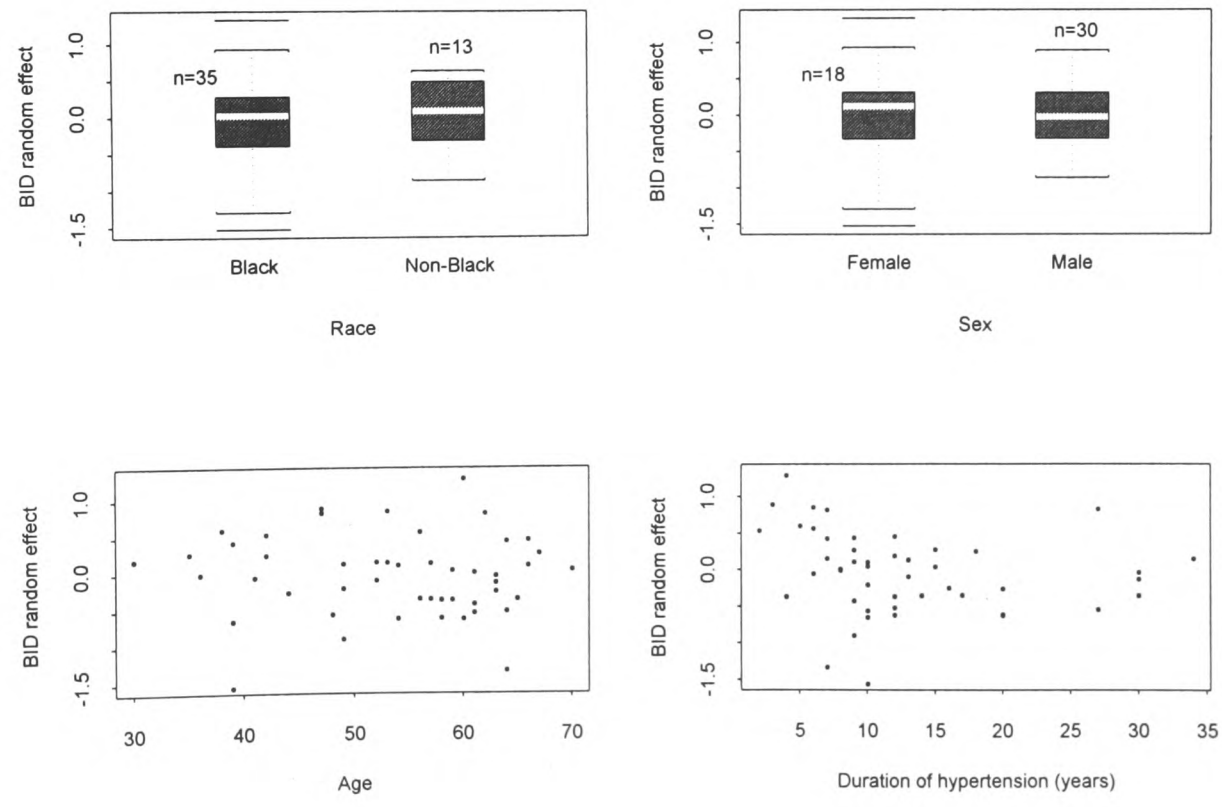

Figure 52. Study 2: Standardised BID random effect.

3.2.5 COMPARISONS

Figure 53 shows the estimated population mean diastolic blood pressure versus dose following the placebo, q.d., and b.i.d. administration of the drug, separately for Black and Nonblack patients according to the final models. Table 60 summarises the findings.

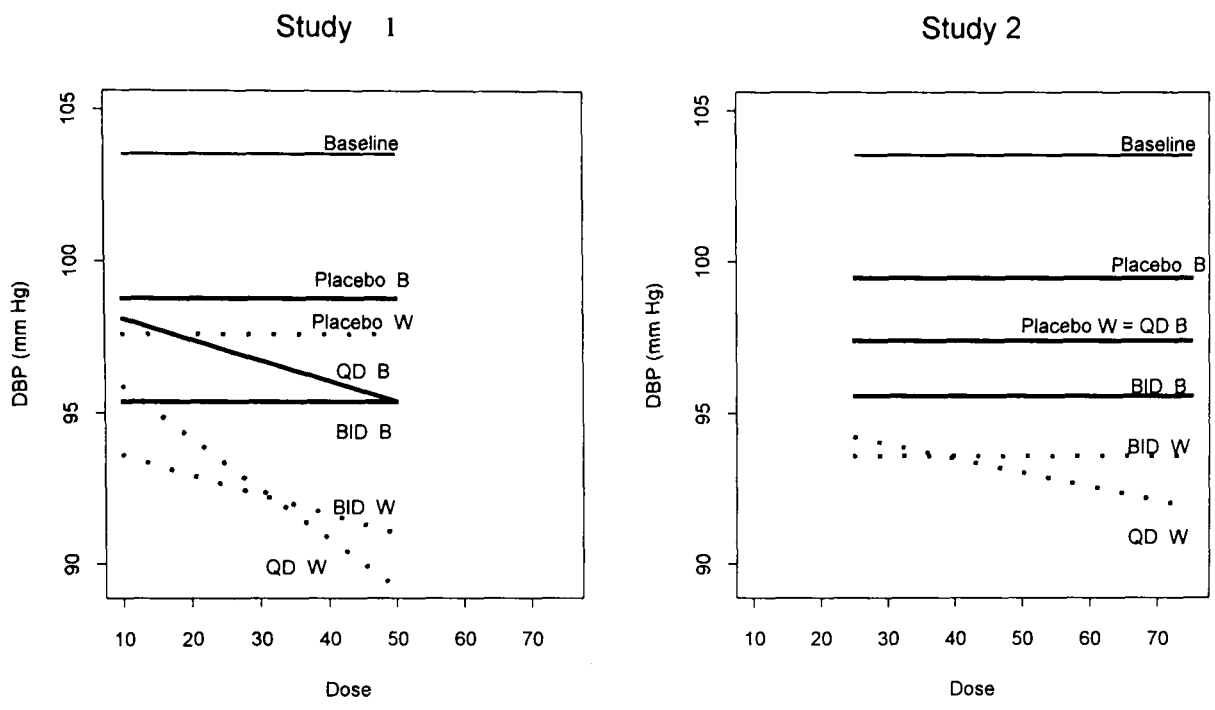

Figure 53. Diastolic blood pressure versus dose.

Table 60. Estimated population mean reduction (mm Hg) in diastolic blood pressure from baseline (103.5 mm Hg) in Studies 1 and 2 for the Black and Nonblack population.

Treatment	Study 1		Study 2	
	Nonblack	Black	Nonblack	Black
Placebo	5.9	4.8	6.1	4.1
Once-a-day	8.0-14.0[a]	5.6-8.0[a]	9.3-11.7[b]	6.1
Twice-a-day	10.1-12.4[a]	8.2	9.9	8.0

a. Linear reduction for doses from 12 mg to 48 mg;

b. Linear reduction for doses from 25 mg to 75 mg.

In Study 1, where dosing started from the lower dose of 12 mg/day, the response on once-a-day treatment was linear for both race groups, whereas it was linear for Nonblacks and on the plateau for Blacks on twice-a-day dosing. In Study 2, that started from 25 mg/day, only the q.d. response for Nonblacks was linear; the q.d. response for Blacks as well as b.i.d. responses for both races were on the plateau.

Estimates of the baseline and placebo effects, as well as the estimates of the placebo and residual variability were close for both studies. The difference in the parameters for the q.d. drug effect is understandable: the dose response was linear in Study 1 with the greater slope for Nonblack patients. With the higher doses of Study 2, it became almost flat for Nonblacks and completely flat for Blacks. In this case, $\theta_{QdIntcpt}$ was essentially the maximal effect E_{max}. The b.i.d. effect was consistent with the q.d. one: it was still on the rise, but close to a plateau for Nonblacks in Study 1, and it was on the plateau for Blacks in Study 1 and for both race groups in Study 2.

To compare the q.d. and b.i.d. effects across the studies, the 95% confidence bands for

$$\theta_{QdIntcpt} + \theta_{QdSlope}*Dose,$$

and for

$$\theta_{BidIntcpt} + \theta_{BidSlope}*Dose$$

were constructed for both race groups. They are shown in Figure 54. Bands for Study 1 completely contain those for Study 2 (q.d. effect in Blacks, b.i.d effect in Nonblacks) or extensively overlap with them (q.d. effect in Nonblacks, b.i.d. effect in Blacks) over the range of overlapping doses.

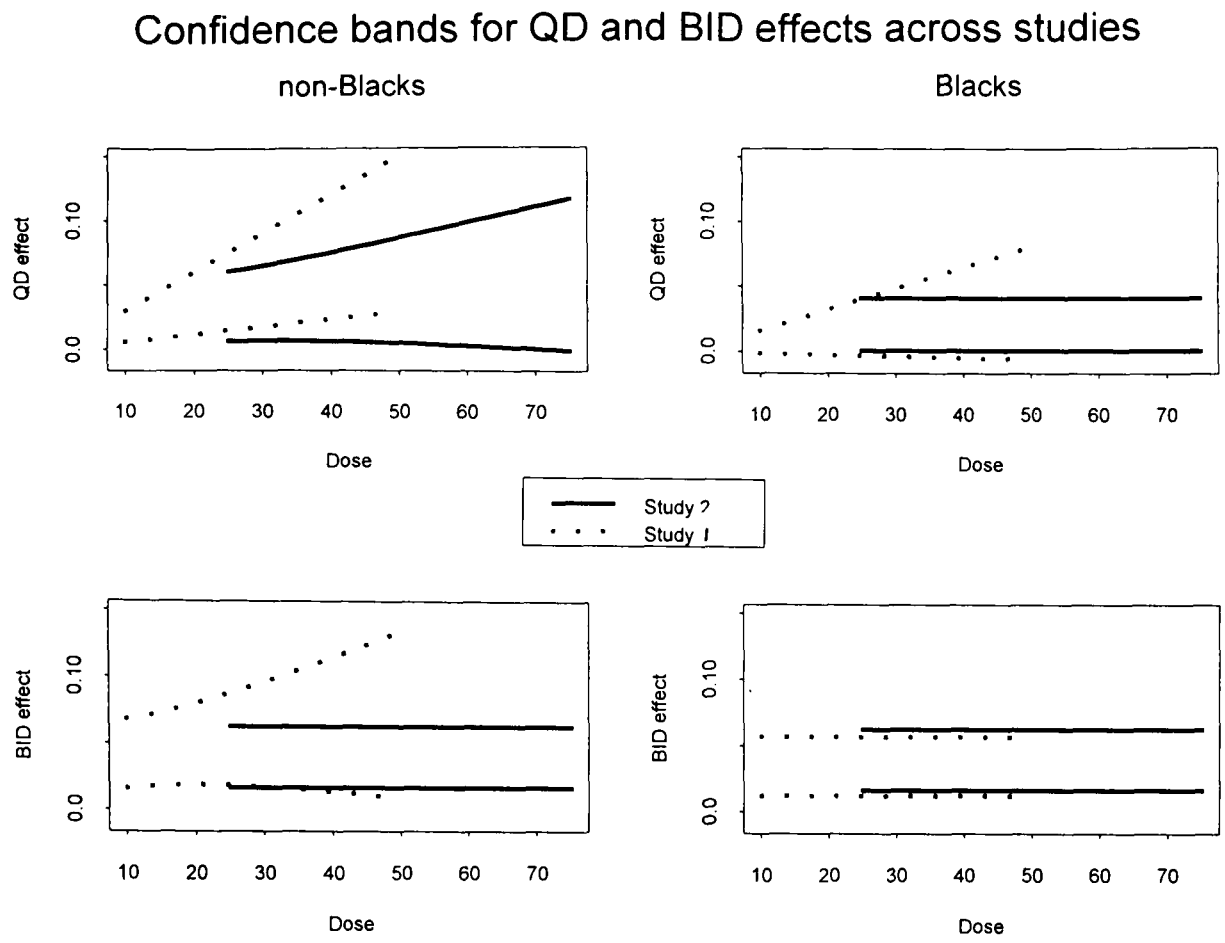

Figure 54. Confidence bands for QD and BID effects across studies.

For Nonblack patients the effect on twice-a-day treatment was larger than for once-a-day dosing at lower doses; however, by a dose level of 32 mg/day (Study 1) or 37 mg/day (Study 2) response on a once-a-day dosing was the same as that for the twice-a-day dosing, and it became superior with increasing doses. In the Black population, the studies gave different results: according to Study 1 the b.i.d. effect was higher at the lower doses, but by the highest dose of 48 mg/day the q.d. effect was equal to b.i.d. According to Study 2 the b.i.d. effect was superior at all the doses. Confidence bands for q.d. and b.i.d. effects, shown in Figure 55 compared the regimens for each study. They support the difference between q.d. and b.i.d. at the low dose of Study 1, but not at the dose of 25 mg/day and higher. In Study 2 the bands for the q.d. effect completely contain b.i.d. bands (Nonblacks) or overlap extensively with them (Blacks) for all the doses.

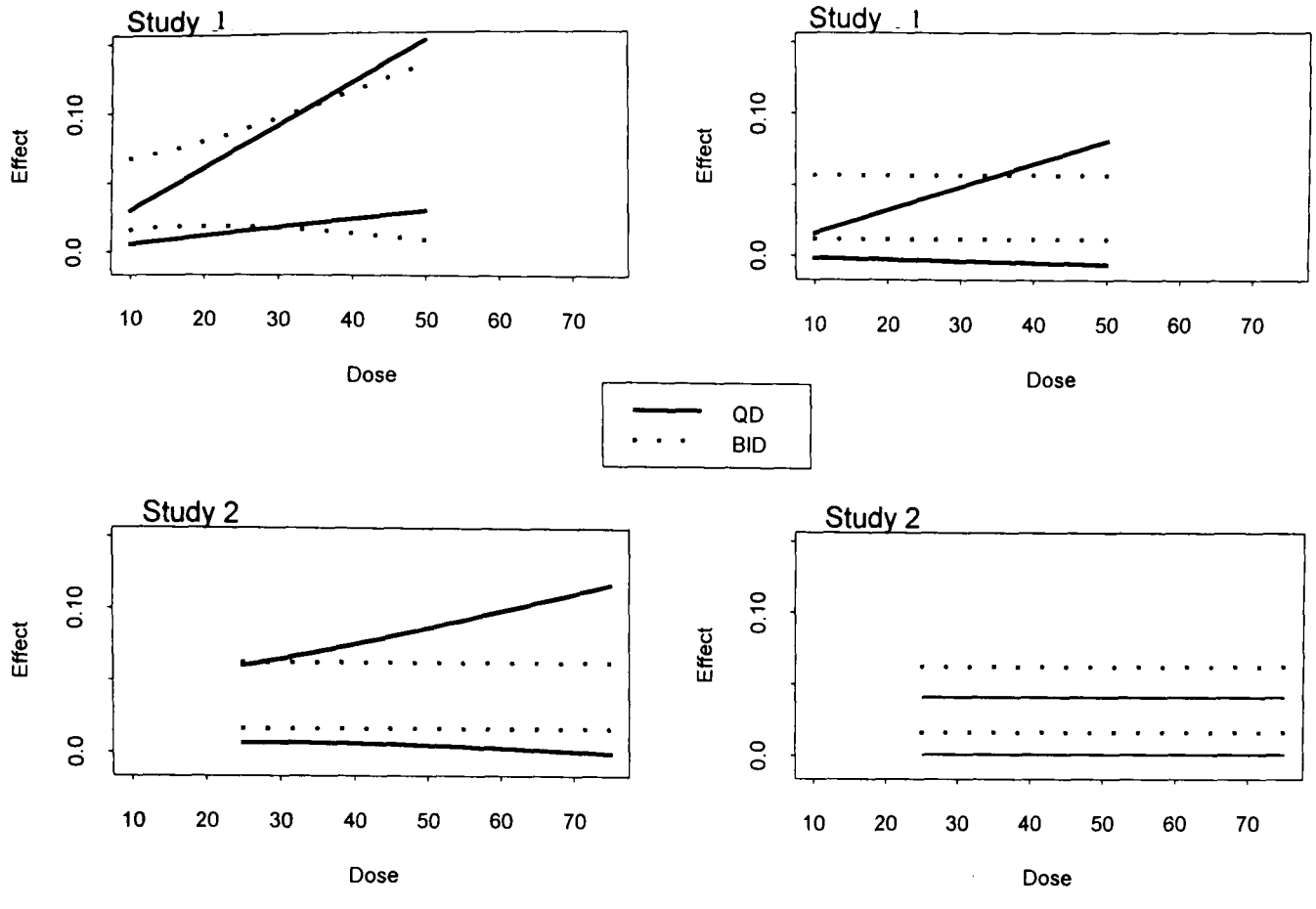

Figure 55. Confidence bands for QD and BID effects within studies.

Efficacy of the drug appeared to be higher for Nonblack population. This effect can be partly attributed to the different placebo effect in these race groups. For Blacks the placebo effect was 33% smaller than for Nonblacks according to Study 2, and 19% smaller according to Study 1. The statistical evidence in favour of the difference was stronger in Study 2.

3.2.6 CONCLUSIONS

Mixed-effects modelling of the data from two studies demonstrated that the linear or step dose-response model could adequately describe the efficacy of the drug. The model depended on the regimen (q.d. versus b.i.d.), on the range of doses (12 to 48 mg/day versus 25 to 75 mg/day), and race (Black versus Nonblack).

Estimated population mean of the maximum reduction in diastolic blood pressure was 14.0 and 11.7 mm Hg for Nonblacks (q.d. treatment), and 8.2 and 8.0 mm Hg for Blacks (b.i.d. treatment) in Study 1 and Study 2, respectively.

Efficacy of the drug was higher in Nonblack than in the Black population. This difference in response was partly attributable to differences in the placebo effect for these race groups.

4 SUMMARY AND CONCLUSIONS

This thesis deals with applications and development of repeated measures analysis, so called 'population approach' methods, in the field of pharmacokinetics.

Chapter 1 describes the organisation of the work and provides a general overview of different types of clinical studies and methods used to analyse them. Chapters 2 and 3 describe further developments and applications of these methods to several drug development projects and to simulated data.
The main goals of the pharmacokinetic investigations described in Chapter 2 were to detect demographic, disease-related and other covariates that alter patient's drug exposure, and characterise their contribution to exposure. The model-free approach has been developed for these situations. The approach is based on partitioning plasma concentrations into observation levels taking into account time of measurements and using all the concentration data from all patients, then assigning each patient an exposure level according to observation levels of his/her concentrations, and then investigating the relationships between the exposure level and covariates.

The approach was applied to three different drugs under development. In the first project partitioning of concentrations into regions was done by piece-wise constant functions within each one-hour interval, the quartiles of dose-normalised concentrations. In the second and third projects, where concentrations were very non-uniformly distributed in time, the data was interpolated: nonparametric smoothing splines separated observation regions. Three spline curves, first of them fitted to all the data and the other two fitted to the points above or below the first curve, divided concentrations into four regions, approximating quartiles of the first project.

In the first two projects, the model-free approach was meant to serve as a screening tool: covariates identified as important were to be incorporated into model building of the nonlinear mixed effects model. Therefore, the interest was mostly in qualitative results. To find predictors of exposure, exposure levels were related to demographic covariates by standard statistical techniques: contingency-table analysis for the categorical covariates and one- or two-way ANOVA for the continuous covariates. Area Under Quartile (AUQ) was used in the first project as a crude measure of individual exposure.

In both projects the nonlinear mixed effect model confirmed the results of the model-free investigation (Section 3.1 of Chapter 3 and Nedelman, *et al.* [1995, 1996]): covariates found to be important by the model-free approach were found significant by the modelling approach, too. Also, model derived individual AUCs showed a strong linear correlation with model-free AUQs computed in the first project.

In the third project the model-free approach was the only technique used for the analysis of the data. Also, there were many more covariates to investigate. This necessitated both, a considerable change in choice of statistical techniques, and the development of quantitative measures of exposure for subpopulations. Classification tree models (CART) were built to find the predictors of exposure, and weighted locally linear regression was used to compare Areas Under Population Curves (AUPC) for subpopulations of interest.

The performance of the method was investigated in the fourth project: the simulation study. It examined the capability of the method to detect subpopulations with altered exposure and investigated the change in AUPC as its measure. Data was simulated using the linear one compartment model with first order absorption and first order elimination. Parameter values, times of concentration measurements, distributions of covariates and correlation between them were based on the data of the third project. Influence of variability, relative contributions and distributions of covariates, and choice of smoothing parameters on the results were examined.

Simulation demonstrated that the model-free method was capable of identifying predictors of exposure in a wide range of variability in the data. Tree models of the data simulated with no intra-subject variability (but with 30% inter-subject variability in both clearance and volume of distribution) always correctly detected the covariates: all influential covariates appeared in the models, and the false covariate was never chosen. As could be expected, with increasing intra-subject variability capability of the tree models to detect influential covariates decreased. But the decrease was slow: the tree model could not detect covariates only when intra-subject variability became as high as twice the contribution of those covariates to AUPC.

Change in AUPC for subpopulations, identified by the tree models was a good approximation of change in exposure for the respective populations. Change in AUPC was very close to the true change in all the cases except in the case of the highest intra-subject variability (CV=50%). Even in this case the difference did not exceed 6% when assessed in appropriate subpopulations.

The application of the Nonlinear Mixed Effect methodology to population pharmacokinetics and dose-response analysis is the theme of Chapter 3. In the first project a population pharmacokinetic model was developed for the drug described in the second project of Chapter 2. The objectives here were broader than in Chapter 2: the aim was not only to find the covariates that altered drug exposure, but also to completely characterise the drug's pharmacokinetics (extent and duration of exposure, in particular), and its relationships to safety and efficacy.

To find a form of the structural model, individual compartmental models were first developed with available data from data-rich phase I studies. Two-stage analysis of pharmacokinetic parameters provided the initial estimates of population parameters. Linear three-compartment mammillary model with zero-order absorption and first-order elimination best described the data. The effect of food was evident in the food-interaction Study H1: absorption was delayed and bioavailability increased with food.

Phase I data (only in fed state) was then combined with sparse data of Chapter 2 for the development of the population model. The developed pharmacokinetic model of the drug described diverse pharmacokinetic data. It obtained pharmacokinetic parameters of the drug and characterised extent and duration of exposure. The model identified demographic predictors of the drug exposure and quantified their influence on pharmacokinetic parameters, extent, and duration of exposure. It confirmed the results of the model-free approach: all the covariates identified by the model-free approach were retained in the final model.

The simulation showed that the first-order estimation method of NONMEM did an adequate job at estimating population parameters of the model. It overestimated half-life and underestimated bioavailability parameters by about 10% with no bias in the

other parameters. It did less well at the estimation of the variability of the terminal half-life: coefficient of variation was underestimated by about 25%.

The developed model was used to explore the relationships between the exposure and demographic predictors of exposure, and safety and efficacy of the drug. Individual average steady-state concentrations and individual inter-visit AUCs based on Bayes posthoc estimates of the model parameters were divided into ordered groups and were used as measures of exposure. Frequency and severity of adverse events and abnormal tests of liver function were the safety measures; and three categories of cure from the disease were the measure of efficacy. Association tests did not find any significant relationships between exposure groups and safety indicators. No relationships between demographic predictors of exposure and safety indicators of the drug were also found. Also, none of the tests of association between efficacy outcomes and exposure, and demographic predictors of exposure was significant.

Finally, the developed model was used to guide the design of a new study, with different doses and different dosing regimens of the drug. Simulation of the study answered the questions of duration and monitoring of dosing, needed to satisfy the objectives of the study. It estimated the time necessary for median AUC and C_{max} to reach 90% of steady state, and for the 25% percentile to exceed 80% of steady state in both once-a-day and twice-a-day regimens; estimated the wash-out monitoring period until disappearing of drug from plasma for half or 75% of subjects. It also explored the alternative ways to speed up the attainment of steady state: loading doses and "pre-treatment".

In the second project of Chapter 3 the Nonlinear Mixed Effects Methodology was used to develop a population dose-response model of a drug. Data for this project came from titration studies in hypertensive patients, where the dose and the regimen of the drug administration depended on patient response (i.e. change in diastolic blood pressure). The developed linear and step dose-response model adequately described the efficacy of the drug. The model depended on the regimen (once versus twice a day), on the range of doses, and race (Black versus Nonblack). Efficacy of the drug was higher in Nonblack than in the Black population. This difference in response was shown to be partly attributable to differences in the placebo effect for these race groups.

The model-free approach developed in this thesis was successfully applied to several drugs. Nonlinear Mixed Effects population models developed for the same data agreed with its results; simulations showed it to be an accurate and reliable tool. But goals that can be achieved with the approach are limited: one can identify covariates – predictors of steady state or near steady–state exposure, quantify their contribution, and obtain estimates of quasi-individual steady-state exposure (AUQ) in some cases. This is equivalent to characterising clearance of the drug. One can't say anything about volume of distribution or other pharmacokinetic parameters. Also, this approach does not allow estimating the variability of pharmacokinetic response, neither between nor within subjects. This is an empirical approach: it helps little in understanding of underlying physiological phenomena, and can't be used for extrapolating or interpolating into different experimental conditions (doses, regimens, populations). The basic assumption of the approach is that patient's concentrations are consistent across weeks of dosing. That limits the applicability of the approach to steady-state or single dose situations. Also, concentration response should be proportional to dose, or all patients should have the same dose and dose regimen. On the other hand, the approach is able to answer important questions. It does not depend on the functional form of underlying pharmacokinetic model or covariate relationships. Nor it depends on the distributional assumptions. It is fast, elegant, non-iterative and easy to implement. Once developed, it can be readily applied with almost no adjustment to different projects.

One of the unanswered questions of the model-free approach is the sensitivity of the results to the number and definition of the observation levels and the exposure measure. In the present work, boundaries of the observation levels are based on smoothing splines. Instead, they might have been drawn by a number of other smoothing functions, for example by lowess regression, or kernel estimators, or smoothed running median. It was shown that the results did not depend on the splines' smoothing parameter. It is natural to expect that the precise number of the observation levels and details of rules for subject's assignment to the exposure levels should not influence the main dependencies uncovered by the method. Though the assignment of points to the observation levels and subjects to the exposure levels may change slightly depending on the assignment algorithms, the change will not affect the results of clinical importance. On the other hand, if a continuous exposure measure is

developed and shown to be robust to outliers, this would open the way to obtaining more quantitative results concerning the relationship of the drug's pharmacokinetics to covariates.

Model–based approach characterises all pharmacokinetic parameters of interest. It is not limited to any particular regimen or state of drug administration: regimen of any complexity can be incorporated into the model. It allows quantifying the variability of the response: inter-subject variability in pharmacokinetic parameters and intra-subject residual error that can be attributed (if data allows) to different sources of variation. It can provide estimates of individual exposure and relate them to safety and efficacy. PK/PD models can go beyond description of the data. They can help in understanding the underlying physiological mechanisms and can be used to answer what-if questions: they can be interpolated and extrapolated (with caution) into different experimental conditions, can be used for design of new studies.

The power and flexibility of the modelling approach comes with a cost. Models are highly dependent on the assumptions: on choice of functional forms used to fit the data and on distributions of random effects.

An exhaustive list of the implicit and explicit assumptions used in the population modelling of a drug's pharmacokinetics or pharmacodynamics by means of nonlinear mixed-effects methodology is presented by Karlsson, *et al.* [1998]. The assumptions may be roughly divided into several groups.

The first group of assumptions is concerned with the data. One assumes that:

a. Dose history, that is, the dosing times and amounts of doses, is error-free;
b. Sampling times, that is, the times of the pharmacokinetic and pharmacodynamic measurements are error-free;
c. Values of covariates are error-free.

One of the directions of the modern pharmacokinetic modelling is to estimate validity of these assumptions, and develop the ways to take into account errors that result from their violation. These are especially important for phase III studies that are the most difficult to control. The rate of non-compliance (that is, violation of the protocol by either the patients, skipping the doses or incorrectly recording dosing time, or by the

nurses, incorrectly recording sampling time) is greater in these studies than that in the smaller phase I and phase II studies.

The other group of assumptions is concerned with the model. Firstly, it is assumed that a subject's pharmacokinetics can be described by the system of ordinary differential equations resulting in the compartmental model. Usually, this is the linear system, although non-linear elimination, absorption, or kinetics can also be considered. Secondly, it is assumed that the same structural model can be used to describe all the subjects (with the parameters that vary from subject to subject). Thirdly, it is usually assumed that the distribution of the subject-specific model parameters in the population is normal. The validity of these assumptions can be justified by the fact that for the vast majority of drugs on the market the modelling procedure based on these assumptions led to reasonable results. Normality of the parameter distribution for the final model can and should be assessed after the modelling. Large deviation from the normal distribution is often the evidence of model misspecification, but may also represent a genuine non-normality of the distributions of inter-individual parameters. In the latter case, an appropriate transformation of inter-individual random effects may solve the problem [Fattinger, *et al.*, 1995], or methods that do not make that assumption [Davidian & Gallant, 1992; Mallet, 1986] may be tried.

Yet another group of assumptions is concerned with the residual error. It is assumed that all the dependencies unexplained by the model result in the residual errors that are independently, symmetrically (possibly after transformation) distributed with a mean zero. The validity of this assumption for the final model can and should always be checked. Diagnostic plots of residuals and weighed residuals versus predicted concentrations for the whole population and each of the subjects help to identify outliers, check the fit, and reveal an existence of autocorrelation. If found, the autocorrelation can be modelled explicitly [Karlsson, *et..al.*, 1995, 1998; Seber & Wild, 1989].

Finally, it is assumed that the sophisticated software used for modelling (such as SAS, NONMEM, S-PLUS) is able to find the solution of the posed minimisation problem. Although it is impossible to verify absence of bugs in any large and complex

computer program, the extensive industry and academic experience with these programs delivers sufficient level of confidence in their output.

Model-based approach is a very powerful tool. One should realise, however, that this power has its limits. Indeed, study of the drug effect on the heterogeneous population is a very complex problem. One cannot hope to explain all the variability in the response that is inherent to complex biological systems via a simple mathematical model. Therefore, the goals of the modelling are more limited, namely to explain the main effects and be able to predict, with reasonable accuracy, the main features of the pharmacokinetic and pharmacodynamic response.

Pharmacokinetic models are usually nonlinear, often ill-conditioned with all the problems of nonlinear optimisation algorithms. Algorithms are iterative, their convergence depends on initial estimates, on model parameterisation, identifiability of parameters, etc. Model building is an iterative procedure: the choice of models depends both on the notion of physiological meaning of the data and on the results of the already tried models. All this requires a wide range of skills, thought, analyst's time, and computer time and power.

The population modelling is a fascinating field of application for modern statistical methods. The combination of complex non-linear statistical and mathematical models with heuristic exploratory techniques offers exiting challenges to modellers and new research tools to the pharmaceutical sciences.

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6 APPENDICES

Appendix A. Differences in inclusion and exclusion criteria across the studies.

Table A.1 Baseline treadmill test inclusion criteria

Study	Treadmill[a] setup	Initial claudication distance (ICD)[b] (m)	Absolute claudication distance (ACD)[b] (m)
I	12.5% incline	30 - 200	--
II	0% incline increasing by 3.5% every 3 min	--	stable[c]
III	12.5% incline	30 - 200	320, stable[c]
IV	0% incline increasing by 3.5% every 3 min	54	805, stable[c]

a) constant speed of 3.2 km/h;

b) in two consecutive tests;

c) two consecutive tests with an ACD within 20% of the first value.

Appendix B Exclusion of samples from analysis

Reasons for exclusion of samples:

1. Missing time post dose due to missing time of blood draw or missing time of dose administration;
2. Negative time post dose;
3. Missing concentration;
4. Time post dose greater than 20 hours;
5. Samples of noncompliant patients

Table B.1 Number of samples/patients analyzed and excluded from the analysis by study and reason

	Study				Total
	I	II	III	IV	
Initial number of patients[a]	95	171	133	119	518
Initial number of samples[b]	1229	594[c]	325[d]	351	2499
Missing time post dose	168	30	3	-	201
Negative time post dose	-	18	-	2	20
Missing concentrations	4	-	1	43	48
Non-compliant patients	1	2	4	2	9
Observations from non-compliant patients	5	12	6	3	26
Samples taken later than 20 hours after last dose[fg]	38	11	18	-	67
Observations used in analysis	1014	547	297	303	2161

a) Randomised to 100 mg BID group;

b) Does not include samples at baseline, prior to 1st dose;

c) There were 594 plasma samples, 606 records of drug administration. Merging them created 570 usable observations, 30 and 18 observations with missing and negative times post dose, respectively;

d) Plasma times were assumed to be the same as times of blood draws for laboratory values, stored in the Lab data set. They were merged with plasma concentrations by visit dates;

f) 28 samples taken between 20 and 50 hours, and 39 samples taken more than 50 hours post dose;

g) Two observations were common to the list of non-compliant patients and the list of times post dose > 20. They are not included here.