Variability in the pharmacokinetics of rifapentine in South African tuberculosis patients: a classical and population approach

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"The ideal of democracy fulfils itself only if and when society fails to suppress the individual"

e e cummings

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Abstract

Tuberculosis is recognised as one of the leading public health problems in sub-Saharan Africa. The treatment and control of the disease depends largely on a limited number of chemotherapeutic agents, most of which have been used for the past 30 years. Discovery and development of new antimycobacterial agents has been relatively stagnant. New impetus from the Global Alliance for Tuberculosis Drug Development aims to register a new faster-acting and affordable drug by 2010. Until such an agent is freely available, though, it is necessary to use the available means at our disposal. Treatment regimens based on rifapentine, with its less demanding schedule, lack of autoinduction and increased absorption following food intake could make it an excellent candidate to anchor an intermittent chemotherapy regimen.

The studies thus far on patients receiving proven bioavailable preparations of rifapentine do not comment on the respective plasma levels but have concentrated on specific outcomes. As a result little is known as to the magnitude of variability in plasma levels amongst patients receiving rifapentine. This degree of variability may prove to be important as rifapentine continues to be investigated as an alternative to rifampicin.

This thesis describes the pharmacokinetics of rifapentine in a South African tuberculosis patient population with special reference to variability in serum drug levels between patients and between occasions. Forty-five patients received rifapentine doses of 600, 750 and 900 mg based on body weight. Doses were administered on study days one and five. All patients had already received not less than four weeks and no more than six weeks of standard antimycobacterial therapy (isoniazid, rifampicin, pyrazinamide and ethambutol). In total, twenty blood samples were collected per patient from 0 – 168 hours. Rifapentine and 25-desacetyl rifapentine concentrations were determined using validated high pressure liquid chromatography methods. Median peak plasma concentrations, time to reach this peak, plasma elimination half-lives and area under the concentration-time curve were calculated for both parent drug and metabolite on both occasions using noncompartmental methods. The pharmacokinetics of the parent drug was best described using a one-compartment model, with a lag time and first-order absorption and elimination. Estimated population parameters were absorption rate constant, lag time on absorption, clearance/bioavailability, and volume of distribution/bioavailability. Between subject and between occasion variability was below 25%

for all parameters except KA which showed a between subject variability of 52%. The pharmacokinetics of the metabolite in this study were best described by a one-compartment model with no first-pass metabolism and a clearance value that declined in a nonlinear fashion over time. Parameters estimated were, volume of distribution, induced metabolite clearance, baseline metabolite clearance, and slope of decline in clearance over time.

In South African tuberculosis patients the 15 mg/kg dose of rifapentine was well absorbed and well tolerated. The variability observed between individuals and between occasions was small and similar to that seen in data from previous studies in healthy volunteers.

Table of Contents

List of Figures

CHAPTER 1

CHAPTER 2

CHAPTER 3

CHAPTER 4

List of Tables

CHAPTER 1

CHAPTER 2

CHAPTER 3

CHAPTER 4

Chapter 1

Introduction and study motivation

1.1 MYCOBACTERIUM TUBERCULOSIS

1.1.1 Introduction

Mycobacterium tuberculosis is a virulent pathogen that knows no ethnic, social or economic boundaries. Globally it is estimated that there are 8 million new suffers each year resulting in 2 million deaths. That equates to one person each 15 seconds! The last decade has seen the number of tuberculosis (TB) cases grow by 20% with impoverished communities carrying the highest burden (Global Tuberculosis Control, WHO Report 2003). This is particularly applicable to sub-Saharan Africa where the emergence of the HIV epidemic and the increase in the number of individuals living in poverty and under extreme conditions has facilitated the spread of the disease. The problem however is not limited to Africa with large parts of Asia also afflicted (Figure 1.1).

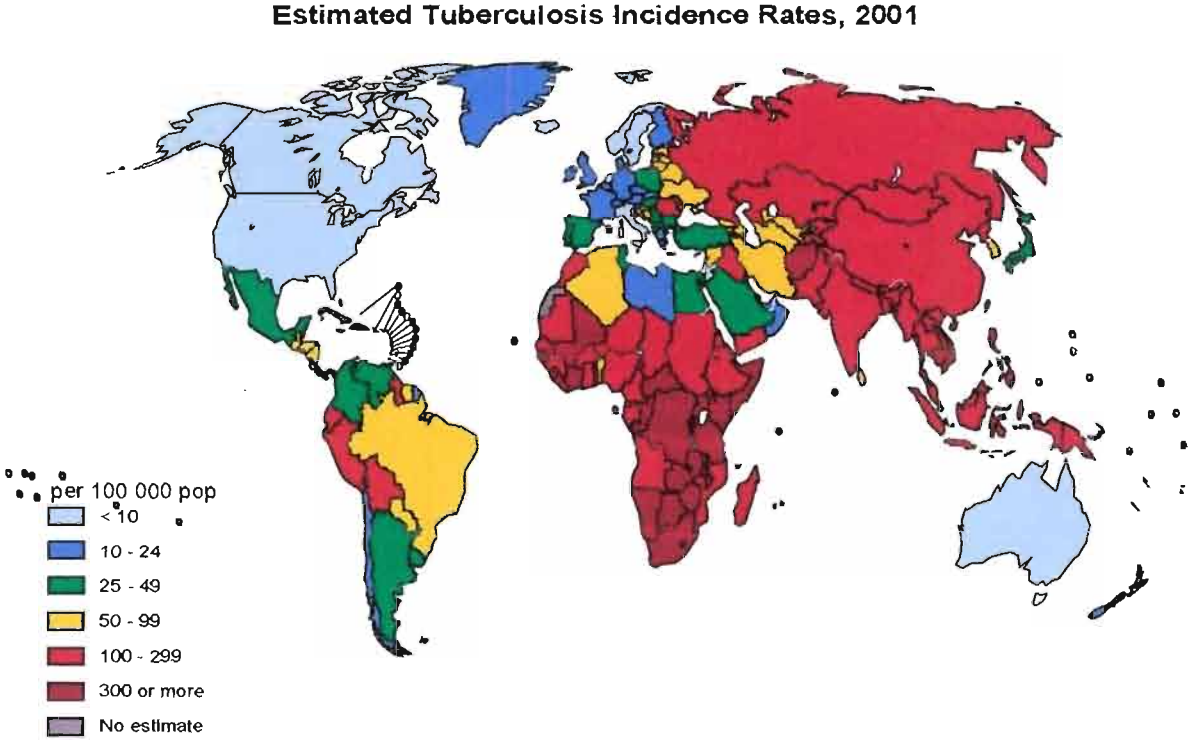


Figure 1.1 World Health Organisation estimated global tuberculosis incidence rates per country for 2001 (Global Tuberculosis Control, WHO Report 2003).

Twenty-two countries have been classified as having a high tuberculosis burden by the World Health Organisation (WHO). All but one of these classified countries have incidence rates above 100 per 100 000 population, and seven have rates above 300/10 000. South Africa is one of

these (556/100 000) and is topped only by Zimbabwe (628/100 000) and Cambodia (585/100 000) (Figure 1.2).

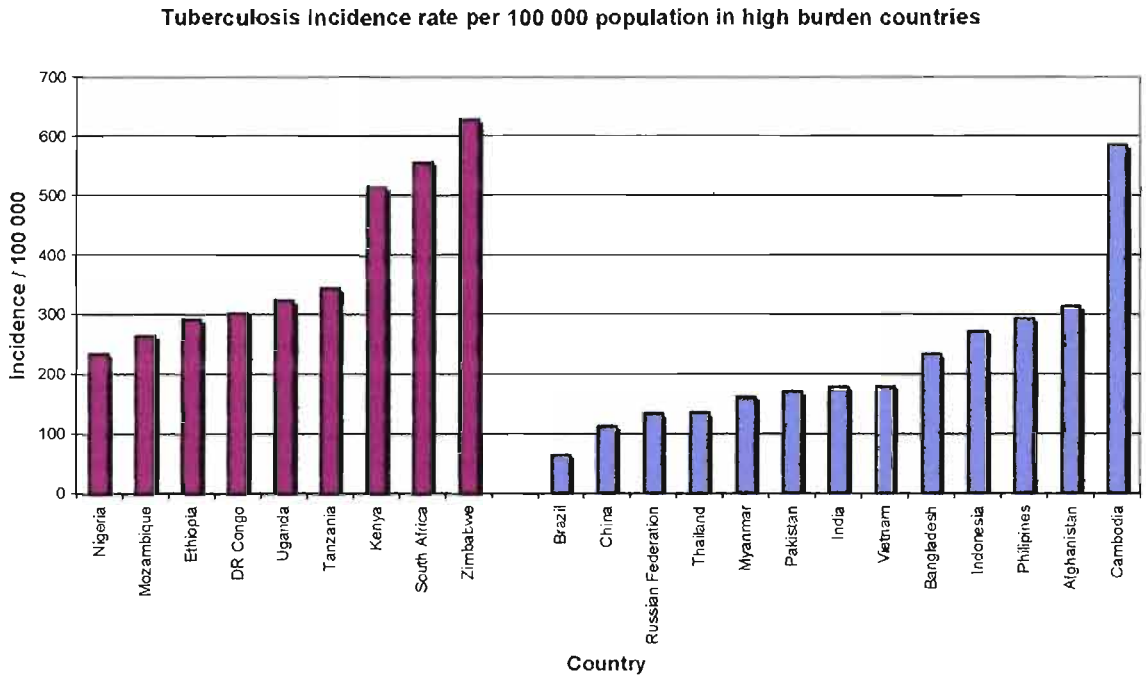


Figure 1.2 Estimated incidence of tuberculosis in 22 high-burden countries for 2001 (Global Tuberculosis Control, WHO Report 2003).

As a result controlling TB is one of the national health priorities in South Africa. A national control plan was implemented in 1996 and incorporated the Directly Observed Therapy Short course (DOTS) strategy. This strategy emphasizes the need for:

a) Political commitment
b) Case detection amongst self-reporting patients with symptoms using sputum smear microscopy
c) Short course chemotherapy under proper management and with direct observation of drug ingestion
d) Assurance of uninterrupted drug supply
e) Strong surveillance and monitoring system

By the end of 2002 DOTS coverage in South Africa was at 77% and prompted new political commitment from the Minister of Health to achieve the goal of 100% coverage. This has led to a steady increase in the detection of new cases to 72% although the percentage of treatment successes (66%) is still well below the 2005 target of 85% (Country Profile South Africa, WHO Report 2003).

The above figures raise intriguing questions as to why we are not able to adequately cure patients when treatment is readily and freely available. These questions are unfortunately beyond the scope of this thesis but highlight the need for increased political and social commitment to the current strategies and control programmes.

1.1.2 Treatment of tuberculosis disease

1.1.2.1 Antimycobacterial agents

A combination of four or five frontline drugs with varying modes of action (Table 1.1) is administered to patients diagnosed as 'new cases' for a period of two months in order to target actively dividing mycobacteria. This is followed by a two drug combination for a period of four months in the continuation phase as illustrated in Table 1.2.

Table 1.1 Frontline agents used in the treatment of tuberculosis and their mode of action (Webb and Davies, 1999)

Antibiotic	Mode of Action	Mode of resistance
Isoniazid	Inhibitor of cell-wall synthesis	*inhA*, *katG* (mycolic acid synthesis)
Ethambutol	Inhibitor of cell-wall synthesis	*embB*
Rifampicin	Inhibitor of RNA synthesis	B-subunit of RNA polymerase (*rpoB*)
Streptomycin	Inhibitors of protein synthesis	All mutations in rRNA
Pyrazinamide	Unknown	*pncA* (pyrazinamidase)

Table 1.2 Current regimens for treatment of drug susceptible tuberculosis (South African Medicines Formulary, 2003)

Regimen	Intensive phase	Continuation phase
New Case	2 months of isoniazid (INH), rifampicin (RIF), and pyrazinamide (PZA), and ethambutol (EMB) ≤ 50kg: INH (240mg), RIF (480mg), PZA (1200mg), and EMB (800mg) ≥ 50kg: INH (300mg), RIF (600mg), PZA (1500mg), and EMB (1000mg)	4 months of isoniazid and rifampicin ≤ 50kg: INH (300mg), RIF (450mg) ≥ 50kg: INH (300mg), RIF (600mg)
Retreatment	2 months of isoniazid, rifampicin, and pyrazinamide, ethambutol and streptomycin. Streptomycin removed for the third month. ≤ 50kg: INH (240mg), RIF (480mg), PZA (1200mg), EMB (800mg) and STREP (750mg) ≥ 50kg: INH (300mg), RIF (600mg), PZA (1500mg), EMB (1000mg) and STREP (1000mg)	5 months of isoniazid, rifampicin and ethambutol ≤ 50kg: INH (300mg), RIF (450mg), and EMB (800mg) ≥ 50kg: INH (300mg), RIF (600mg), and EMB (1200mg)

1.1.2.2 Resistance to treatment

M. tuberculosis is a hardy organism and possesses various intrinsic mechanisms to combat the effect of the drugs. The first is the almost impenetrable waxy outer cell wall. Porin channels allow smaller hydrophilic molecules to pass through while larger, hydrophobic compounds need to diffuse across the lipid bilayer. The reluctance of the mycobacterial cell wall to allow molecules to pass through it results in extended equilibration times following drug exposure. These times however are much shorter than the mycobacterial generation time (≈ 24 hrs) and does not solely account for the resistance to treatment (Liu *et al.*, 1999).

Molecules that are able to cross the first line of defence are then subjected to hydrolysis by β-lactamase, present in most species of mycobacteria. A third defensive mechanism exists to shield themselves from attack; a multi-drug efflux pump. The *lfi*A gene codes for the protein that catalyses the active efflux of molecules (Liu *et al.*, 1996) and demonstrates surprisingly broad specificity (Nikaido H., 1996).

In conjunction to the synergistic relationship that exists between cell-wall barrier, hydrolysis and active efflux, the organism has also evolved and developed resistance to the specific mode of action of the various frontline agents (Table 1.1).

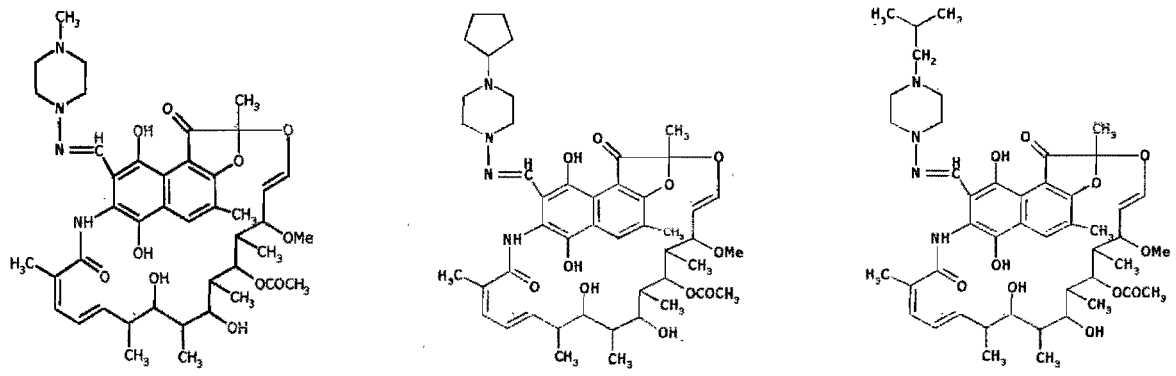
Point mutations in the *rpoB* gene encoding the β-subunit of the RNA polymerase enzyme complex accounts for 90% of mycobacteria resistant to the rifamycin family (Cole S.T., 1994). About 5% of clinically isolated rifampicin (RIF) resistant strains of M. tuberculosis do not have this mutation and an inactivation mechanism is thought to be responsible (Cole S.T., 1996). Clinically isolated INH resistant strains may either have a deletion or mutation in the *katG* gene encoding catalase peroxidase (Zhang *et al.*, 1992; Heym *et al.*, 1995). This prevents the conversion of INH to the active drug and thus renders it ineffective (Webb and Davies, 1999). Mutations on codon 306 of the *embB* genes disrupt the ability of ethambutol (EMB) to inhibit the arabinosyltransferases involved in cell-wall synthesis, although the exact mechanism is still under investigation (Sreevatsan *et al.*, 1997). Pyrazinamide (PZA), like INH, is a prodrug that must be activated to pyrazinoic acid by a bacterial pyrazinamidase. Loss of activity due to mutations in the structural gene (*pncA*) has been correlated to resistance (Sreevatsan *et al.*, 1997).

Despite the intrinsic and mutational resistance exhibited by *M. tuberculosis,* treatment with the multidrug regimen will kill the majority of the actively dividing population within the first two weeks (Waters *et al.*, 1974). The small remaining group of persistent organisms may still be sensitive to the antimycobacterial agents but survive by entering into a state of dormancy. This ability to survive in a dormant or semi-dormant state makes complete eradication of the disease extremely difficult. It is the unique ability of the rifamycin antibacterials to penetrate into macrophages and retain activity against the residing semi-dormant organisms when they undergo sporadic bursts of growth and metabolism that make them such critical components of treatment.

1.1.3 Absorption and elimination of the rifamycin antibacterials

1.1.3.1 Absorption

The bioavailability of an orally administered drug depends on its dissolution profile and the ability of the drug to cross the intestinal mucosa and be absorbed into the bloodstream. Despite small structural differences (Figure 1.3) the three rifamycins (rifampicin, rifapentine and rifabutin) have differing absorption profiles.



A) B) C)

Figure 1.3 Comparative structures of the three rifamycins: A) rifampicin, B) rifapentine, C) rifabutin

In a study by Ranaldi et al. (1992) an in vitro intestinal transport model was designed to compare the transport of drugs with varying physiochemical and pharmacokinetic properties across an intestinal epithelial cell line. In this study both rifampicin and rifapentine were successfully transported across the epithelial monolayer showing linear first-order kinetics up to 60 minutes at concentrations of 100 mg/L. Rifapentine demonstrated more efficient transport and exceeded the rate of passage of rifampicin by 3-fold (18% vs. 5% of the initial concentration in 60 minutes). Transport across the mucosal layer was by passive diffusion down a concentration gradient, which reflects the hydrophobicity of the relatively large rifamycin molecules.

In clinical studies rifampicin administered at a dose of 600 mg is the most rapidly absorbed taking between 1.5 and 2.5 hours to reach maximal concentrations (C_{max}) of 8 - 20 mg/L (Acocella G., 1978; Peloquin *et al.*, 1999). This is followed by rifabutin with time to C_{max} (T_{max}) values between 2.5 and 4 hours although the C_{max} is markedly less at 0.2 – 0.6 mg/L with the current licensed dose of 300 mg (Narang *et al.*, 1992; Skinner and Blaschke, 1995). Rifapentine dosed at 600 mg achieves similar maximal concentrations as rifampicin although the T_{max} is extended to between 5 and 6 hours (Chan *et al.*, 1994; Peloquin *et al.*, 1999).

Ingestion of food prior to drug administration has shown to impact on the absorption kinetics of the three agents (Table 1.3). Food has no significant effect on the area under the concentration curve (AUC) or the C_{max} of rifabutin although the extent to which the Tmax was delayed did reach significance (Narang *et al.*, 1992). The bioavailability, as measured by C_{max} and AUC, is reduced when rifampicin is dosed with food both in healthy volunteers (Polasa and Krishnaswamy, 1983; Peloquin *et al.*, 1999) and patients with active pulmonary disease (Siegler *et al.*, 1974; Zent and Smith, 1995).

The study by Polasa and Krishnaswamy (1983) enrolled six healthy young male volunteers. Rifampicin (10 mg/kg) was administered orally following a wheat-based breakfast and compared to the fasting state. Seven blood samples were drawn over a period of 8 hours and pharmacokinetic measures derived. C_{max} and AUC were reduced by 30% and 26% respectively while the T_{max} was doubled to 4 hours. A more recent study by Peloquin *et al.* (1999) also investigated the pharmacokinetics of rifampicin under fasting conditions and after food intake. Fourteen healthy subjects underwent extensive sampling with 18 blood samples collected up to 48 hours post-dose. The results were similar to those of Polasa and Krishnaswamy with a 36% reduction in C_{max} from 10.54 ± 3.18 mg/L to 7.27 ± 2.29 mg/L with a concomitant high-fat meal. AUC was only marginally reduced by 6%.

Administration of anti-tuberculosis medication after ingestion of a meal was proposed to overcome pyrazinamide induced nausea and to improve compliance in patients with active disease. This was found, however, to have an adverse effect on the kinetics of both rifampicin and isoniazid (Siegler *et al.*, 1974; Zent and Smith, 1995). Siegler *et al.* (1974) administered rifampicin to 17 patients and blood was collected at five time points over 12 hours. Their results showed a 25% reduction in C_{max} and a 23% reduction in AUC when rifampicin was administered together with a high-fat meal. A three way crossover study by Zent and Smith (1995) compared high-fat and high-carbohydrate meals to the fasted state. A relatively large cohort of 27 patients was enrolled and blood sample collection up to 8 hours post-dose was intensive. The results, while in agreement with the study by Polasa and Krishnaswamy (carbohydrate-rich meal), contradicts the findings of Siegler and Peloquin (both high-fat meals). The carbohydrate-rich meal produced moderate although significant reductions in C_{max} (15%) and AUC (4%). The lipid-rich meal had no significant effect on the C_{max} and actually increased the AUC by 8%.

Table 1.3 Summary of the effect of concomitant food intake on the pharmacokinetics of the rifamycins

Reference	Drug (Dose)	Meal type	Cmax	AUC	Tmax
Narang et al.	Rifabutin 300 mg	High-fat	⇩ 17%	⇩ 5%	⇧ 80%
Siegler et al.	Rifampicin 450 mg	High-fat	⇩ 25%	⇩ 23%	⇧ 100%
Polasa and Krishnaswamy	Rifampicin 10 mg/kg	High-carbohydrate	⇩ 30%	⇩ 26%	⇧ 100%
Zent and Smith	Rifampicin	High-fat	⇩ 4%	⇧ 8%	⇧ 2%
	< 50 kg 450 mg > 50 kg 600 mg	High-carbohydrate	⇩ 15%	⇩ 4%	⇧ 19%
Peloquin et al.	Rifampicin 600 mg	High-fat	⇩ 36%	⇩ 6%	⇧ 103%
Keung et al.	Rifapentine 600 mg	High-fat	⇧ 49%	⇧ 56%	⇧ 32%
Keung et al.	Rifapentine 600 mg¶	High-fat	⇧ 50%	⇧ 46%	⇧ 10%

¶ HIV positive subjects

⇧ Increase in values ⇩ Decrease in values

The bioavailability of rifapentine is positively impacted by concomitant food intake. This was first shown by a Chinese research group (Chan *et al.*, 1994) and later confirmed by an American research group that used rifapentine of Western manufacture (Keung *et al.*, 1995). The American group studied the effect of food on absorption in 20 healthy young male volunteers. C_{max} and $AUC_{0-\infty}$ increased by 49% and 56% respectively over the fasted state. A more recent study by the same group fed a high-fat breakfast to HIV seropositive volunteers and showed increases in C_{max} of 50% and $AUC_{0-\infty}$ of 46% (Keung *et al.*, 1999). Meals with varying caloric composition have been shown to have considerable impact on the absorption of rifapentine (Lepetit Research Centre, 1988; Chan *et al.*, 1994). A study in 1999 by the Division of Pharmacology, University of Cape Town on 31 uninduced healthy male volunteers (unpublished data) investigated this variable effect in more detail. The five-way single dose cross-over study produced the following results:

Table 1.4 Table comparing the difference in Cmax and AUC0-72 after administration of 900mg of rifapentine following the ingestion of various meals.

M	Cmax ± SD (mg/L)	SD (mg.hr/L)	Cmax % Increase	AUC % Increase	Significance (Paired t-test)
Fasting	16.37 ± 1.28	422.49 ± 36.44	-	-	-
English Breakfast	28.28 ± 1.17	729.75 ± 23.62	42.11	42.10	$p < 0.01$
High fluid content (soup)	23.42 ± 1.13	578.25 ± 34.08	30.10	26.94	$p < 0.01$
High bulk, high fat	22.84 ± 1.19	563.04 ± 36.14	28.33	24.96	$p < 0.01$
High bulk	19.83 ± 0.84	482.01 ± 20.64	17.45	12.35	$p < 0.2$

The meals for this crossover study were chosen based on previous research and also in recognition of the differing needs and diets of an African population. The Chinese study (Chan *et al.*, 1994) used a fast-food sandwich containing ham and eggs and showed a favourable result. This led to the inclusion of an English breakfast in one arm. The high-bulk meals were maize-based which is the staple food in a large majority of South Africans. The soup-based meal was included to emulate an outpatient clinic setting whereby a meal could easily be distributed to a large number of individuals to possibly aid absorption and improve nutritional status. The results from the study were very encouraging, particularly the soup-based meal which showed increases in C_{max} of 30% and in AUC of 27%.

1.1.3.2 Elimination

Both rifampicin and rifabutin undergo extensive hepatic and intestinal metabolism and are deacetylated to their main metabolites by β-esterases (Jamis-Dow *et al.*, 1997). The further oxidation of 25-o-desacetyl rifabutin is catalysed by CYP3A4 (Iatsimirskaia *et al.*, 1997; Trapnell *et al.*, 1997) while rifampicin is subject to nonoxidative acid-mediated degradation to formyl derivatives (Acocella G., 1978). The suggested primary metabolic pathways for rifapentine are deacetylation and non-enzymatic hydrolysis although the enzymes responsible have not yet been identified. This results in one primary enzymatic metabolite, 25-desacetyl rifapentine, and two secondary non-enzymatic metabolites, 3-formyl rifapentine and 3-formyl-desacetyl rifapentine (Reith *et al.*, 1998). The primary route of elimination for the rifamycins is via biliary excretion, although gastrointestinal secretion and renal clearance also play a role (Acocella G., 1978; Battaglia *et al.*, 1990; Reith *et al.*, 1998). The structural differences again result in widely differing elimination half-lives. Rifampicin is eliminated from the plasma the fastest with a half-life of 2 – 5 hours (Acocella G., 1978) followed by rifapentine with half-life

estimates of between 14 and 18 hours (Keung *et al.*, 1999). Rifabutin has the longest half-life with reported values of between 32 and 67 hours (Skinner *et al.*, 1989; Skinner and Blaschke, 1995).

Neither rifampicin nor rifapentine have been shown to be cytochrome P450 substrates but both are confirmed inducers of CYP3A4 and CYP2C8/9 (Acocella G., 1978; Kenny and Strates, 1981; Aventis, 2000). The induction potential of rifapentine is similar to rifampicin when both agents are dosed daily at 600 mg but is weaker when rifapentine is dosed intermittently i.e. every three days or once-weekly (Keung et al., 1999). Enzyme induction occurs within 4 days of the initial dose and returns to baseline levels 12-14 days after the last administration (Keung et al., 1999).

Repeated daily administration of rifampicin results in a substantial decrease in AUC and terminal-half-life (Acocella G., 1978) while repeated administration of rifabutin reduces AUC with no apparent change in half-life (Strolin Benedetti *et al.*, 1990). Induction of presystemic extrahepatic metabolism, which seems to be important in the availability of rifabutin, should be mainly responsible for the decrease in the AUC observed. Unlike the other two rifamycins rifapentine does not induce its own metabolism. This result was concluded in a 1999 study by Keung and colleagues who investigated the pharmacokinetics of rifapentine after daily and intermittent doses and showed that no significant differences ($p=0.307$) exist between $AUC_{0-\infty}$ for a single dose and AUC_{0-24} at steady state following eight daily doses of 600mg. These results indicate a lack of autoinduction and also point to the fact that steady exposure may be predicted from single-dose pharmacokinetics.

Rifampicin is currently the rifamycin of choice for the treatment of active pulmonary tuberculosis. As we have seen healthy volunteers produce maximal serum levels of approximately 10.5 mg/L in the fasted state (Peloquin *et al.*, 1999). The combined effect of autoinduction (Acocella G. 1978) and drug administration after food intake (Siegler *et al.*, 1974; Zent and Smith, 1995) in tuberculosis patients could result in a decrease in serum levels of close to 60% from those seen in healthy volunteers. This already places patients in the so-called suboptimal or grey-therapeutic treatment bracket of serum levels between 4 and 8 mg/L (Peloquin C.A., 1997). Various other factors have also been linked to this grey-therapeutic and also to possible subtherapeutic (< 4mg/L) serum rifampicin levels. These include malabsorption in immunocompromised hosts (Berning *et al.*, 1992; Peloquin *et al.*, 1993; Gordon *et al.*, 1993; Patel et al., 1995; Sahai et al., 1997), poor bioavailability of available formulations (McIlleron *et*

al., 2001; Van Crevel *et al.*, 2002), gastrointestinal disease and poor nutritional status (Mehta *et al.*, 2001). All factors considered it seems patients face an uphill battle in the quest to achieve levels above 8 mg/L and the need exists to define a therapeutic level and optimise therapy to achieve this.

1.1.4 Delayed response to treatment and inferior bioavailability

Kimerling *et al.* (1998) studied a group of non-HIV infected individuals that were slow to respond to drug treatment, showed early relapse, had reactivated disease, or acquired drug resistance while undergoing directly observed therapy. Once patients were identified, serum levels were drawn and assayed for levels of rifampicin at two hours. These case studies (n=22) were compared demographically to a randomly selected control group (n=43). There was no difference between the two groups with respect to extent of tuberculosis disease. For a 600mg dose of rifampicin, 14/22 patients in the case study group had levels below 8mg/L and five of those had levels below 1 mg/L. Isoniazid serum levels measured also showed that only 7/22 patients had therapeutic levels. Patient non-compliance was not responsible for these results as drug administration was fully supervised and adequate levels of pyrazinamide were present. Unfortunately serum levels were not drawn from the control group so the question remained: Were suboptimal levels only present in a small subset of patients that were slow to respond or was the problem more widespread?

Recent studies by McIlleron *et al.* (2002) and Van Crevel *et al.* (2002) in developing countries show that low plasma concentrations may not be restricted to a small subset. In the South African study (McIlleron *et al.* 2002) full pharmacokinetic profiles were determined for RIF, INH, PZA and ETB in 118 patients that had been receiving therapy for a period of two months. Patients were fasted and drugs administered under direct supervision of the investigators. The mean peak concentration for rifampicin was well below the published recommended level of 8 mg/L (Peloquin C.A., 1997) and just crept above 4 mg/L. Close to 40% (44/118) of patients had measured concentrations below 4 mg/L. Inferior bioavailability as a result of poor drug quality was seen as the biggest culprit here and the need for efficient enforcement of regulatory requirements was emphasized.

The Indonesian study (Van Crevel *et al.*, 2002) enrolled a total of 62 patients and only two hour post dose serum levels were measured. Only two patients of the cohort reached the previously defined 'therapeutic' levels (8 mg) while 43 individuals had 'subtherapeutic' levels

(<4 mg). Despite this 80.6% of the patients were cured, with three patients showing delayed response. Once again reduced bioavailability was indicated as the major point at issue. Two formulations were used in the study and while both contained similar amounts of rifampicin (93% and 100% when compared to reference) formulation B produced serum concentrations that were 2.35 times higher than formulation A. Regrettably the majority of the patients in the study received the inferior product and this further stresses the need for strict enforcement of bioavailability and bioequivalence requirements.

Individuals involved in the treatment of tuberculosis therefore need to be acutely aware that small changes in product formulation or concomitant food intake can negatively impact on rifampicin kinetics. This sensitivity to change may contribute to inter-subject variability in the absorption of rifampicin as well as the possibility of variability in the serum levels between consecutive days of treatment (Ellard and Fourie, 1999).

1.1.5 Interindividual and interoccasional variability

Considerable inter-subject and inter-occasional variability in the pharmacokinetics of rifampicin has been recognised (Ellard and Fourie, 1999). Healthy volunteer studies with rifampicin have shown inter-subject variability to be in the order of 30% for peak concentration (C_{max}) as well as area under the curve (AUC) although interoccasional variability is reported as negligible (Peloquin *et al.*, 1997; Peloquin *et al.*, 1999). In a more recent study with healthy Asian Indian volunteers (Pargal and Rani, 2001) the variability between subjects for C_{max} was 54% and AUC 62%. The studies had similar inclusion/exclusion criteria although the Peloquin studies used a dose of 600mg and the Pargal study a dose of 450mg. The disparity in results highlights the intrinsic variability of rifampicin pharmacokinetics. A pilot study conducted by the Division of Pharmacology, University of Cape Town in 1999 (unpublished data) in tuberculosis patients showed a large degree of variation in rifampicin C_{max} and AUC levels between both individuals and occasions. Variations in gastrointestinal bioavailability, concomitant medication, food intake, and the induction of hepatic enzymes and transporters could all account for the high degree of variability in this patient group.

A sub-study by the Division of Pharmacology in the same patient group as mentioned above evaluated the interoccasional variability between different dosing occasions (unpublished data). Three full pharmacokinetic profiles were collected from each patient separated by one week.

The results (Table 1.5) indicate a considerable amount of variability in rifampicin measures that were not present with isoniazid or pyrazinamide.

Table 1.5 Interoccasional variability in rifampicin pharmacokinetic measures of tuberculosis patients over a three-week period.

Patient	Cmax (mg/L)			AUC0-∞ (mg.hr/L)		
Number	Mean ± SD	%CV	Range	Mean ± SD	%CV	Range
1	4.54 ± 5.60	123.36	1.20 – 11.01	25.12 ± 29.27	116.52	5.75 – 58.80
2	6.01 ± 4.98	82.80	2.15 – 11.63	28.80 ± 11.96	11.96	26.37 – 31.24
3	1.32 ± 0.91	69.04	0.43 – 2.24	16.95 ± 20.19	119.11	2.67 – 31.23
4	5.63 ± 3.84	68.22	1.90 – 9.56	17.62 ± 12.11	68.74	6.71 – 30.65
5	12.30 ± 1.45	11.79	10.65 – 13.37	87.23 ± 30.17	34.59	66.37 – 121.83
6	1.97 ± 1.13	57.30	1.10 – 3.24	7.14 ± 2.05	28.72	5.69 – 8.59
7*	0.22 ± 0.04	18.86	0.19 – 0.25	2.10 ± 1.27	60.61	1.20 – 3.00
8	6.47 ± 3.84	59.33	2.04 – 8.85	25.25 ± 17.11	67.79	6.50 – 29.20
9	3.11 ± 3.17	101.85	1.25 – 6.77	11.96 ± 13.87	115.95	3.59 – 27.96
10	11.02 ± 2.57	23.31	8.59 – 13.71	52.74 ± 36.43	36.43	33.58 – 72.00
11	8.44 ± 1.74	20.65	6.67 – 10.16	65.50 ± 34.91	53.30	30.66 – 100.49
12	4.19 ± 1.91	45.64	2.14 – 5.93	16.90 ± 8.18	48.42	7.92 – 23.93

* Only sampled on two occasions SD – standard deviation CV – coefficient of variation

The degree of variability, both intersubject and interoccasional, is of importance because with increased variability the efficacy and safety of the drug may become compromised and subtherapeutic levels may become a likely scenario. This could lead to delayed or incomplete responses to treatment and increase the risk of drug resistance.

1.1.6 Rifapentine-based treatment as an alternative to rifampicin

The current rifampicin-based treatment regimen is demanding and complex. Asking a patient to ingest upwards of six tablets everyday for a minimum of six months is stretching the limit of tolerance and will invariably lead to a proportion of patients defaulting, especially after a significant improvement in overall health is observed during the first eight weeks. These patients may not be cured and could well continue to spread the disease as well as deadly drug-resistant strains.

Details regarding the compliance of South African patients adhering to a full six months of therapy are scant, although the problem of non-adherence is widely recognised. Solutions to the issue of non-compliance have been sought and the effectiveness of a modified DOTS programme was investigated (Wilkinson D., 1994). Adult patients received isoniazid 900mg, rifampicin 600mg, pyrazinamide 3g, and ethambutol 2g, twice weekly. Supervision sites were identified, starting with clinics, community health care workers, schools, and extending to stores and even tea rooms. Emphasis was placed on patient convenience and each patient was assigned a supervisor of their choice. Monthly visits by a TB health worker to the supervisor ensured compliance. Of the cohort of 814, 83% completed treatment, 7% died, and the remaining individuals were lost to the programme. This is an improvement in compliance of 65%, as retrospective analysis showed that prior to the study only 18% had definitely completed treatment.

This was a well structured programme that maximised available community resources to ensure full supervision of treatment. The cohort however was relatively small (814) and the up-scaling of this programme from a provincial to national level would no doubt face logistical problems. Nevertheless valuable conclusions can be made. The DOTS strategy has been labelled authoritarian and alienating (Dick and Pekeur, 1995), but by including members of the community, motivating staff, and by putting patient's convenience first a more favourable environment was created. The simplified intermittent treatment strategy also played a key role in the success and therein lays the challenge; developing curative regimens that require patients to take drugs less frequently or for a shorter period of time (Chan and Iseman, 2002).

In recognition of this the Global Alliance for TB Drug Development was established in 1998. The aim is to accelerate the discovery and development of faster-acting and affordable drugs to fight tuberculosis. The current strategy is to register a new compound by 2010 that will:

- Shorten the duration of treatment or simplify its completion
- Be effective against multi-drug resistant TB
- Improve the treatment of latent TB infection

Advantages of such an agent may be greater efficacy, improved patient compliance, less resistance, and decreased healthcare costs (Temple and Nahata, 1999). Until such an agent is freely available, though, it is necessary to use the available means at our disposal. Treatment

regimens based on rifapentine, with its extended half-life, will not shorten the treatment period but will offer the possibility of less frequent dosing and therefore a less demanding dosing schedule. This could improve adherence to therapy and could also potentially make direct observation more efficient and cost-effective.

Further advantages of rifapentine lie in the positive impact on absorption with concomitant food intake. Maintaining adherence to the current therapy on an empty stomach is difficult, particularly when one of the most frequent adverse events is pyrazinamide induced nausea and vomiting. The proposed idea of ingesting a meal prior to drug administration did not have the desired outcomes (Zent and Smith, 1995) with significantly reduced levels of rifampicin and isoniazid. This problem can be overcome with rifapentine as the prior ingestion of a meal promotes absorption and boosts serum levels by up to 30% with a soup-based meal. While food intake could limit nausea and aid absorption of rifapentine it still creates unfavourable absorption conditions for isoniazid. However, the time over critical concentration rather than Cmax is the critical pharmacodynamic parameter for isoniazid treatment and as long as this is still achieved giving doses of rifapentine and isoniazid with food is not unreasonable (Burman *et al.*, 2001).

Rifapentine with its less demanding dosing schedule, lack of autoinduction and increased absorption following food intake could make it an excellent candidate to anchor an intermittent chemotherapy regimen.

1.2 RIFAPENTINE

1.2.1 in Vitro Activity

Rifapentine is a semisynthetic rifamycin derivative that differs from rifampicin by the substitution of a methyl group with a cyclopentyl ring. The chemical formula of rifapentine (Figure 1.3b) is rifamycin, 3-[[(4-cyclopentyl-1-piper-azinyl) imino] methyl] and its molecular formula $C_{47}H_{64}N_4O_{12}$ (Aventis Pharmaceuticals, 2000).

To be effective, antimicrobial agents need to reach and be active within the site of infection. Both rifapentine and its primary metabolite, which is active against *M. tuberculosis*, localize within monocyte-derived macrophages (Easmon and Crane, 1984; Mor *et al.*, 1995) thus promoting intracellular inhibition of the organism. This results in an enhanced kill rate compared to either the parent or metabolite acting alone. Rifapentine uptake into neutrophils is rapid and intracellular/extracellular ratios are 5-fold higher than rifampicin at physiological pH and temperature (Easmon and Crane, 1984; Mor *et al.*, 1995; Pascual *et al.*, 1987). The intracellular accumulation for each agent was independent of drug concentration with the intracellular/extracellular ratio reaching 24.0 ± 3.9 for rifapentine and 4.4 ± 1.9 for rifampicin (Mor *et al.*, 1995). The postulated mechanism of uptake is by simple diffusion across the cell wall due to good lipid solubility. Neither oxidative nor glycolytic metabolism is required to provide energy for uptake (Easmon and Crane, 1984).

Various studies have compared the minimal inhibitory concentrations (MIC), minimum bactericidal concentrations (MBC) (Table 1.6), and pulsed drug exposures of rifampicin and rifapentine on different resistant and susceptible clinical strains of *M. tuberculosis*. Dickinson and Mitchison (1987) demonstrated that the MIC of rifapentine was 2-3 times lower than rifampicin in sensitive isolates, although rifampicin showed greater bactericidal activity at concentrations of 0.5 and 1.0 mg/L. Log phase cultures were exposed to the two agents for periods of 6, 24 and 96 hours. Each exposure was followed by a period of slow growth, lasting 2-3 days, after which the growth rate would gradually return to normal. Rifapentine and rifampicin produced identical effects with respect to pulsed drug exposure. Resistance developed in 5 of 12 strains after 10 days of exposure. A separate study examining the MICs of rifampicin and rifapentine for a collection of 24 rifampicin-resistant clinical isolates showed

them to be virtually indistinguishable. All mutants resistant to rifampicin were also resistant to rifapentine (Moghazeh *et al.*, 1996).

Table 1.6 Comparison of the inhibitory and bactericidal activities of rifapentine and rifampicin against various strains of *M. tuberculosis* both intra-and extracellularly (Mor *et al.*, 1995).

Strain	Minimal Inhibitory Concentrations (mg/L)					
	Extracellular bacteria			Intracellular bacteria		
	Rifapentine	Metabolite*	Rifampicin	Rifapentine	Metabolite*	Rifampicin
H37Rv	0.06	0.06	0.12	0.015	0.03	0.25
Erdman	0.06	0.06	0.12	0.06	0.12	0.12
Atencio	0.06	0.12	0.25	0.03	0.06	0.25
Strain	**Minimal Bactericidal Concentrations (mg/L)**					
	Extracellular bacteria			Intracellular bacteria		
	Rifapentine	Metabolite*	Rifampicin	Rifapentine	Metabolite*	Rifampicin
H37Rv	0.25	0.5	0.5	0.06	0.06	1.0
Erdman	0.5	1.0	0.5	0.25	0.25	0.5
Atencio	0.5	1.0	0.25	0.12	0.25	0.5

* The primary metabolite of rifapentine is 25-desacetyl rifapentine

Both rifampicin and rifapentine significantly reduced the number of colony forming units (cfu) of bacteria within the macrophage when administered in pulse doses of 600mg weekly (rifapentine) and 600mg every three days (rifampicin). After two weeks of treatment those in the rifampicin treated group had a higher number of cfus than those treated with rifapentine. These preliminary studies suggest that rifapentine possesses a greater ability to reduce the bacterial count for a longer period (Mor *et al.*, 1995; Pascual *et al.*, 1987).

1.2.2 Animal Efficacy Studies

After rifapentine penetration into macrophages was established, its role in treating and preventing tuberculosis was studied.

The three rifamycins: rifampicin (RIF), rifapentine (RPT), and rifabutin (RBT) were compared for the preventative treatment of tuberculosis in immunocompetent mice (Ji *et al.*, 1993). The mice were initially vaccinated with BCG (Bacillus Calmette Guérin) and then inoculated with the

virulent H37Rv strain of *M. tuberculosis*. After six weeks the various treatment regimens were evaluated and compared.

After a single oral dose of 10mg/kg, RPT had the highest Cmax value (15.24 ± 0.32 mg/L), highest AUC (area under the curve) value (640.6 ± 21.6 mg/L), and the longest half-life (23.35 ± 2.62 hr), almost six times longer than RIF. RPT given once weekly reduced the cfu count more significantly than the same dose of RIF given three times a week. The cfu counts in mice treated with RPT three times a week were comparable to RIF dosed 10 mg/kg six times a week. When RIF was given at the same dosage and rhythm as RPT it always resulted in significantly higher cfu counts. The results indicate that during the first six weeks of treatment, at similar doses and dosage intervals, RPT has superior bactericidal activity against M. tuberculosis in mice than RIF.

The use of rifapentine in mice for preventative therapy of *M. tuberculosis* infection was studied to assess the efficacy of rifapentine versus other non-rifamycin anti-tubercular agents in immunocompromised and normal hosts (Chapuis *et al.*, 1994). Two reference drug regimens (INH alone for a period of 26 weeks, or RIF-PZA daily for 13 weeks) were tested against two rifapentine containing regimens (13 weeks of once weekly dosing of rifapentine either alone or in combination with INH). Normal mice treated with the INH only regimen produced spleen and lung cultures negative in 7/10 mice treated. The combination of RIF and PZA improved outcomes; 9/10 mice were spleen and lung culture negative at 13 weeks. In the two rifapentine groups the addition of INH to the regimen significantly increased effectiveness. All spleen and lung cultures were negative compared with 4 spleen-culture negatives in the RPT only group. This is a notable difference and highlights the effectiveness of the combined regimen. Furthermore, no resistance was noted. Nude mice treated with INH alone responded poorly with the entire group lung-culture and spleen-culture positive at completion. A mortality rate of 100% was observed in the RIF-PZA group at 9 weeks. The nude mice did not respond well to treatment with RPT alone at extended dosing intervals and mortality was related to the length of the interval. Administration of RPT once weekly showed real bactericidal activity but was unable to render cultures completely negative. Addition of INH to the regimen once again significantly improved results and was the only regimen to produce negative cultures in nude mice. Eight of nine mice treated with RPT-INH survived until the end of follow-up (26 weeks).

The studies described above evaluate the performance of intermittent regimens. The study by Lenaerts *et al.* (1999) evaluates the efficacy of RPT in providing a sterilising cure without

relapse for three months post-dose. Daily treatment of normal mice with RPT-INH-PZA cleared the lungs and spleen of mycobacterial growth after 10 weeks, while the RIF-INH-PZA combination required an additional 14 weeks to achieve the same results. Twice weekly therapy of INH-RPT with or without PZA resulted in the same total bacterial clearance after nine weeks. After a follow-up observation period of 12 weeks both RPT and RIF based treatment groups showed relapse of infection. While initial findings were positive this study highlights the importance of long-term experiments in establishing differences between efficacies of new drug candidates.

Recently moxifloxacin (MOX) has been shown to have strong antimycobacterial properties in murine tuberculosis models (Ji *et al.* 1998; Miyazaki *et al.*, 1999) and the extended half-life (Stass and Kubitza, 1999) makes it a more suitable partner to rifapentine than isoniazid. Lounis *et al.* (2001) examined the sterilising activity of moxifloxacin both during the initial and once-weekly continuation phase of therapy by comparing it to streptomycin. The results from the study are presented below:

Table 1.7 CFU counts in mouse lungs after two weeks and six months of treatment and after three months of follow-up without treatment (Lounis *et al.*, 2001).

Treatment group[b]	**Log_{10} CFU at 2 weeks**	**% of positive cultures at**[¶]	
		6 months	**9 months**
A: 6 mnths of P alone 1/7	-	7/10*	-
B: 2 mnths of HRZ 5/7 + 4 mo of HR 5/7	5.60 ± 0.34	0/20	1/16
C: 2 wks of SHRZ 5/7 + 5.5 mo of SHP 1/7	4.94 ± 0.32**	2/16	11/18
D: 2 wks of SHRZ 5/7 + 5.5 mo of HP 1/7	4.94 ± 0.32**	0/16	10/18
E: 2 wks of MHRZ 5/7 + 5.5 mo of MHP 1/7	5.30 ± 0.45	0/19	2/13
F: 2 wks of MHRZ 5/7 + 5.5 mo of HP 1/7	5.30 ± 0.45	0/18	7/14
G: 6 mnths of SHPM 1/7	5.56 ± 0.25	1/19	7/12

b H, isoniazid; R, rifampicin; Z, pyrazinamide; P, rifapentine; S, streptomycin; M, moxifloxacin; 1/7, once weekly; 5/7, five times weekly, mnths – months, wks - weeks.

¶ A culture was considered positive if any number of colonies was detected.

* five of seven mice harboured rifampicin-resistant mutants

** significantly different from groups B, E, and F ($p<0.01$)

While streptomycin exhibited marginally better activity during the initial daily phase, the inclusion of moxifloxacin strongly increased the sterilising activity during the continuation phase with only 2/13 mice relapsing three months after treatment compared with 60% in the streptomycin group. The strongest result to emerge from this study is the comparable effectiveness at 6/9 months of the standard regimen of two months INH-RIF-PZA followed by four months of INH-RIF and the two weeks of INH-RIF-PZA-MOX followed by 5.5 months of INH-RPT-MOX. By increasing the RPT dose to 15 mg/kg and the MOX dose to 400mg/kg, the antimicrobial activity of the regimen may be further enhanced with improved longer term outcomes and as such could become a candidate regimen for evaluation in clinical trials in patients with pulmonary tuberculosis.

1.2.3 Clinical efficacy studies

Three separate studies on three different continents evaluated the efficacy of a continuation phase treatment of once-weekly therapy of rifapentine in conjunction with isoniazid.

The first study began in December 1991 in the People's Republic of China (Tam *et al.*, 1998; Tam *et al.*, 2000; Tam *et al.*, 2002). Rifapentine of Chinese manufacture was used and a total of 592 patients were included in the study. After receiving an initial two months of therapy (RIF-INH-PZA-STREP) patients were randomly assigned to one of three different regimens:

a) INH-RIF three times weekly (which served as a control)
b) INH-RPT once weekly
c) INH-RPT once weekly with every third dose omitted to simulate poor compliance

Drug dosages were 600 mg INH for patients under 43 kg, 800 mg for those between 43 and 57 kg, and 1000 mg for those over 57 kg. RIF and RPT were administered at doses of 600 mg irrespective of body weight. The bioavailability of the Chinese manufactured rifapentine was found to be inferior to the drug manufactured by Merrel Dow Lepetit in the United States, and the dose was increased to 750mg for the last 38% of patients. Monthly sputum tests were carried out for 24 months after the start of treatment and then at three and six monthly intervals until a five-year follow-up had been completed (Table 1.8).

Table 1.8 Final outcomes from Hong Kong Trial

Months from start of treatment	INH-RIF		INH-RPT		INH-RPT (2/3)*		Total Patients n (%)
	Available	Relapse n (%)	Available	Relapse n (%)	Available	Relapse n (%)	
6	172	0 (0)	179	1 (0.5)	183	0 (0)	534 (100)
12	166	5 (3.0)	169	10 (5.6)	163	17 (9.4)	498 (93.3)
18	160	6 (3.6)	163	14 (7.9)	158	19 (10.5)	481 (90.1)
30	152	7 (4.2)	151	18 (10.2)	149	20 (11.1)	452 (84.6)
42	141	7 (4.2)	145	19 (10.8)	143	21 (11.7)	429 (80.3)
60	133	7 (4.2)	140	19 (10.8)	129	21 (11.7)	402 (75.3)

* INH-RPT regimen with every third dose omitted

It is evident that the rate of relapse in the rifapentine based regimens increased substantially after 12 months and was close to three times higher than the rifampicin based regimen at 5 years. An intriguing question is why rifapentine performed worse than expected despite its promising in vitro results and satisfactory serum levels (Tam *et al.*, 1997). One possible explanation is that rifapentine is highly protein bound (Reith K *et al.*, 1998) with only a small unbound fraction circulating that is not able to satisfactorily sterilise the lesions during the continuation phase.

The final report after 5 years of follow-up (Tam *et al.*, 2002) assessed the prognostic influence of various factors. Independently significant factors were the regimens (rifampicin vs. rifapentine), gender, and the extent of radiographic disease. A positive result to emerge from this study is the small difference in relapse between the two rifapentine regimens. It would appear that while the dose may not be optimal, the once-weekly regimen is robust enough to maintain a degree of efficacy even in the presence of simulated poor compliance.

The second study was licensing study 008 conducted by Hoechst Marion Roussel in South Africa (Aventis Pharmaceuticals, 2000). Patients were randomised to receive the standard therapy of RIF-INH-PZA-ETB daily for the first 60 days followed by 120 days of RIF-INH twice weekly or, daily doses of INH-PZA-ETB for 60 days in conjunction with RPT twice weekly followed by RPT-INH once weekly for 120 days. 600 mg of RPT was used in all cases. Patients in either group were followed-up for a period of 24 months following the 180 day treatment period. Data from 570 patients are presented (Table 1.9).

Table 1.9 Clinical outcomes from Study 008

	Rifapentine Combination	**Rifampicin Combination**
Status at the end of treatment		
Converted	87% (249/286)	81% (229/284)
Not-converted	1% (4/286)	3% (8/284)
Lost to follow-up	12% (33/286)	17% (47/284)
Status at the end of follow-up		
Relapsed	10% (25/249)	5% (11/229)
Sputum negative	81% (201/249)	90% (205/229)
Lost to follow-up	9% (23/249)	6% (13/229)

The above results show a positive response to rifapentine at the end of treatment, but an unacceptable rate of relapse in the follow-up to 24 months. Most of the relapses were attributed to poor compliance with the companion drugs during the intensive phase but this did not account for all relapses. Further risk factors associated with relapse included failure to convert sputum at two months, extent of radiographic disease, and gender, with males more predisposed to relapsing than females, a result in accordance with the Hong Kong Trial. Relapse was not associated with the development of rifampicin/rifapentine resistance.

The third study was initiated by the Tuberculosis Trials Consortium and began enrolment in April 1995 from a variety of sites across North America (Vernon et al. 1999; Benator et al., 2002). A similar study design to the Hong Kong trial was employed and similar prognostic factors were assessed, although only results from HIV negative subjects are presented here. After the intensive two month treatment period 502 patients were randomised to the rifapentine arm. Patient health was assessed on a monthly basis during treatment and three monthly after that to complete 24 months after treatment completion. In agreement with the previous two studies relapse rates were in the order of 9% (46/502) for the rifapentine group and 6% (28/502) for rifampicin (p=0.04). Patients randomised to receive rifapentine were more likely to have a positive sputum smear or culture and a greater extent of disease at the end of the intensive phase. These factors were identified as being associated with relapse and if patients with pulmonary cavitation are excluded from the analysis the relapse rates between the two groups becomes non-significant (p=0.81). Of great concern was the emergence of rifamycin monoresistance in 4/30 HIV positive patients who were initially randomised to receive rifapentine. This led to the decision not to include any further HIV positive subjects. The

shorter half-life of isoniazid essentially results in rifapentine monotherapy for six of seven days and this could be a major contributing factor. Nonetheless this extensive study proved that the once-weekly regimen of INH-RPT in the continuation phase is effective in HIV negative patients that do not present with pulmonary cavitation or positive culture at two months.

These clinical studies focus on the effectiveness of rifapentine as part of an appropriate drug regimen for the treatment of tuberculosis disease but did not investigate the pharmacokinetic characteristics. These pharmacokinetic characteristics are important to try and understand if the difference in treatment outcomes become the rifapentine and rifampicin based treatment is related solely to serum levels or if other factors that influence exposure are at play (e.g. increased pharmacokinetic variability). As a result there is a paucity of published data regarding the plasma levels of rifapentine as well as the levels of variability in this target population.

1.2.4 Classical versus the population pharmacokinetic approach

The traditional method of analysing pharmacokinetic data uses the two-stage approach where frequent sampling in a small, homogenous group of individuals produces a rich data set (Aarons L., 1992; FDA, 1999). The first stage involves calculation of individual pharmacokinetic parameters by nonlinear regression of concentration time-data followed by the calculation of descriptive statistics, typically means and coefficients of variation, during stage two. Incorporation of relationships between parameters and subgroups bound by a common covariate can also be included during the second stage (FDA, 1999). This method has been employed in all cases for the analysis of data from healthy volunteer studies with rifapentine. The major drawback is that study conditions are often artificial and subjects studied are rigidly standardised and homogenous and are not representative of the target population.

Population PK offers a more robust and efficient technique because all individuals in the experimental data set are analysed simultaneously (Sheiner and Ludden, 1992), and more complex models can be applied because information is shared amongst individuals. A further advantage is the application of the same model to all individuals which enables parameter estimates and profiles to be calculated in those individuals for which only sparse data is available (Friberg L., 2003).

The population modelling approach involves the application of nonlinear mixed effect models to repeated measurements data from a group of people to quantify the variability in drug absorption, distribution, metabolism and excretion. It further aims to describe the variability in terms of genetic, environmental and pathophysiological patient factors (Steimer J.-L., 1992; Pérez-Urizar *et al.*, 2000). These population models can allow better understanding of the underlying physiology and can enhance the search for the optimal dose, serum drug level, or observed effect (Friberg L., 2003).

One of the main aims however of population PK analysis is the quantification and description of the relationship between parameters defined by the model and patient-specific characteristics (covariates). The parameter-covariate relationship can explain, to a degree, the variability of an individual from the population mean, but more importantly can identify subgroups that are at risk of subtherapeutic or toxic serum drug levels. This subgroup may then in turn benefit from therapeutic drug monitoring (TDM) and consequent dosage adjustment to provide optimal therapy with minimum toxicity.

1.3 MOTIVATION FOR STUDY

1.3.1 Brief summary

The benefit of full supervision to aid adherence to treatment for tuberculosis was recognised as early as 1958 (Fox W., 1958). Full supervision, or more recently directly observed treatment, could be more efficient if drug dosing was intermittent (Mitchison D.A., 1998). Earlier studies combining INH and RIF in an intermittent regimen showed positive results with an increased dose of RIF (900 mg) (Singapore Tuberculosis Service/British Medical Research Council, 1975). The down side was the emergence of an immunological type of toxicity or flu-like syndrome which was related to RIF. In view of this further development of rifapentine was discussed and in 1986 the go ahead was given for phase II/III trials, nearly ten years after the original patents were taken (Mitchison D.A., 1998). At this stage the optimal dose of rifapentine was unknown but the view was that a 600 mg dose would provide a 100-fold margin between the MIC of M. tuberculosis (0.2 mg/L) and blood concentrations. Merrel Dow Lepetit were having difficulties in producing a formulation with reproducible bioavailability and a decision was made in 1990 to stop production. For that reason the first trials investigating the efficacy of once-weekly regimens of INH and RPT in the continuation phase were conducted in Hong Kong

using rifapentine of Chinese manufacture (Mitchison D.A., 1998). As we have seen the bioavailability of that formulation was also not reproducible (Tam *et al.*, 1997). The dose was increased from 600 mg to 750 mg to overcome this but only for the last 38% of patients enrolled. The inferior preparation combined with a possible sub-effective dose could have accounted for the poor performance of rifapentine (Mitchison D.A., 1998). No doubt the high protein-binding properties of rifapentine also played a role with only 2% of drug being unbound (Reith *et al.*, 1998) and able to circulate and sterilise within the lesions (Tam *et al.*, 1998). So to date the results of clinical studies using rifapentine in an intermittent regimen have not been as promising as expected. Despite this though the advantages that rifapentine holds over rifampicin with regard to less frequent dosing is clear to see. Secondly the increased absorption after a meal may help to overcome nausea commonly associated with concomitant intake of pyrazinamide.

Furthermore rifampicin has been shown to have significant variability between individuals and also possibly between occasions. The studies thus far on patients receiving proven bioavailable preparations of rifapentine do not comment on the respective plasma levels but have concentrated on the specific outcomes. As a result little is known as to the magnitude of variability in plasma levels amongst patients receiving rifapentine. This degree of variability may prove to be important as rifapentine continues to be investigated as an alternative to rifampicin.

1.3.2 Aim

To describe the pharmacokinetics of rifapentine in a South African pulmonary tuberculosis patient population with special reference to variability in serum drug levels between patients and between occasions.

1.3.3 Research Questions

1. What are the peak serum drug levels of rifapentine in a South African tuberculosis patient population?
2. What is the degree of variability in serum drug levels between individuals and between occasions?

3. How does this compare to the degree of variability observed in a healthy volunteer patient population?
4. What are the factors that contribute to the variability?

1.3.4 Objectives

1. Obtain full pharmacokinetic profiles of patients receiving rifapentine on two occasions
2. Develop and validate a high pressure liquid chromatography assay for the estimation of plasma concentrations of rifapentine and 25-desacetyl rifapentine
3. Describe plasma levels of rifapentine and the desacetyl metabolite using traditional pharmacokinetic analysis
4. Develop a multi-compartmental model to describe the pharmacokinetics of rifapentine
5. Develop a multi-compartmental model to describe the pharmacokinetics of 25-desacetyl rifapentine
6. Characterise the degree of interindividual variability in rifapentine and 25-desacetyl rifapentine serum levels present in a patient population
7. Compare the degree of variability in patient levels to the variability observed in healthy volunteers for both the parent drug
8. Identify potential factors influencing variation and quantify and describe their role

Chapter 2

Rifapentine High Pressure Liquid Chromatography Assay Development and Validation

2.1 INTRODUCTION

Earlier pharmacokinetic studies of rifapentine made use of microbiological assay techniques to measure the concentrations of rifapentine in biological samples (Birmingham et al., 1978; Arioli et al., 1981). These microbiological assays used Petri dishes filled with antibiotic medium inoculated with 0.5% *Sarcina lutea* ATCC9341. Three standard concentration solutions (0.1, 0.2, 0.4 mg/L) were placed in wells made in the dishes and incubated overnight at 33°C. The diameter of the inhibition zones from the well were then measured to the nearest 0.1 mm and plotted against the known concentrations. Rifapentine content was measured by linear regression analysis of the standard curve (Riva et al., 1991). A similar microbiological method is described by Kenny et al. (1997) although *Bacillus subtilis* was used as the test organism. These methods for measuring rifapentine concentrations while accurate and reproducible, are labour intensive and time consuming. A further disadvantage is the inability of the assay to differentiate between the parent compound and active metabolite. The demand for the establishment of a simple, sensitive and efficient assay grew with the increasing number of pharmacokinetic studies involving rifapentine. As a result five different high-pressure liquid chromatography (HPLC) assays have been published since 1990. The extraction procedures and chromatographic conditions are summarised Table 2.1

Table 2.1 Published methods for the quantification of rifapentine in biological samples

Reference	RPT¶	DRPT‡	Extraction procedure			Chromatography	
			Method	Solvent	Time§	Mobile Phase	Run time
Riva *et al.*	✓	✓	Filtration: Direct Injection	-	-	MES*-acetonitrile (pH 6.5): MES-tetrahydrofuran	34 min
Lee *et al.*	✓	-	Direct Injection	-	-	Acetonitrile-tetrahydrofuran-0.05 M KH2PO4 (pH 7.0)	25 min
He *et al.*	✓	-	Liquid-liquid	Ethyl acetate	115 min	0.01 M NaH2PO4 (pH 5.5)-methanol	8 min
Reith et *al.*	✓	✓	Liquid-liquid	Methanol	20 min	Methanol-acetonitrile-water	5 min
Panchagnula *et al.*	✓	-	Liquid-liquid	Methanol	75 min	0.01 M NaH2PO4 (pH 5.2)-methanol	17 min

¶ RPT – rifapentine, ‡ dRPT – 25-desacetyl rifapentine, § time to process 10 samples, * MES – morpholinoethanesulphonic acid

The HPLC assay methods described by Riva et al. (1991) and Lee et al. (1992) share the common advantage of no extraction procedure. The former only incorporates the filtration of the plasma sample through a 0.45 µm acetate membrane. This filtration step may become tedious if large numbers of samples with small volumes are to be assayed. The latter method requires a three-step on-line sample clean-up using a column-switching technique that may run up to 25 minutes per sample. The first step (0-5 min) requires a dilute serum sample to be injected onto a C18 precolumn that retains the drug while polar serum components are washed to waste. Step two (6-9 min) elutes the retained components from the precolumn through the C8 guard column. Eluted drugs are then separated on the C18 analytical column during step three (10-25 min). Although both these methods save on labour intensity by not requiring extraction procedures, the extended run times limit the throughput to a maximum of two samples per hour which is not ideal. The chromatographic section of the assay method employed by He and colleagues (1996) utilises a simple isocratic mobile phase that elutes and separates rifapentine from the desacetyl metabolite in under 8 minutes. This however is negated by the lengthy and complex extraction procedure. Eight steps, including a 5 minute vortex-mix per sample, adds up to a total of approximately 115 minutes per batch of 10 samples. Substituting the vortex mixing for shaking could dramatically reduce the extraction time allowing multiple samples to be mixed over a 5 minute period although this possibility was not addressed by the authors. Panchagnula et al. (1999) used rifapentine as an internal standard when developing a HPLC assay for the structural analogue rifampicin. The method required a methanol liquid-liquid extraction with the drying down of the supernatant following centrifugation. The dried sample was reconstituted in mobile phase and injected onto the analytical column. A similar method developed and validated by Hoechst Marion Roussel, Inc. (Kansas City, Mo.) was first employed by Reith et al. (1998) and then later by Keung et al. (1999) and Conte et al. (2000). Plasma proteins were precipitated by the addition of methanol directly into the autosampler vial. After samples were mixed and centrifuged the supernatant was inject directly onto the analytical column by programming the depth of the auto-injection needle to avoid the protein pellet. Because of the short run time and the ability to process large numbers of samples in a short period of time attempts were made to reproduce this method. Unfortunately efforts were hampered by the interference of serum components and could not be overcome. As a result a new assay for the quantification of rifapentine and its desacetyl metabolite was developed and validated.

2.2 MATERIALS AND EXPERIMENTAL METHODS

2.2.1 Reagents

Rifapentine (RPT) and 25-desacetyl rifapentine (DRPT) were obtained courtesy of Aventis Pharmaceuticals Inc. (Bridgewater, NJ, USA) and were stored at -4°C under desiccation. Methanol (Scharlau, Barcelona, Spain) and acetonitrile (Burdick and Jackson, MI, USA) were HPLC grade. All water used was prepared using Millipore ion exchange and filtration apparatus (Millipore, MA, USA). Trifluoroacetic acid (TFA) was obtained from Riedel-de Häen, South Africa (SA). Chromic acid was made by dissolving 25 g of potassium dichromate (Merck, SA) in 500ml of concentrated sulphuric acid (Merck, SA) and was used to rid glassware surfaces of any protein contaminants prior to use.

2.2.2 Analytical instruments

The development, validation and analyses were performed using a Spectra Physics HPLC system consisting of two high-pressure solvent delivery pumps (SP8800 Ternary), a UV-Vis detector (SP8450), an autosampler (SP8450) and an integrator (SP4290). The analytical column used was a reversed phase Supelco Discovery C8 silica based column (15 cm x 4.6 cm, 5 µm) preceded by a guard column packed with pellicular Perisorb C8 material (30 – 40 µ). Solid- phase extraction cartridges (Oasis Bond Elut 3ml, 500mg) were purchased from Anatech (SA). Other instruments used included a Heraeus Centrivac and Biofuge-13, a Sartorius analytical scale and Eppendorf pipettes.

2.2.3 Mobile phase

The final optimised mobile phase composition was acetonitrile – TFA (0.1% solution) (50:50, v/v). Elution was performed at room temperature at a flow-rate of 2 ml/min and resulted in well resolved peaks for parent drug and metabolite. The TFA solution was filtered and degassed by passing it through Millipore 0.45 µm HAWP filters (Millipore, MA, USA). Mobile phase solutions were further degassed by continually bubbling helium through the solutions during chromatography. Peaks for RPT and DRPT were detected and resolved by UV absorption at 270 nm and an integrator attenuation of 4 over a 5 minute run time.

2.3.3 Calibration and quality control sample preparation

Standard solutions of RPT and DRPT were prepared by accurately weighing 5 mg of each and dissolving separately in 5 ml of methanol. These solutions were then further diluted with distilled deionised water to give working solutions of 1000, 100, and 10 mg/L. Calibration standard samples were prepared by spiking 15 ml blank plasma pools with the appropriate amounts RPT and DRPT to yield seven-point standard curves in the concentration range 0.6 – 30 mg/L for RPT and 0.25 – 20 mg/L for DRPT. Calibration concentration ranges for the parent drug and metabolite were selected on the basis of their expected concentration levels over the study period. Volumes of 50 ml of blank plasma were spiked with appropriate amounts of RPT and DRPT to obtain quality control samples containing 25, 10, and 1.5 mg/L for RPT and 17, 7.5, and 1 mg/L for DRPT. Calibration and quality control samples were divided into aliquots of 600 µl into tightly capped vials and frozen at -80°C to be thawed as needed at room temperature. Control blank serum samples were also spiked with concentrations of isoniazid, pyrazinamide, ethambutol, rifampicin, para-amino salicylic acid, ethionamide, kanamycin, amikacin, thiocetazone, caffeine, paracetamol, pseudoephedrine and ibuprofen to check for interference. All stock solutions underwent one freeze-thaw cycle before analysis.

2.3.4 Sample handling and preparation of patient plasma samples

Patient's blood samples were collected into plastic tubes containing lithium-heparin and centrifuged at 3000 rpm for 10 minute and the plasma separated. The plasma was transferred to 2.5 ml polypropylene vials and kept frozen at -80°C until analysis.

2.3.5 Solid-phase extraction procedure

To determine the optimal plasma volume needed for extraction, different volumes of plasma (100-500 µl) were tested and 500 µl was found to yield the best extraction efficiency for the concentration ranges used. Samples were prepared as detailed above and extracted using Oasis Bond Elut C18 cartridges (3ml, 500mg) with an Analytichem extraction vacuum manifold as follows:

1. Pre-wash: 3 ml of methanol preceded and followed by 3 ml of distilled deionised water.

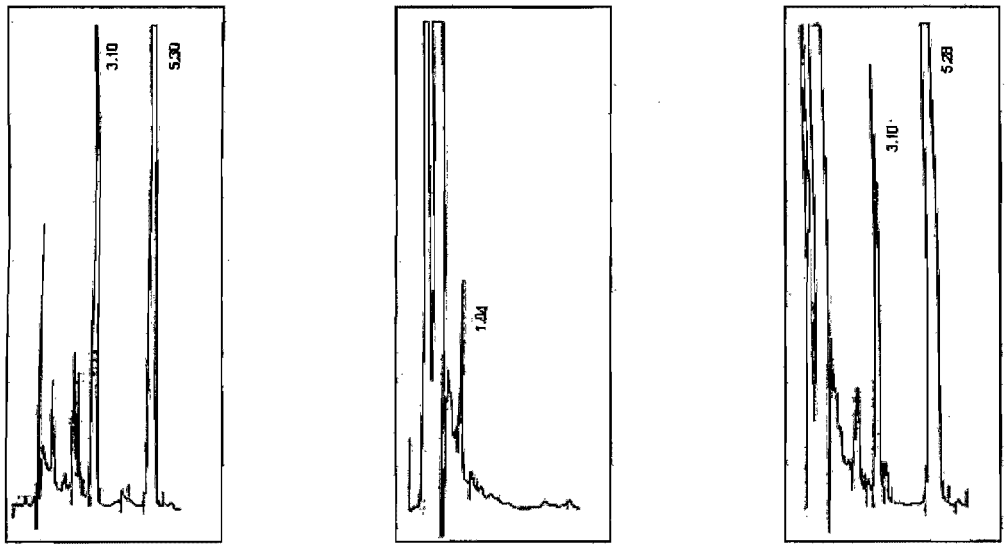
2. Load: 500 µl plasma sample. Allow to stand and bind to the cartridge matrix for 10 minutes.
3. Rinse: 6 ml distilled deionised water pulled through to waste.
4. Elute: load the following volume of solvents and allow to stand for two minutes before pulling through into autosampler vial:
   a) 200 µl methanol
   b) 200 µl methanol
   c) 100 µl methanol
   d) 200 µ acetonitrile.
5. Injection: briefly vortex eluent and inject 60 µl into the chromatographic system for quantification.

2.3.6 Assay validation

Two standard curves were run on each of the three validation days. The slope, intercept and correlation coefficient were determined by linear regression (Prism 4, GraphPad Software, USA) of the area under the peak against concentration. Five replicates of each of the high, medium and low quality control samples were analysed on each of the validation days (n=5 at each concentration) to determine the inter- and intraday precision as well as the accuracy. The concentrations used for quality control analysis of RPT were 25 mg/L (high), 10 mg/L (medium) and 1.5 mg/L (low) and for DRPT 17 mg/L (high), 7.5 mg/L (medium) and 1.0 mg/L (low). Precision was expressed as the coefficient of variation (%CV). Accuracy was calculated by transforming the observed areas under the peak for the quality control samples according to the linear regression equation; $y = mx + c$ where m is the slope of the regression line and c is the intercept value. This resulted in predicted concentrations for the high, medium and low quality control samples from the standard curve. These predicted values where in turn divided by the true concentration values and expressed as a percentage nominal concentration. It is recommended that the precision and accuracy determined at each concentration level should not exceed 15% (Bioanalytical Method Validation, FDA, 2001). The extraction efficiency was calculated by dividing the area under the peak of the extracted analyte by the area under the peak for the unextracted standard over the concentration range. Means and standard variations of the quality control samples were compared on days 1, 2, 5, 10, 15, 20, and 25 to check stability of the analytes over the 28-day analysis period.

2.3 RESULTS AND DISCUSSION

Figure 2.1a shows that the parent drug (RPT) is clearly separated from the desacetyl metabolite (DRPT) with retention times in the region of 5.30 min and 3.10 min respectively. The peaks obtained were homogenous, well separated and sharp. Figure 2.1b shows the extracted blank plasma used for the preparation of calibration and quality control samples. The chromatogram shows no interferences at the retention times of RPT and DRPT with the majority of plasma artefacts eluting out during the first 1.8 minutes. None of the additional agents tested for interference with the peaks of interest were detected with this assay method. Figure 2.1c shows the extracted plasma of a patient 4 hours after receiving doses of rifapentine (900 mg), isoniazid (300 mg), pyrazinamide (1500 mg) and ethambutol (1000 mg).



a) b) c)

Figure 2.1 a) Unextracted standard in mobile phase b) Extracted blank plasma sample c) Extracted patient sample 4 hours post-dose

The extracted plasma calibration curve was linear across the concentration range for RPT (0.6 – 30 mg/L) and DRPT (0.25 – 20 mg/L) (Fig 2.2). Correlation coefficients were 0.9894 (n=6) for the parent drug and 0.9692 (n=6) for the metabolite. The limit of quantitation was 0.6 mg/L for rifapentine and 0.25 mg/L for 25-desacetyl rifapentine.

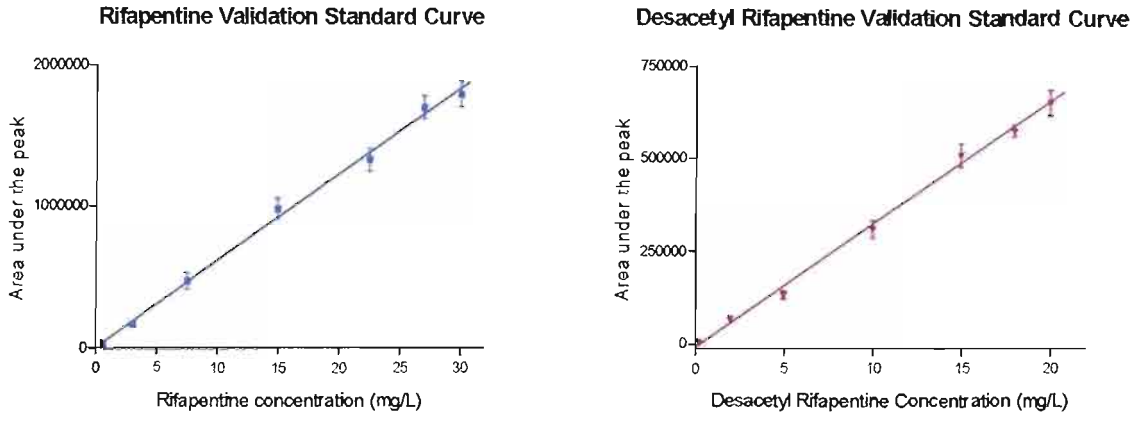


Figure 2.2 Linear regression curves for rifapentine and 25-desacetyl rifapentine from extracted plasma

The values obtained during validation for precision and accuracy are summarised in Table 2.2 and graphically presented in Fig 2.3 – 2.4. All values for precision were within the recommended limits and all those for accuracy except the medium quality control sample for the metabolite. The predicted value from the area under the peak was only 50% of the true value and this discrepancy was thought to be due to an error during sample preparation. Both the parent and metabolite concentrations are determined simultaneously. Since the precision of the desacetyl metabolite control sample was good and the accuracy of the parent drug (in the same sample) was within the recommended limits, it was decided not to revalidate the assay. The medium quality control sample for rifapentine would therefore serve as control for both the parent and metabolite.

Table 2.2 Inter- and intraday precision and accuracy for rifapentine and desacetyl-rifapentine

Concentration (mg/L)	Rifapentine		
	Precision		Accuracy
	Mean ± SD	%CV	MRE*
Intraday			
1.5	1.52 ± 0.07	4.43	-7.3
10	9.70 ± 0.30	3.09	-4.2
25	27.25 ± 0.77	2.84	7.2
Interday			
1.5	1.37 ± 0.04	2.92	-12.8
10	9.98 ± 0.81	8.11	-5.3
25	28.8 ± 1.80	6.25	13.4

Concentration (mg/L)	25-Desacetyl rifapentine		
	Precision		Accuracy
	Mean ± SD	%CV	MRE*
Intraday			
1.0	0.98 ± 0.05	5.27	-6.0
7.5	4.58 ± 0.30	6.51	-41.6
17	16.56 ± 0.74	4.46	12.0
Interday			
1.0	1.05 ± 0.09	8.77	-9.3
7.5	3.77 ± 0.36	9.69	-52.0
17	18.59 ± 1.18	6.34	-8.7

* Mean relative errors – percentage difference between the true and predicted concentration value

%CV – coefficient of variation

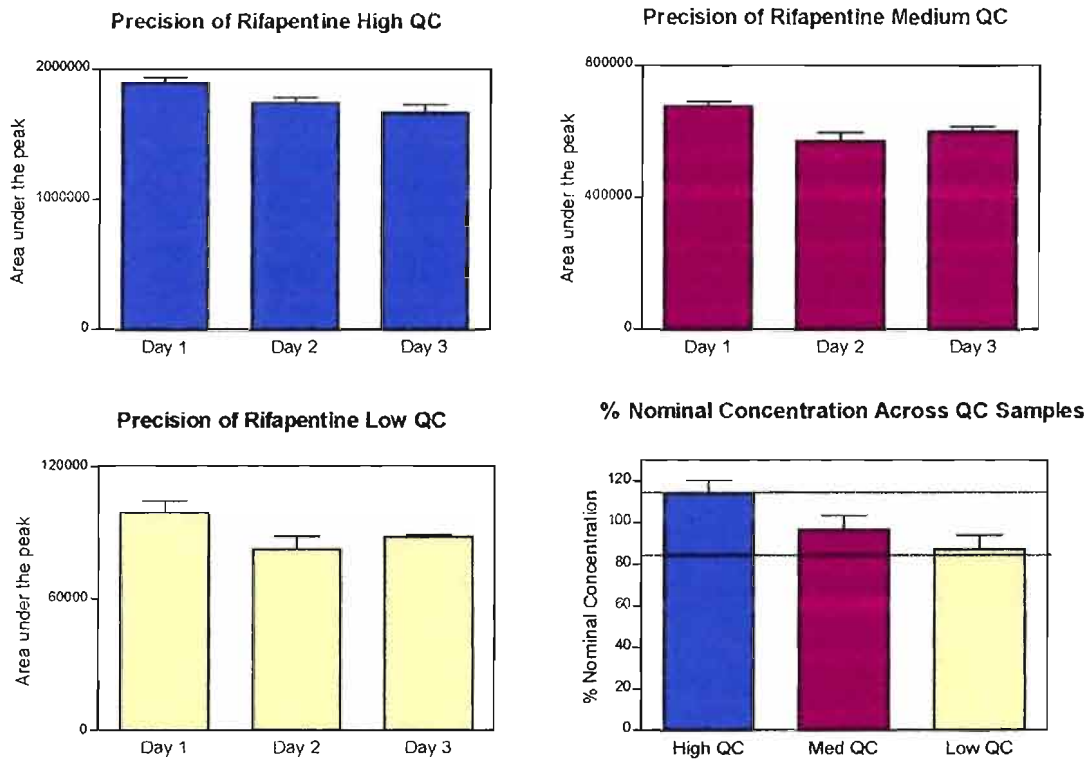


Figure 2.3 Graphical presentations of precision and accuracy for rifapentine over the 3-day validation. Dotted line represents ±15% recommended limits.

*QC – quality control

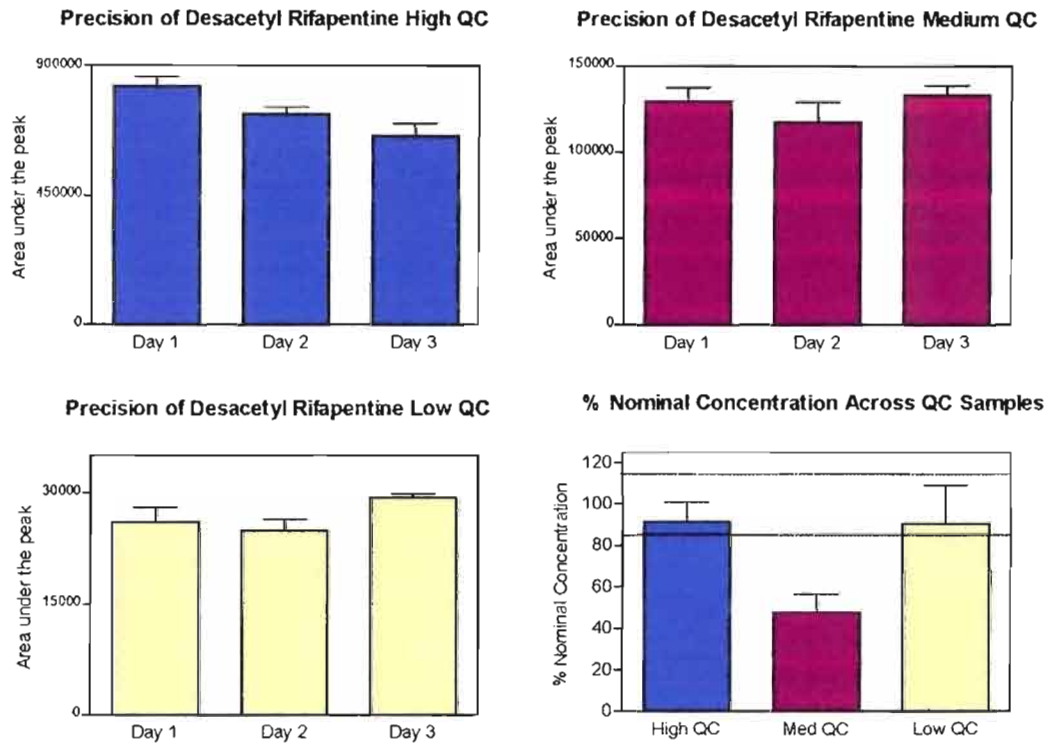


Figure 2.4 Graphical presentations of precision and accuracy for 25-desacetyl rifapentine over the 3-day validation. Dotted line represents ±15% recommended limits.

Recovery of rifapentine ranged between 94.4% and 102.1% and for 25-desacetyl rifapentine between 83.9% and 93.4%. No significant change was seen in the mean area under the peak values of the quality control samples over the 28 day sample analysis period confirming stability of the analytes at -80°C.

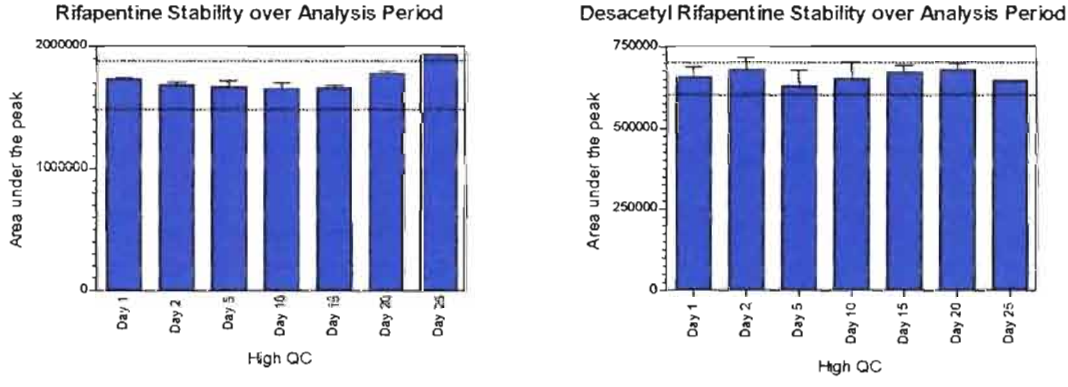


Figure 2.5 Stability of quality control sample (high) over the sample analysis period. Dotted line indicates ±10% of the mean area under the peak value.

2.4 CONCLUSIONS

Of the number of chromatographic methods reported for the estimation of rifapentine and 25-desacetyl rifapentine in plasma all, apart from the method developed by Hoechst Marion Roussel, suffer from limitations such as low throughput, lengthy and complex extraction procedures, or complex mobile phase compositions. The main advantages of the assay we have developed and validated are the simplicity (5 step extraction), efficiency (5 minute run time), sensitivity (0.6 mg/L for RPT and 0.25 mg/L for DRPT) and reproducibility (< 15% CV). The method is suitable for the quantification of parent and metabolite in plasma with concomitant intake of other frontline antimycobacterial agents (INH, PZA and ETH). Further advantages of this method include a simple mobile phase composition, single wavelength detection (270 nm), and fast extraction procedure (20 minutes per 10 samples). This combined with the short run time allows for a cost-effective high-throughput analysis (>10 samples/hour) which was of particular benefit to this pharmacokinetic study which required extensive blood sampling in a large number of patients.

3.5 METHOD SUMMARY

Method name	Rifapentine and 25-desacetyl rifapentine in human plasma
Column	Supelco Discovery C8 silica based column (15 cm x 4.6 cm, 5 µm)
Column Temperature	Room
Mobile Phase	Acetonitrile – TFA (0.1% solution) (50:50 v/v)
Injection volume	60 µl (200 µl partial loop)
Flow Rate	2 ml/min
Detection wavelength	270 nm
AUFS (sensitivity)	0.02
Attenuation	4

Reference standard	Rifapentine	25-desacetyl rifapentine
Reference standard supplier	Aventis Pharmaceuticals Inc.	Aventis Pharmaceuticals Inc.
Reference Standard Product Nr.	MDL 473-069	MDL 27-718
Storage Location	-20°C dessicator	-20°C dessicator
Typical retention time	5.30 min	3.20 min
Concentration curve range	0.6 mg/L – 30 mg/L	0.25 mg/L – 20 mg/L
Limit of quantisation	0.6 mg/L	0.25 mg/L
Quality Control Concentrations:		
High	25 mg/L	17 mg/L
Med	10 mg/L	7.5 mg/L
Low	1.5 mg/L	1.0 mg/L
Extraction Method type	Solid phase extraction	
Volume of sample applied	500 µl	
Dilution factor	1.4x (elute with 700 µl solvent)	

System suitability: Test and FDA Recommended Limits			
ANALYTE	**Rifapentine**	**Desacetyl-R**	**FDA Limits**
Capacity Factor	7.67	5.71	>2
Selectivity	0.56		-
Resolution	5.71		> 2
Theoretical Plate number	2044	2844	> 2000
Tailing factor	0.96	1.0	< 2

Chapter 3

RIFAPENTINE NONCOMPARTMENTAL PHARMACOKINETICS

3.1 Introduction

Rifapentine is approved by the Food and Drug Administration for the treatment of non-HIV infected tuberculosis patients in the United States but is not yet registered for use in South Africa. In the intensive phase of treatment rifapentine has been shown to be as effective as rifampicin when measured by sputum conversion at two months (Aventis, 2000). However, when administered once a week together with isoniazid in the continuation phase of treatment an unacceptable rate of relapse is evident (Benator *et al.*, 2002; Tam *et al.*, 2002; Tam *et al.*, 2000; Vernon *et al.*, 1999; Tam *et al.*, 1998).

There is little published information available describing the pharmacokinetics of rifapentine in tuberculosis patients. As a result, little is known regarding the variability in plasma levels amongst patients receiving the drug. The magnitude of variability is of importance because with increased variability the safety of the drug may become compromised, and the likelihood of subtherapeutic drug levels is also increased. This phenomenon can be seen with rifampicin, where several studies have reported low levels in tuberculosis patients (Peloquin *et al.*, 1996; Choudhri *et al.*, 1997; Kimerling *et al.*, 1998). A recent study by McIlleron *et al* (2002) amongst tuberculosis patients demonstrated a high degree of variability existed in the pharmacokinetic measures of rifampicin, and many in the study demonstrated 'subtherapeutic' levels. Such low levels may lead to a delayed or incomplete response to treatment, and the selection for drug resistant strains.

3.2 Scope of Noncompartmental Data Analysis

This chapter aims to describe the pharmacokinetics of two consecutive doses of rifapentine equivalent to 15 mg/kg in pulmonary tuberculosis patients and investigate whether the values of the PK measures obtained are reproducible between individuals and between occasions. Descriptive statistics from patient data will also be compared to data from healthy volunteers.

3.3 Patient Study design and outline

A prospective cohort pharmacokinetic study was carried out by the Tuberculosis Unit of the Department of Pharmacology, University of Cape Town.

Our method required that subjects who had been on a multiple drug regimen including rifampicin have a single 600mg rifampicin dose replaced with a 15 mg/kg dose of rifapentine on the day of the study and have multiple venous blood samples drawn at fixed times. The patients then received their standard tuberculosis treatment minus rifampicin for the next three days. On day four they would be required to repeat the procedure and allow for sampling on a second occasion.

3.3.1 Selection of subjects

Patients, who had been receiving daily antimycobacterial treatment containing rifampicin for a period of not less than 4 weeks and not more than 6 weeks, were recruited from DP Marais Hospital, Cape Town, South Africa. This allowed for the assumption that the patients' hepatic enzymes were maximally induced and that their overall health had improved to a stable level.

A group of 46 patients were recruited over an eighteen-week period. The study protocol was discussed with them at least three weeks after they had been diagnosed with pulmonary tuberculosis and had started taking their antimycobacterial therapy.

3.3.1.1 Inclusion criteria

Patients meeting all of the following criteria were considered for admission to the study:

- Diagnosis of pulmonary tuberculosis
- Receiving antimycobacterial therapy (including rifampicin) for not less than 4 weeks and no more than 6 weeks
- Between 18 and 65 years of age
- Given written informed consent to participate in the study.

Informed consent was obtained for all subjects before enrolment in the study.

3.3.1.2 Exclusion criteria

- History of hypersensitivity to any rifamycin antibiotics
- Known resistance to rifampicin prior to study initiation
- Contraindication to multiple blood sampling, e.g. mental confusion or poor venous access
- Blood donation of more than 500ml during the previous month
- Mental capacity limited to the extent that the subject could not provide legal consent or understand information regarding the side effects or tolerance of the study drug
- Subject unlikely to comply with protocol, e.g. uncooperative attitude

No subject was allowed to enrol in the study more than once.

3.3.2 Study treatments

INN:	Rifapentine	
Trade Name:	Priftin®	
Dosage form:	150mg film coated tablet	
Dose:	28-35kg	450mg
	36-45kg	600mg
	46-55kg	750mg
	>56kg	900mg
Batch:	A0002	Exp 31/07/2002
Route:	Oral	
Duration of treatment:	Single dose	

At each of the two treatment periods each subject received a single dose of Priftin® after ingestion of a low fat, high fluid content breakfast. The breakfast was prepared by simply dissolving a brand name instant packet soup in boiling water and administering it to the patients after it was allowed to cool slightly.

3.3.3 Dosage schedule

Each subject received two treatment doses, 3 days apart. After an overnight fast, subjects ate a standardised soup-based meal on the day of drug administration. A single dose of Priftin®

was swallowed with ±100ml of water 30 minutes later under direct supervision of a study investigator.

Details of the exact dose and time of medication were documented in the case report form.

3.3.4 Study procedure and schedule

3.3.4.1 Data collection

During screening the study procedure was explained and informed consent sought. Once consent was obtained medical history was gleaned from patient folders and documented in the case record form.

3.3.4.2 Description of study days

The following tests and examinations were carried out on the first study day:

- Haematological status (haemoglobin, haematocrit, red blood cell count, white blood cell count, differential count and platelet count)
- Blood chemistry (alanine transaminase, aspartate aminotransferase, alkaline phosphatase, total bilirubin, total protein, albumin)
- HIV test

Pre-test counselling which included information regarding the HI virus/AIDS, ways of spreading, risk factors and consequences was provided verbally by a trained counsellor on the day or prior to the first sampling occasion. Patients were informed of their HIV status at the end of the second sampling occasion (study day eight), by a trained professional. For individuals testing positive, follow-up was made available.

On the morning of the first evaluation day subjects had the study schedule explained to them by the investigator. The subjects received the soup-based breakfast at ±07h30. Subjects consumed the whole breakfast and then received the test drug 30 minutes after beginning their breakfast.

Blood samples (8ml) for the determination of the plasma concentration-time profiles of rifapentine and its primary metabolite 25-desacetyl rifapentine were collected via an indwelling cannula into lithium-heparin coated tubes at the following times:

- 60 to 15 minutes prior to drug administration
- 2.0 hours post-drug administration
- 3.0 hours post-drug administration
- 4.0 hours post-drug administration
- 5.0 hours post-drug administration
- 6.0 hours post-drug administration
- hours post-drug administration
- 24.0 hours post-drug administration
- 48.0 hours post-drug administration
- 72.0 hours post-drug administration

3.3.4.3 Methods of evaluation

Between blood sample collection and centrifugation, tubes were kept on crushed ice. The blood samples were centrifuged at 3000 rpm for 10 minutes within 1 hour of collection. After being transferred into dry polypropylene tubes samples were frozen on dry ice and later stored at –80°C out of light until assay.

Haematology, blood chemistry, and urinalysis were carried out according to standard operating procedures by Chemical Pathology and Haematology Laboratory of the University of Cape Town.

3.3.5 Approval of study protocol

Before the start of the study, the study protocol, informed consent document, and any other appropriate documents were submitted to the Medicines Control Council (MCC), the Ethics Committee (EC) of the University of Cape Town, and the Ethics Committee of DP Marais Hospital (ECDP) for approval. The study commenced only once written approval had been granted from all three bodies.

The full study protocol, patient information leaflet and consent form are attached as Appendices 1-3.

3.4 Healthy Volunteer Study Outline

32 healthy, non-smoking male volunteers aged between 18 and 46 and weighing an average of 74 kg (56-110 kg) were enrolled into an earlier cross-over study conducted by the Division of Pharmacology, University of Cape Town (1999) and will serve as a comparison group. The study design is briefly described. After an overnight fast subjects were randomized to receive their drugs on an empty stomach or with one of the following meals: 1) English breakfast 2) high-fluid content meal 3) high-bulk meal 4) high-bulk high-fat meal. Each subject received 900 mg of rifapentine 30 minutes after finishing their meal, together with 200 ml of water. Blood samples were drawn at 0, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 24, 36, and 72 hours post-dose. Patients refrained from exercise, alcohol and caffeine consumption prior to and during the study period. No concomitant medication was allowed. Only data from the soup-based meal subgroup will be presented here.

3.5 Statistical and Pharmacokinetic Analysis

WinNonLin version 4.01 (Pharsight Corporation, CA, USA) was used to derive pharmacokinetic parameters from concentration-time data by non-compartmental methods. Missing samples and samples more than 10% below the limit of quantitation were excluded from the data set. C_{max} was defined as the maximal observed concentration and T_{max} as the time to reach this concentration. Both were determined directly from the plasma concentration-time profiles for both parent and metabolite. The elimination half-life ($T_{1/2}$) was calculated by dividing 0.693 by elimination rate constant (λ_z). The linear trapezoidal rule was applied to calculate the area under the concentration–time curve (AUC) from 0 to 72 hours. Absolute bioavailability of rifapentine has not been determined and thus all PK parameters and derived parameters were estimated assuming 100% bioavailability (F = 1). Volume of distribution (V/F) was calculated by dividing the dose by the product of the AUC and λ_z. Descriptive statistics (median, 25th and 75th percentile, range) were calculated for pharmacokinetic measures within each group using Prism version 4.01 (GraphPad Software, CA, USA). To allow for a graphical comparison between patient data and healthy volunteer data, individual values from each data group – 1) patient PK values: occasion one 2) patient PK values: occasion two 3) healthy volunteer

parameter values – were transformed to a nominal percentage scale by dividing the true value by the median parameter value for that specific data group and multiplying by a 100 to obtain a transformed value. Interquartile ranges (25^{th} and 75^{th} percentile) for the transformed data were calculated for all three data groups. The distribution of the pharmacokinetic measures was non-normal, and the two-sided Wilcoxon Rank Sum Test was used to determine differences amongst groups. A p-value of <0.005 was regarded as significant.

3.6 Results

In the patient subset one patient was withdrawn prior to commencement of the study due to poor venous access. Blood samples were collected from 0 to 72 hours in 41 tuberculosis patients on both occasions and from 0 to 8 hours in four patients on both occasions. Three patients – out of the cohort of 45 - were withdrawn after occasion one. The first due to an adverse reaction (rash), the second due to poor venous access, and the third subject was unavailable for the second dosing occasion. Patient and healthy volunteer demographic characteristics are listed in Table 3.1.

The dose of 15 mg/kg was well tolerated by the patient cohort. Reported adverse events included nausea (n=2), stomach cramps (n=2), vomiting (n=1), light-headedness (n=1), increased appetite (n=5) and rash (n=1). All adverse events were followed-up immediately by the on-site physician after they were reported by the patient. In the cases where nausea (n=2), vomiting (n=1) and stomach cramps (n=2) were reported the symptoms resolved spontaneously and did not require further follow-up. For the subject who presented with a rash (n=1), treatment was withdrawn and the subject was followed-up for one month after the initial reporting of the adverse event. The rash did not resolve following withdrawal of study medication and the one-site physician was of the opinion that the adverse event was more likely linked to one of the concomitant medications.

The median pharmacokinetic measures of the patient study and healthy volunteer study are summarized in Table 3.2. The healthy volunteers showed significantly higher C_{max} and AUC values than the patients although direct comparisons are difficult because of differing dosing regimens and the lack of prior dosing with rifampicin in the healthy volunteer group. The differences in variability, as represented by the transformed 25^{th} and 75^{th} percentile ranges, for both patient and healthy volunteer data are presented in Figure 3.1. In the majority of PK

measures, the patient data either demonstrates a narrower or equal quartile range to those found in the healthy cohort.

The median peak plasma concentrations of rifapentine were similar on occasions 1 and 2 (15.19 and 15.56 mg/L respectively) in the patient study. Times to reach these peak concentrations were also similar. This is further illustrated in Figure 3.2, which shows almost identical concentration-time profiles for each occasion. In fact the only significant interoccasional difference was observed with the elimination half-life, which increased on occasion 2. This increase is clearly observed in Figure 3.6 where the majority of patients showed a positive increase from occasion one to occasion two. Further interoccasional differences in pharmacokinetic measures between individuals for the patient cohort are presented in Figures 3.4 – 3.8. Mean interoccasional variation for the individual PK measures were C_{max}-17%, AUC-18%, elimination half-life-16%, CL/F-22% and V/F-24%. Four patients had greatly inflated interoccasional differences in their pharmacokinetic measures. The first was patient number 1 whose elimination half-life increased by 72%. The V/F in this patient increased by 45% and this coupled with the decrease in CL/F (15%) resulted in the much extended half-life. The second was patient number 7 whose C_{max} value was 2.5 fold higher on occasion two. Unfortunately data for this patient was only available up to 8 hours post-dose and no conclusions regarding the interoccasional differences in the other parameters could be made. Patients 32 and 33 both had large differences in CL/F and V/F values and the individual data plots of these patients are presented on a logarithmic scale in Figures 3.9 – 3.10. The AUC and elimination-rate constant in patient number 32 were reduced by 24% and 34% respectively. This combined reduction led to the increase in V/F in excess of 70%. The product of the reduction in elimination-rate constant and increase in V/F would naturally reduce the CL/F to the value of 30% which we see in Figure 3.8. This situation is also evident with patient 33 although the extent of the decrease in AUC and elimination-rate constant (63% and 38% respectively) are much greater and result in the much greater interoccasional differences in V/F and CL/F.

The major metabolite, 25-desacetyl rifapentine, showed increased serum concentrations on occasion 2 (Table 3.2, Figure 3.3) in the patient study, with AUCs up to 72 hours significantly different from those measured on the first occasion ($p = 0.002$). Conversely the elimination half-life was fractionally reduced on the second occasion but the difference did not reach significance ($p=0.582$).

The presence or absence of HIV infection in patients with tuberculosis and its effect on pharmacokinetic measures, as well as the influence of gender is presented in Table 3.3. HIV status did not appear to influence the pharmacokinetics while significant gender differences were noted.

Table 3.1 Patient and healthy volunteer characteristics grouped according to the different dose sizes

Dose Group (mg)	Mean Age (SD)	Gender	Mean Mass (SD)	Mean BMI (SD)	Mean dose/kg (SD)	Smoking*	Alcohol¶	HIV Positive	HIV status Unknown	Previously treated for TB
600 (n=10)	35.5 years (11.05)	4 Males 6 Females	43.8 kg (2.48)	16.5 kg/(m^2) (1.72)	13.7 mg/kg (0.85)	9	8	5	2	5
750 (n=19)	36.0 years (10.21)	12 males 7 females	50.0 kg (2.44)	17.9 kg/(m^2) (1.52)	15.0 mg/kg (0.75)	18	13	6	3	12
900 (n=16)	32.5 years (9.70)	13 males 3 females	60.5 kg (5.23)	21.5 kg/(m^2) (1.22)	14.9 mg/kg (1.22)	14	16	3	4	12
All Patients (n=45)	35.0 years (10.03)	29 males 16 females	50.0 kg (7.97)	19.7 kg/(m^2) (2.27)	15.0 mg/kg (1.04)	41	37	14	9	29
Healthy Volunteers (n =32)	24.0 years (4.98)	32 males	74.4 kg (12.13)	23.5 kg/(m^2) (2.83)	12.4 mg/kg (1.85)	0	0	0	0	0

* Evaluated by direct questioning, smoking while an in-patient

¶ Evaluated by direct questioning, alcohol consumption during or prior to admission

SD = standard deviation, BMI = body mass index, HIV = human immunodeficiency virus

Table 3.2 Median pharmacokinetic measures (interquartile range) of rifapentine and 25-desacetyl rifapentine on both occasions

Parameter	Rifapentine			25-Desacetyl rifapentine		
	Occasion 1 (n=45)	Occasion 2 (n=42)	Healthy Volunteer (n=32)	Occasion 1 (n=45)	Occasion 2 (n=42)	Healthy Volunteer (n=32)
Cmax (µg/ml)	15.19 (13.37-18.57)	15.56 (13.38-17.75)	23.64 (18.94-27.98)	5.66 (4.04-7.23)	6.58 (4.50-8.43)	12.41 (9.14-18.10)
Tmax (hr)	5.00 (4.05-6.08)	5.52 (4.11-7.87)	4.00 (3.00-5.00)	8.05 (7.83-23.49)	15.60 (8.00-24.15)	24.00 (13.00-24.00)
AUClast (µg.hr/ml)	355.8 (296.6-457.9)	373.7 (314.8-485.3)	583.7 (428.5-662.0)	174.57 * (151.3-253.9)	259.35 * (179.0-327.1)	507.9 (383.2-836.2)
AUCinf (µg.hr/ml)	360.6 (305.1-468.2)	381.8 (319.7-501.61)	599.8 (440.0-704.0)	183.93 * (156.8-263.8)	271.38 * (195.5-341.2)	551.7 (415.5-891.6)
$T_{1/2}$ (hr)	11.63 * (10.18-12.84)	12.05 * (11.25-14.00)	13.610 (11.76-17.55)	14.71 (12.42-16.34)	13.98 (12.73-16.66)	12.39 (10.42-18.57)
CL/F (L/hr)	1.93 (1.61-2.25)	1.89 (1.61-2.39)	1.51 (1.28-2.05)			
V/F (L)	32.10 (26.26-37.31)	34.57 (28.57-41.72)	27.82 (25.00-38.46)			

Cmax = maximum plasma concentration

Tmax = time to reach maximum plasma concentration

AUClast = area under the plasma concentration-time curve from time 0 to the last quantifiable sample

AUCinf = area under the plasma concentration-time curve from time 0 extrapolated to infinity

$T_{1/2}$ = elimination half-life

CL/F = apparent oral clearance

V/F = apparent volume of distribution

* Significant differences exist in PK parameters between the two occasions

Table 3.3 Median pharmacokinetic measures of rifapentine in various population subgroups on both occasions

Parameter	Occasion	HIV			Gender		
		Positive (n=14)	Negative (n=22)	P-value	Male (n=29)	Female (n=16)	P-value
Cmax (µg/ml)	1	16.42 (11.31 – 17.54)	15.18 (13.41 – 18.66)	0.490	14.36 (12.76 – 17.56)	16.80 (15.23 – 19.23)	0.176
	2	16.91 (14.36 – 17.96)	15.81 (13.16 – 17.63)	0.383	15.26 (13.16 -17.04)	17.63 (15.04 – 18.08)	0.007
Tmax (hr)	1	6.00 (4.30 – 6.94)	6.00 (4.94 – 6.19))	0.541	5.03 (4.00 – 6.17)	4.98 (4.93 – 6.02)	0.937
	2	5.95 (5.05 – 8.03)	6.01 (4.05 – 6.02)	0.945	5.08 (4.05 – 7.82)	6.02 (5.01 – 6.04)	0.423
AUClast (µg.hr/ml)	1	334.29 (297.71 – 453.09)	363.32 (302.51 – 433.72)	0.492	331.13 (290.36 – 416.56)	432.40 (357.69 – 479.22)	0.092
	2	413.65 (328.72 – 478.37)	373.69 (304.02 – 439.76)	0.313	339.01 (300.05 – 420.00)	446.53 (377.18 – 506.33)	*0.002*
AUCinf (µg.hr/ml)	1	338.71 (308.92 – 463.31)	370.14 (307.15 – 438.85)	0.492	335.55 (301.67 – 424.69)	436.74 (362.98 – 497.83)	0.110
	2	423.48 (341.58 – 500.98)	381.76 (309.30 – 446.88)	0.313	343.45 (305.40 – 435.76)	457.50 (381.97 – 528.89)	*0.003*
t1/2 (hr)	1	11.27 (10.07 – 12.42)	11.77 (10.21 – 12.62)	0.322	11.67 (10.67 – 12.84)	11.23 (9.96 – 12.41)	0.970
	2	11.85 (11.22 – 14.42)	12.03 (11.59 – 12.87)	0.641	12.55 (11.40 – 13.65)	11.73 (11.34 – 13.46)	0.638
CL/F (L/hr)	1	2.21 (1.73 – 2.39)	2.03 (1.71 – 2.32))	0.492	2.42 (1.65 – 2.75)	1.56 (1.44 – 2.00)	0.027
	2	1.77 (1.44 – 2.30)	1.96 (1.68 – 2.73)	0.461	2.18 (1.74 – 2.67)	1.75 (1.54 – 2.11)	*0.001*
V/F (L)	1	32.64 (25.57 – 33.55)	33.29 (26.41 – 39.19)	0.322	39.97 (26.93 – 47.31)	26.19 (25.27 – 33.73)	0.064
	2	33.04 (30.29 – 38.37)	35.29 (28.75 – 42.06)	0.641	43.02 (31.59 – 45.81)	27.60 (27.39 – 36.49)	*0.001*

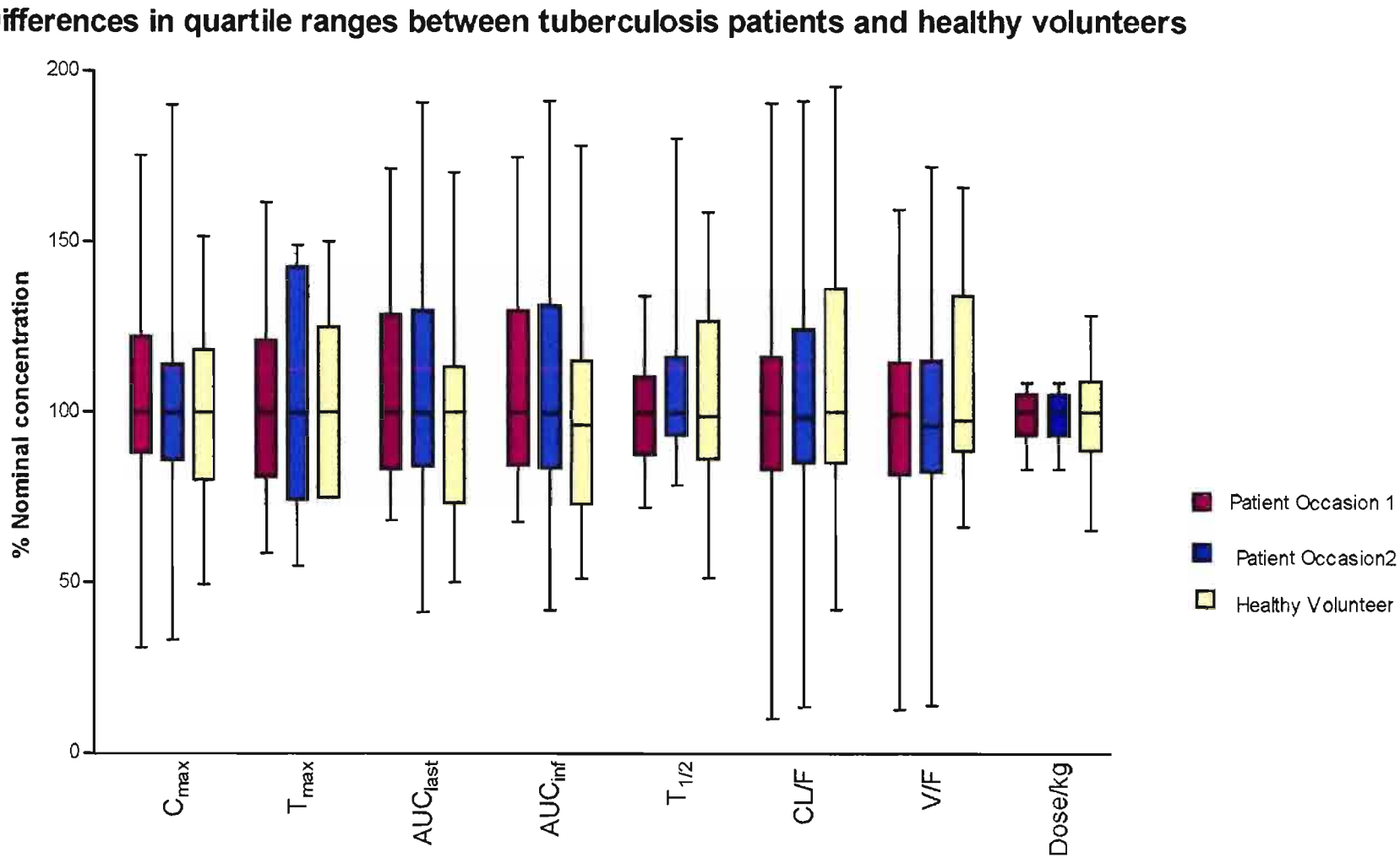


Figure 3.1 Comparative interquartile ranges in pharmacokinetic parameters of tuberculosis patients and healthy volunteers.

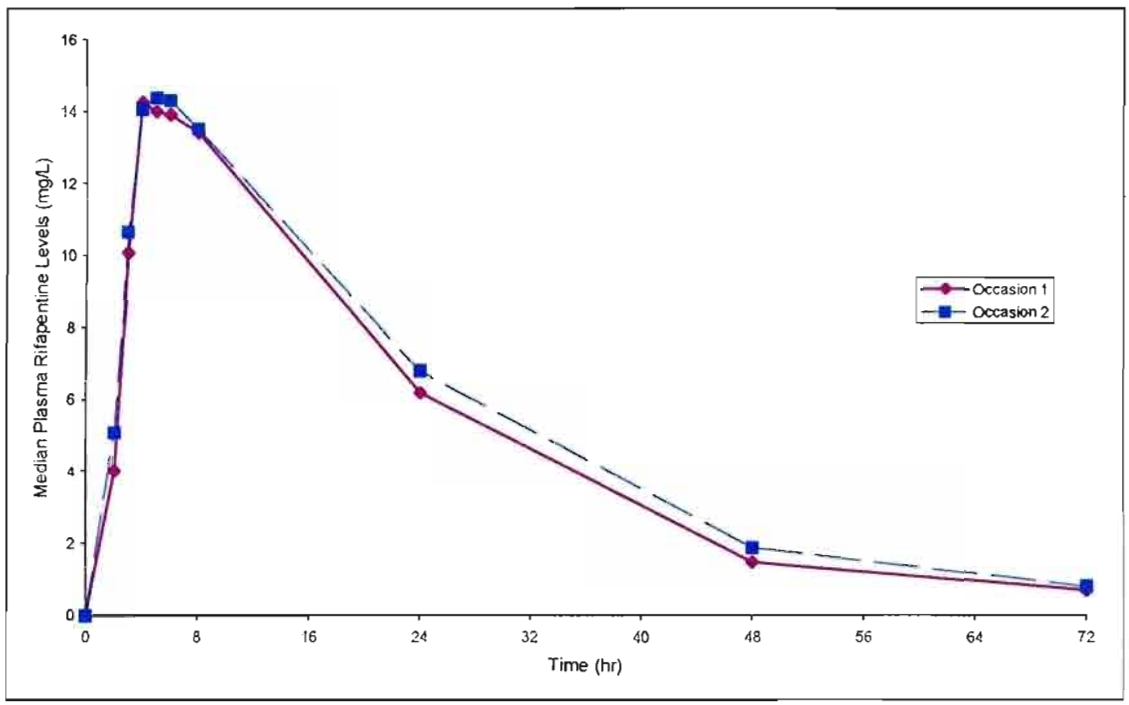


Figure 3.2 Median plasma rifapentine concentration-time profiles for all patients on occasions one (solid line, n=45) and two (dashed line, n=42).

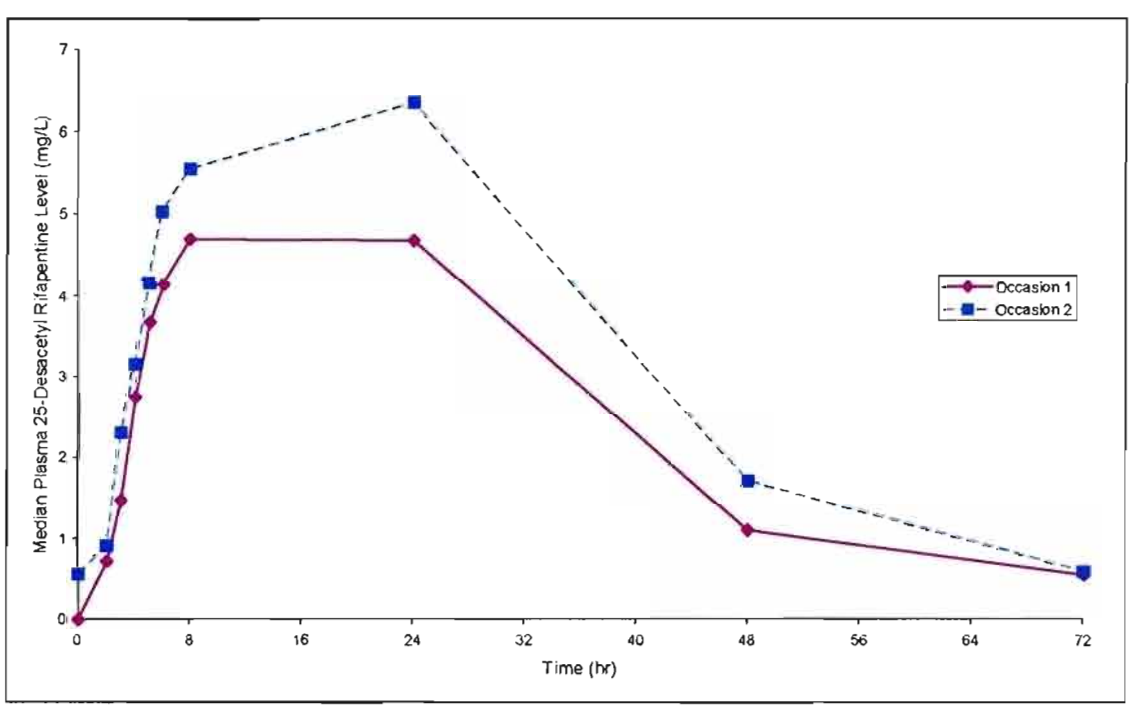


Figure 3.3 Median plasma 25-desacetyl rifapentine concentration-time profiles for all patients on occasions one (solid line, n=45) and two (dashed line, n=42).

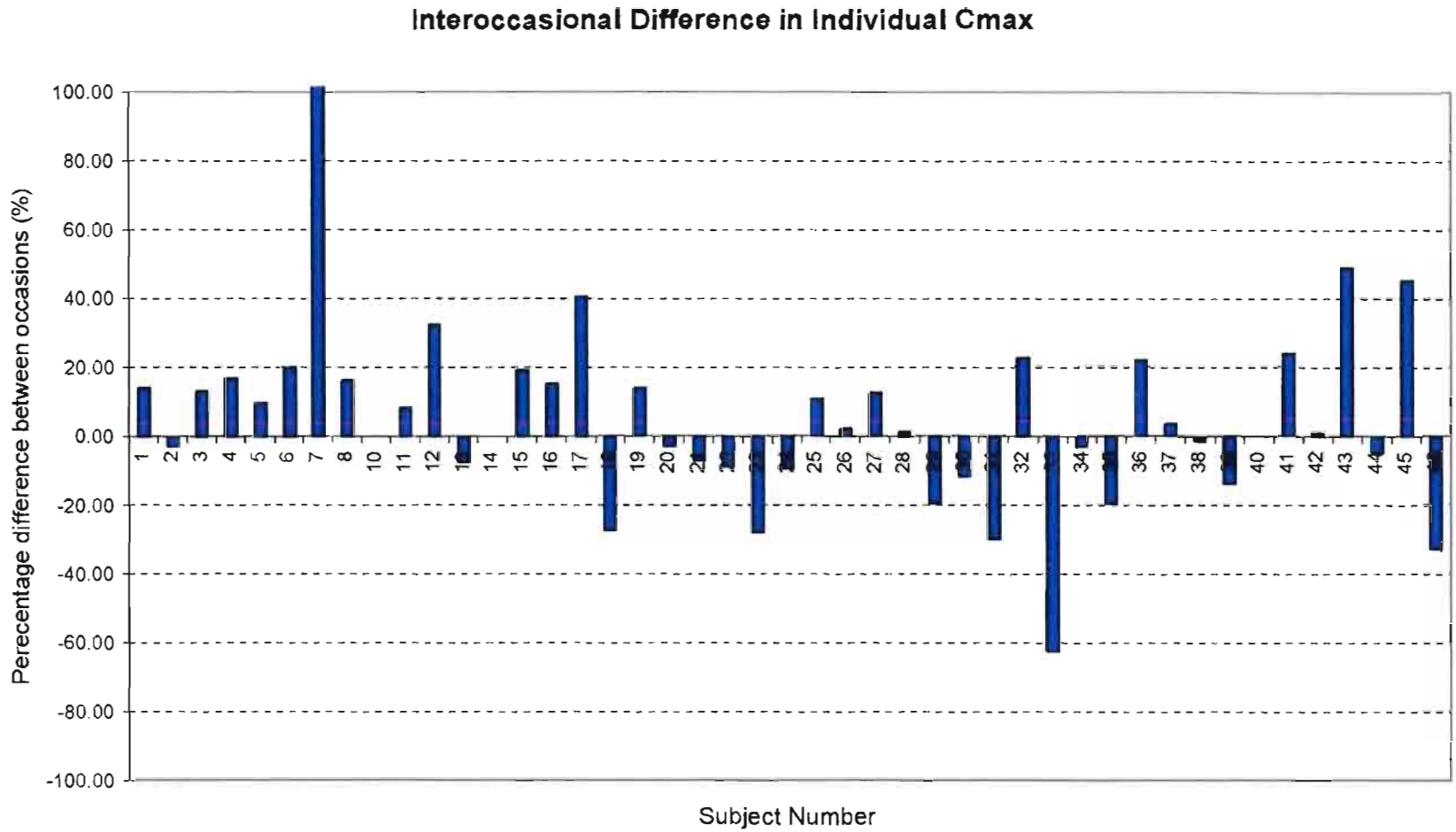


Figure 3.4 Individual differences in the Cmax values of rifapentine between occasions for 42 patients¶

¶ % Difference = ((Occasion 2 value – Occasion 1 Value) / Occasion 1 value)) *100

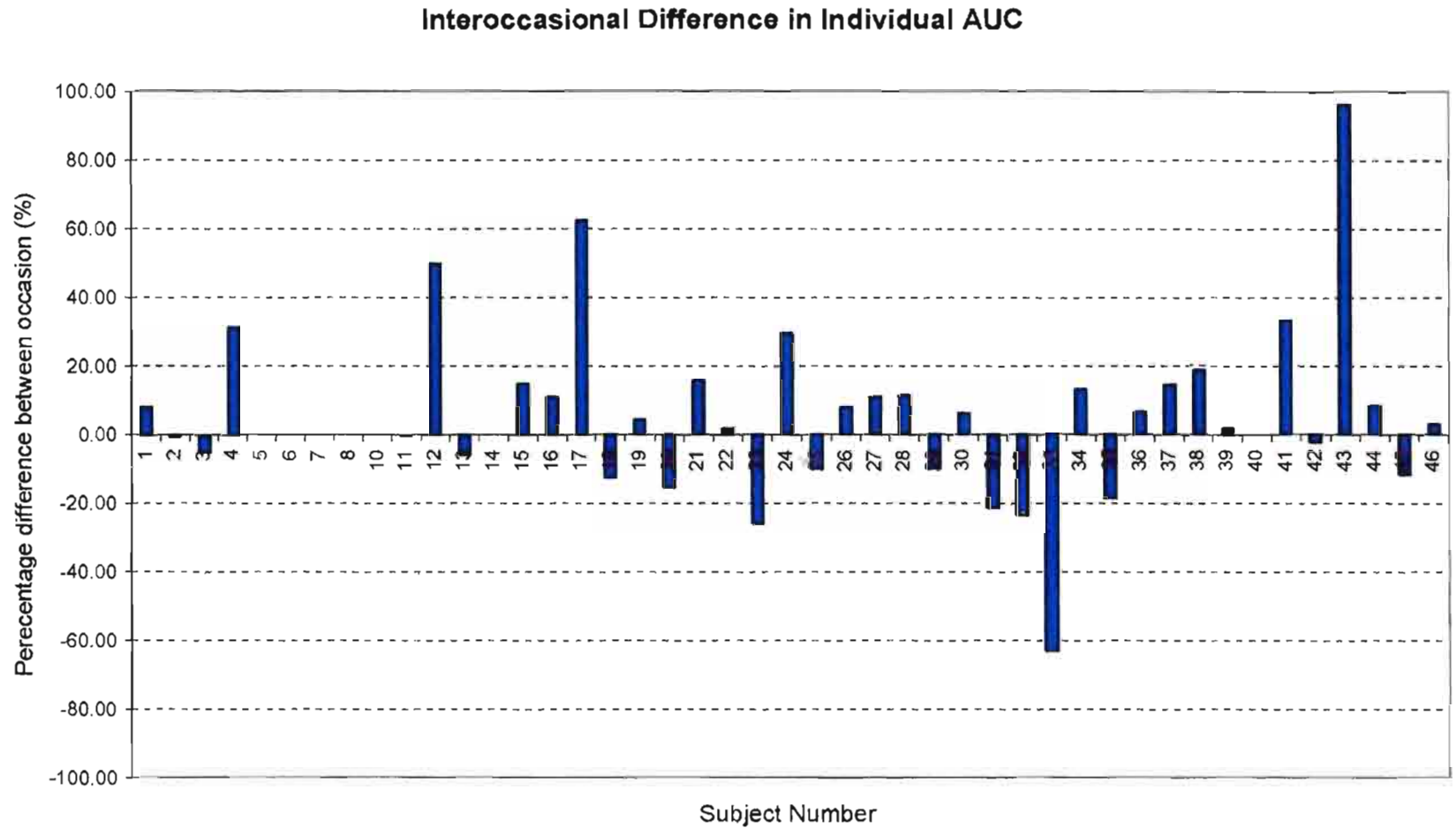


Figure 3.5 Individual differences in the area under the curve (mg.hr/L) values for rifapentine between occasions for 38 patients¶

¶ % Difference = ((Occasion 2 value – Occasion 1 Value) / Occasion 1 value)) *100

Patients with incomplete concentration time profiles (n=4) and patients withdrawn after occasion1 are not included

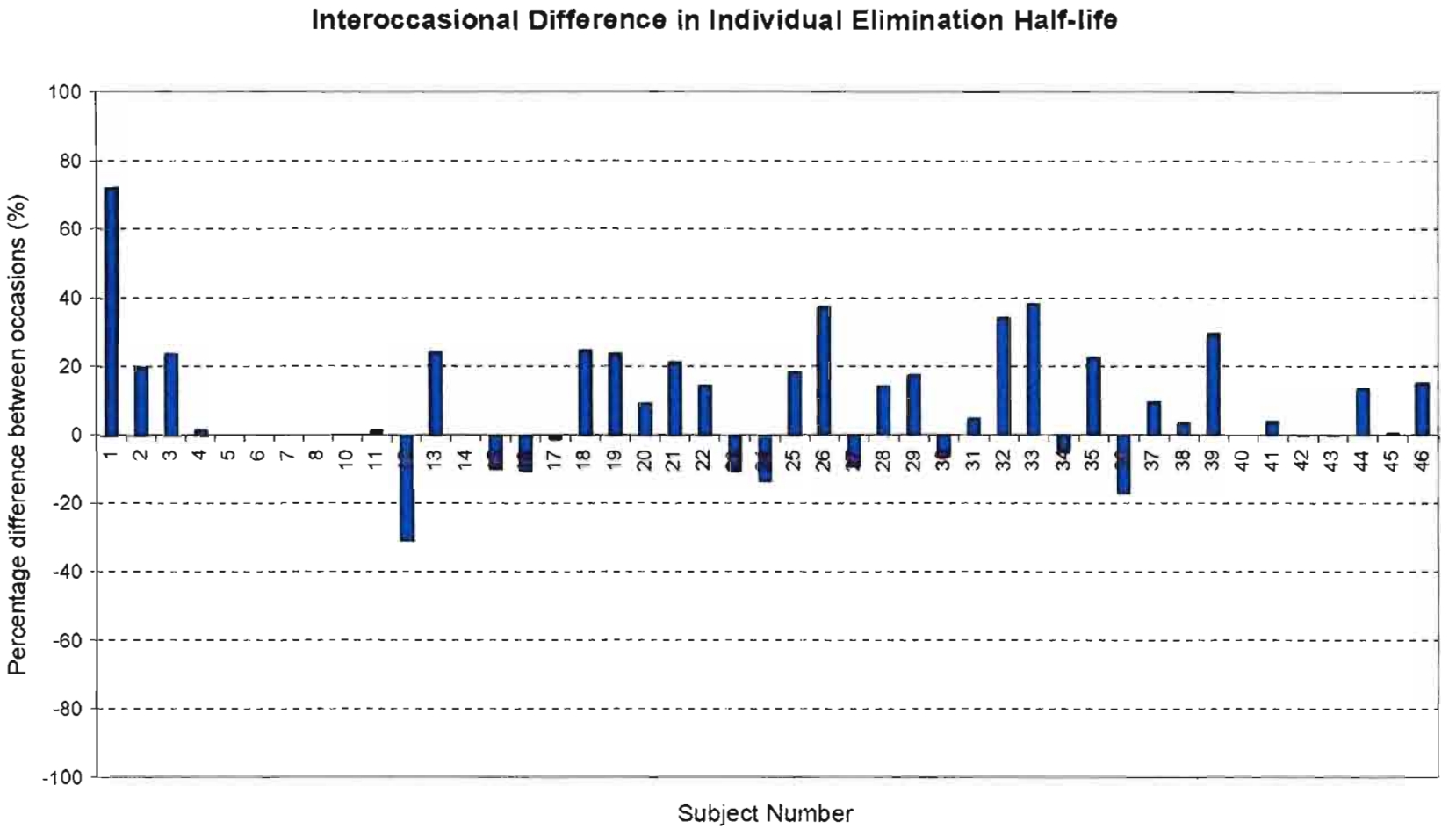


Figure 3.6 Individual differences in the elimination half-life (hr-1) values for rifapentine between occasions for 38 patients¶

¶ % Difference = ((Occasion 2 value – Occasion 1 Value) / Occasion 1 value)) *100

Patients with incomplete concentration time profiles (n=4) and patients withdrawn after occasion1 (n=3) are not included

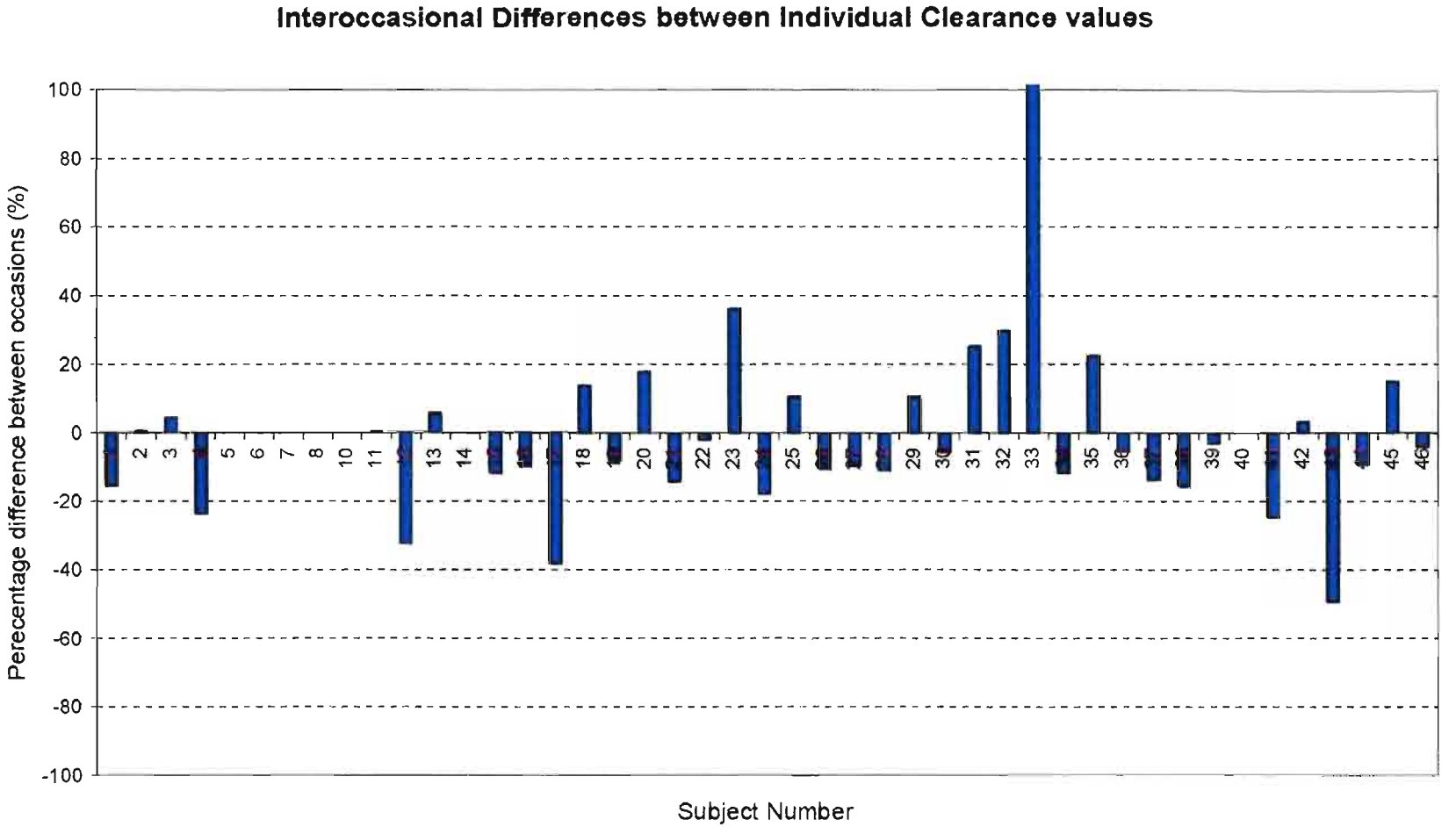


Figure 3.7 Individual differences in the Clearance (L/hr) values for rifapentine between occasions for 38 patients¶

¶ % Difference = ((Occasion 2 value – Occasion 1 Value) / Occasion 1 value)) *100

Patients with incomplete concentration time profiles (n=4) and patients withdrawn after occasion1 are not included

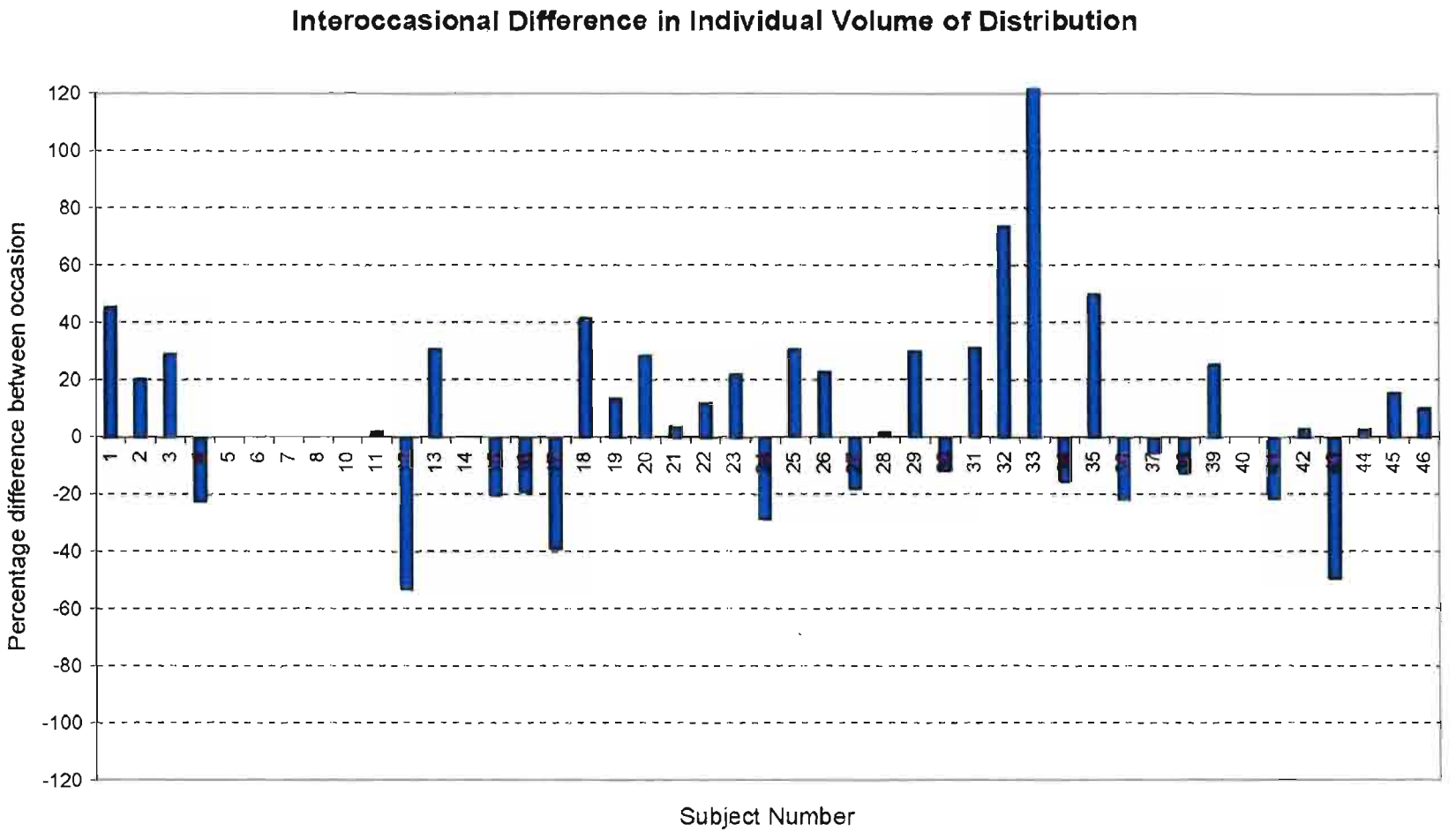


Figure 3.8 Individual differences in the Volume of Distribution (L) values for rifapentine between occasions for 38 patients¶

¶ % Difference = ((Occasion 2 value – Occasion 1 Value) / Occasion 1 value)) *100

Patients with incomplete concentration time profiles (n=4) and patients withdrawn after occasion1 are not included

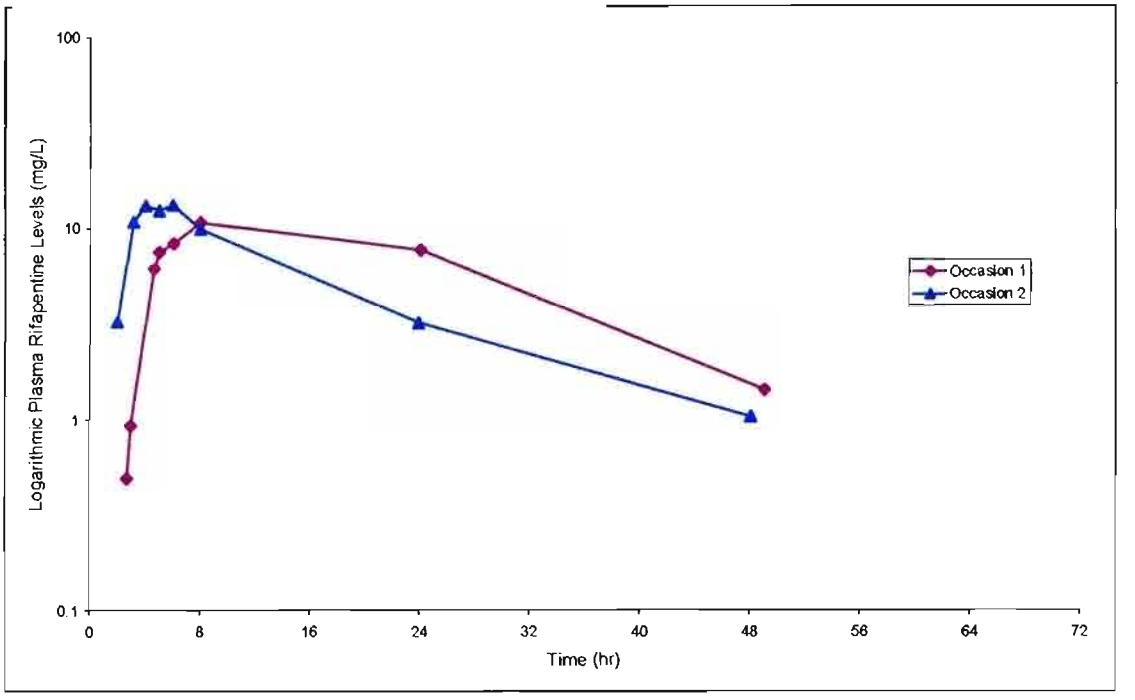


Figure 3.9 Plasma rifapentine concentration-time profile for patient number 32 on occasions one (solid mauve line) and two (solid blue line).

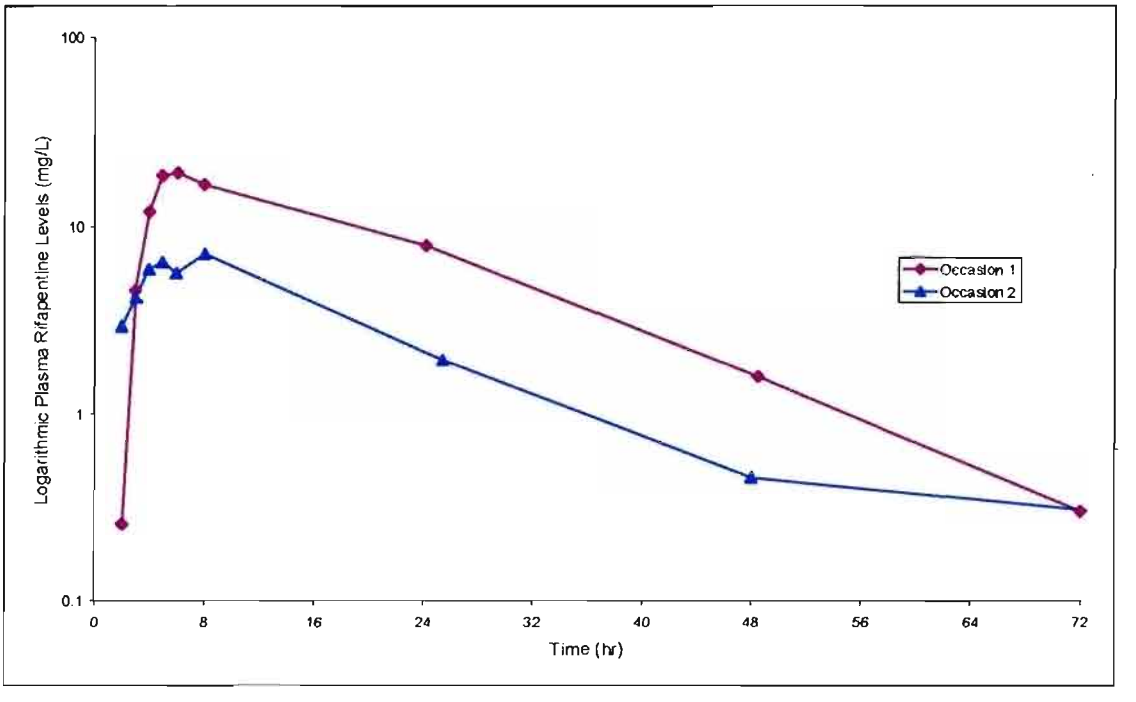


Figure 3.10 Plasma rifapentine concentration-time profile for patient number 33 on occasions one (solid mauve line) and two (solid blue line).

3.6 Discussion

The results from this study clearly show that, in pulmonary tuberculosis patients, rifapentine is well absorbed and shows little variability, both between individuals on a single dosing occasion and in a single individual between dosing occasions. This low variability is also in line with that observed with the healthy volunteer data, which was collected under more standardised conditions with stricter inclusion/exclusion criteria. Of the four patients that demonstrated larger than expected (> 20%) interoccasional differences, none had demographic factors that distinguished them from the rest of the population. They also did not share any common factors that could explain the large differences. The ingestion of the full complement of daily medication was fully supervised at each doing occasion. The counting and packing of the daily medication was independently checked to ensure each patient received the correct dose. Ingestion of the entire meal on the morning of the study dose was followed up as thoroughly as possible although it was not fully supervised. This could account for the differences in pharmacokinetic profiles as rifapentine absorption is sensitive to food intake.

The reduction in the C_{max} and AUC and the increased clearance found in the patient rifapentine data in comparison to the healthy volunteers is thought to be due to the induction of rifapentine metabolism by rifampicin, which was given in daily doses to the patient group prior to participation in the study. Since rifapentine is not autoinductive (Keung *et al.*, 1999) one could expect the plasma levels observed in patients to be even higher if therapy is initiated with rifapentine rather than rifampicin. No statistically significant differences exist in the pharmacokineticss of the parent compound between occasions 1 and 2, although the trend is toward increased values on the second occasion. The enzymes responsible for the biotransformation of rifampicin and rifabutin to their primary 25-deacetylated metabolites are B-esterases (Jamis-Dow *et al.*, 1997). It is not unreasonable to assume that rifapentine, also a member of the rifamycin family, is metabolized by the same enzyme system (Leinweber *et al.*, 1987; Reith *et al.*, 1998; Burman *et al.*, 2001). The inductive effect on mixed-function oxygenases in the liver and autoinductive properties of rifampicin are well known (Acocella G., 1978; Kenny and Strates, 1981) and this suggests possible induction of rifapentine metabolism by rifampicin. Based upon this assumption we would expect to see an increase in AUC from occasion 1 to occasion 2, attributable to the decrease in rifampicin-related inductive effect on the second occasion as no rifampicin was administered for the ten days while subjects received rifapentine only. Although there was a trend toward increased plasma levels on the second occasion it did not reach significant levels. A similar finding by Tam et al. (1997) confirmed that

the induction of rifapentine metabolism by rifampicin does not alter appreciably when rifampicin is replaced by rifapentine. It is however possible that the period between dosing occasions was not sufficient to detect a significant change as enzyme induction usually takes up to 14 days to return to baseline (Acocella G., 1978).

More noticeable is the significant increase ($p=0.021$) in the AUC of the metabolite. Acocella (1978) showed that with consecutive doses of rifampicin, the elimination of the desacetyl metabolite in the bile is increased from day 1 to day 7 with a resultant decrease in serum concentrations. Should this effect also apply to the elimination of 25-O-desacetyl rifapentine, the decreasing biliary inductive effect discussed above would result in a decrease in biliary elimination with time, and account for the increased concentrations of desacetyl rifapentine on occasion 2.

Our patient population showed significant differences in pharmacokinetic parameters between male and female subjects, particularly on occasion 2. These differences were also observed in a population study by Aventis (2000) which estimated oral clearance for males to be 2.51 L/h and for females to 1.69 L/h. This is in stark contrast to a healthy volunteer study by Keung *et al.* which showed no significant gender-related differences in rifapentine pharmacokinetics (Keung et al., 1998). The decreased clearance in females in our patient study is correlated to a decreased volume of distribution with no differences in the apparent half-lives. This can be attributed to the weight differences between the two groups, as gender variation is not known to affect the rifamycin excretory pathways (Keung *et al.*, 1998).

Studies of differences in the pharmacokinetics of rifampicin in HIV-positive and negative individuals have produced mixed results, with some groups showing significant differences (Peloquin *et al.*, 1996; Kimerling *et al.*, 1998), and others not (Choudhri *et al.*, 1997; Taylor and Smith, 1998). Our cohort of HIV-positive patients showed good absorption of rifapentine and no differences in pharmacokinetic measures when compared to HIV-negative patients. Rifapentine was also well tolerated by the HIV-positive group with only one individual experiencing an adverse event (a rash). These findings correspond to the findings of another study by Keung (1999), although direct comparisons are difficult because of differences in dosing regimens.

Considerable inter-subject and inter-occasional variability in the pharmacokinetics of rifampicin have been recognised (Ellard and Fourie, 1999). This variability in pharmacokinetic measures in

healthy volunteers ranges from 30% (Peloquin *et al.*, 1997; Peloquin *et al.*, 1999) to in excess of 60% (Pargal and Rani, 2001). While various groups of patients with low rifampicin plasma levels have been identified (Berning *et al.*, 1992; Peloquin *et al.*, 1993; Gordon *et al.*, 1993; Patel et al., 1995; Sahai et al., 1997; McIlleron *et al.*, 2001; Van Crevel *et al.*, 2002) the degree of variability and potential factors influencing the variability have not been explicitly defined. A pharmacokinetic study involving 109 patients drawn from the same study site as our patient population is currently nearing completion and is addressing these questions surrounding rifampicin. Preliminary findings using a population pharmacokinetic analysis estimate the inter-subject variability to be in the order of 50% (Personal communication, J. Wilkins, 2004). This is considerably higher than the variability observed in our patient population who received rifapentine, and while the role of potential determinants has not been concluded the extensive hepatic, intestinal and autoinductive properties of rifampicin are thought to play a critical role. Rifapentine holds an advantage over rifampicin in this regard as it does not induce its own metabolism (Keung *et al.*, 1999) and induces hepatic enzymes and transporters to a lesser degree due to the intermittent dosing regimen.

In conclusion, a 15mg/kg dose of rifapentine was well absorbed and well tolerated by patients diagnosed with pulmonary tuberculosis. The noncompartmental pharmacokinetics of rifapentine is well described and the variability observed between individuals and between dosing occasions was small and in-line with data from healthy volunteer studies.

Chapter 4

Rifapentine Population Pharmacokinetic Analysis

4.1 Introduction

Population pharmacokinetic (PK) modelling involves the application of nonlinear mixed effect models to repeated measurements data from a group of people to determine the population average pharmacokinetic measures and to quantify the variability in drug absorption, distribution, metabolism and excretion. It further aims to describe the variability in terms of genetic, environmental and pathophysiological patient factors (Steimer J-L., 1992; Pérez-Urizar *et al.*, 2000). These population models can allow better understanding of the underlying physiology and can enhance the search for the optimal dose, serum drug level, or observed effect (Friberg L., 2003).

4.1.1 Mixed effects Model

Mixed effect models are so called because they are comprised of both fixed and random effects. General representation of the model is as follows:

$$Y_{ij} = f\,(\Phi_i, t_{ij}, D) + \varepsilon_{ij} \qquad (1)$$

Where Y_{ij} is the observed data (j = 1, 2,..., n) for the *i*th individual. *f* is the structural model (e.g. 1-compartment, first-order absorption) for predicting the response in the *i*th individual. D is the dose of drug administered at a time t_j. These quantities are measured and are called fixed effects. Φ is a vector of individual parameters and may be expressed as:

$$\Phi_i = g\,(z_i, \theta) + \eta_i \qquad (2)$$

Where g is a structure-type model which is a function of individual specific fixed effects (e.g. weight), and fixed effect parameters θ (e.g. clearance). η is a random effect parameter accounting for the interindividual difference from the typical population value (mean) and is normally distributed with a mean zero and variance ω^2. ε_{ij} is the random residual error and accounts for the difference between the observed measurement and the true predicted value (Sheiner and Beal, 1998).

$$C_{obs,ij} = C_{pred,ij} + \varepsilon_{ij} \qquad (3)$$

In equation (2) the statistical model for describing interindividual variability (η_i) is assumed to be additive. The value remains constant and is independent of the magnitude of the observed value. The error term could also be multiplicative (4) or exponential (5) and will increase proportionally to an increase in the magnitude of the predicted value.

$$\Phi_i = g\,(z_i\,,\,\theta)(1 + \eta_i) \qquad (4)$$

$$\Phi_i = g\,(z_i\,,\,\theta).\exp(\eta_i) \qquad (5)$$

In some situations random variation in a parameter is seen within an individual from the first to the second dosing occasion. This is termed interoccasional variability and can also be quantified in the model by an error term κ_{ik} with a mean zero and variance n^2, where the individual on occasion k differs from the population typical value by an additional random effect, κ_{ik} (Karlsson and Sheiner, 1993; Sheiner and Beal, 1998).

$$\Phi_i = g\,(z_i\,,\,\theta) + \eta_i + \kappa_{ik} \qquad (6)$$

Statistical models to describe the residual random effects (ε_{ij}) can be additive, proportional or a combination of the two. The population model can therefore be described as a collection of models (structural and statistical) from individual observations (Steimer J.-L., 1992).

4.1.2 Model Estimation in NONMEM

Estimation of population parameters in NONMEM is achieved through iterative minimisation of the associated least-squares objective function which is approximately proportional to minus two times the logarithm of the likelihood (-2log likelihood) of the data.

The original estimation method employed by NONMEM was the so-called first-order (FO) method. The derivation of an approximate likelihood relies upon a first-order Taylor series expansion and provides estimates of the population parameters. Individual parameter estimates are obtained as empirical Bayes *posthoc* estimates (Sheiner and Beal, 1998).

The first-order conditional estimation (FOCE) method differs from the FO method in that estimates of the population parameters and random interindividual effects (η) are determined simultaneously rather than *a posteriori*. This method is more time consuming and CPU intensive since individual estimates need to be obtained during each iteration. The role of the FOCE method is better served when the degree of nonlinearity in the data increase and obvious bias is seen when using the FO method (Sheiner and Beal, 1998).

Further methods of estimation include the FOCE method with interaction (FOCE INTER) which includes an interaction of interindividual and residual random effects, and the Laplacian method which employs second-order derivatives rather than first-order derivatives with respect to the η values used (Sheiner and Beal, 1998).

4.1.3 Mixed effects model building

Building the population pharmacokinetic model can therefore be viewed as a three-step process whereby the 1) structural, 2) statistical and 3) covariate submodels are added sequentially to describe the data at hand. The structural model development is exploratory in nature, provided no prior knowledge is available, and describes the rate of transfer and distribution of drug through the physiological compartments that make up the body, although these compartments usually have no physiological or anatomical reality.

Once a suitable structural and statistical model that adequately describes the data is established, the estimation of population parameters and their variability takes place. The next step is exploring the influence of covariates. This can be a time-consuming and laborious task because not only do significant parameter-covariate relationships need to be identified but also the shape of the relationships needs to be characterised (Wählby U., 2002). The identification of candidate covariates begins by screening potential relationships within the basic mixed-effects model. This can be especially tedious if a large number of covariates are to be considered, so a few methods have been employed to ease the process. Use of empirical Bayes (posthoc) estimates of the parameters plotted against covariates assists in identifying potential relationships as well as the functional shape of the relationship (Maitre *et al.*, 1991). Other options include stepwise generalised additive model (Mandema *et al.*, 1992), stepwise multiple linear regression, or tree-based modelling (Verotta D., 1997). Covariates identified to have a

significant influence on the basic model are included using stepwise forward inclusion and are then tested using backward deletion with stricter significance criteria.

It is important to evaluate the goodness of fit, stability and reliability of a model, particularly when it is used for descriptive purposes (Ette *et al.*, 2003) as is the case with this study. There are various techniques available to perform this model validation. These include mapping of the objective function to analyse parameter sensitivity (Holford and Peace, 1992) as well as the reestimation of model parameters using bootstrapping (Ette E.I., 1997).

The pharmacokinetics of rifapentine in a tuberculosis patient population has not been described in detail before using nonlinear mixed effects modelling. The compartmental pharmacokinetics as well as the effect of various covariate factors has however been studied in healthy volunteers.

4.2 Rifapentine compartmental analysis and covariate studies

The compartmental pharmacokinetics of rifapentine has been briefly described previously (Conte *et al.*, 2000) although the study was initially designed to compare the concentrations of rifapentine in plasma, alveolar cells and epithelial lining fluid in normal volunteers. A group of 30 subjects (20 women and 10 men) was subdivided into six groups of five. Each member of the group had three blood samples drawn. The first sample was drawn at 2 hours post-dose in each of the 30 volunteers. The second sample was drawn at a specified time between 4 and 48 hours defined by the group number. This resulted in 5 samples each at times 4, 5, 7, 12, 24, and 48 hours. A third sample was drawn from each subject 20 – 24 hours post-dose (Table 4.1).

Table 4.1 Concentrations of rifapentine and 25-desacetyl rifapentine in plasma from the study by Conte *et al.* (2000).

Group	Drug	Concentration in plasma (mg/L)		
			At group time	At 20-24 hr post-dose
1) 4 hr	Parent	4.5 ± 1.7	13.3 ± 9.2	4.1 ± 2.6
	Metabolite	0.0 ± 0.0	4.9 ± 3.5	4.8 ± 3.6
2) 5 hr	Parent	13.2 ± 3.6	26.2 ± 6.1	7.6 ± 1.1
	Metabolite	1.6 ± 0.4	8.2 ± 2.6	6.8 ± 2.6
3) 7 hr	Parent	13.9 ± 3.6	14.9 ± 4.9	6.7 ± 1.7
	Metabolite	1.5 ± 0.7	6.3 ± 2.1	5.0 ± 2.6
4) 12 hr	Parent	9.7 ± 5.5	15.5 ± 4.4	10.7 ± 4.0
	Metabolite	0.5 ± 0.5	7.6 ± 2.5	7.6 ± 3.1
5) 24 hr	Parent	9.1 ± 4.7	8.2 ± 1.9	7.9 ± 2.9
	Metabolite	0.6 ± 0.7	7.7 ± 4.7	5.8 ± 4.3
6) 48 hr	Parent	9.7 ± 2.2	3.4 ± 3.2	9.8 ± 7.4
	Metabolite	1.1 ± 0.5	5.1 ± 4.7	7.3 ± 5.3

The data were fitted to a two-compartment model and the following parameters were estimated from the model: volume of distribution of the central compartment, the elimination rate constant (0.038 hr^{-1}), the elimination half-life (18.3 hrs), the maximum plasma concentration (26.2 mg/L) and the time to reach this maximum (5 hrs). Although the volume of distribution of the central compartment was estimated from the model no value was reported. A calculation using the dose (600 mg), AUC (580 mg.hr/L) and elimination rate constant (0.038 hr^{-1}) gives an estimate of 27.2 L. These results are quoted as being in line with the previous healthy volunteer studies conducted by Keung *et al.* (1998). No healthy volunteer covariate factors were explored.

Reference is made in the Priftin package insert (Aventis, 2000) to a population pharmacokinetic study using sparse blood samples from 351 tuberculosis patients. Details regarding the structure of the pharmacokinetic model are not described. Results after a 600 mg oral dose estimate the apparent volume of distribution as 70.2 L. Males and females had statistically significant differences in oral clearance with reported values of 2.51 L/hr and 1.69 L/hr respectively, although the clinical relevance of this difference has not been studied further. The value quoted for the apparent volume of distribution is 2.5-fold greater than those reported previously in the literature (Keung *et al.* 1995; Keung *et al.* 1998; Keung *et al.* 1999) while the clearance values lie within a similar range to literature values. It is difficult to explain this discrepancy especially since details regarding the data used for the pharmacokinetic analysis

are not available, nor are any details regarding the model used to describe the data. Calculations made using the above mentioned parameter values yields an estimate of elimination half-life of 28.8 hours. This is also 2.5-fold greater than the value quoted from previous Aventis studies with rifapentine and no further explanation is given in the package insert with regard to the differences in elimination half-life.

The effect of age (Keung *et al.*, 1998), gender (Keung *et al.*, 1998), varying degrees of hepatic dysfunction (Keung *et al.*, 1998), and HIV infection (Keung *et al.*, 1999) on the pharmacokinetics of rifapentine have all been investigated in separate studies. The results show that clearance is reduced in older men and patients with hepatic dysfunction when compared to healthy volunteers although no dosage adjustments are recommended. No significant differences exist with regard to gender although AUC is slightly reduced in HIV positive volunteers but not to a significant level.

The data from these studies were all analysed using model-independent methods and only investigated a single covariate factor at a time. The advantages of a population pharmacokinetic approach are that a compartmental model is used to describe the rate of transfer of drug between the system of compartments that represent the body and that this model implemented in the NONMEM programme, can account for three sources/levels of variability:

1. interindividual variability
2. intraindividual or interoccasional variability
3. residual variability as a result of inaccuracies in recording of sample collection times, assay error or model misspecification

4.3 Scope of Population Pharmacokinetic Data Analysis

The aim of this analysis was to develop a qualified population pharmacokinetic model for rifapentine and the deacetylated metabolite after administration of a 15 mg/kg dose to patients with TB for the purpose of evaluating pharmacokinetic variability and the influence of possible covariates.

4.4 Methods

4.4.1 Data analysis

The initial data set for the building of the pharmacokinetic model for the patient study was constructed using data from all individuals on all occasions. One patient was sampled a third time although the third dose was separated by ten days from the first two consecutive doses with daily doses of rifampicin administered daily between the second and third dose of rifapentine. Data from the third occasion was therefore included as a new individual. The limit of quantification of the assay was 0.6 mg/L for the parent and 0.25 mg/L for the metabolite. Blood samples with concentrations that fell within 10% of this value were not excluded from the data set. Plasma molar concentration-time data of rifapentine and 25-desacetyl rifapentine from all patients was modelled with a nonlinear mixed effects approach using NONMEM (version V, Level 1.1) (Sheiner and Beal, 1998). NONMEM was operated in a LINUX environment (Mandrake Linux 9.1) using a gcc Fortran compiler 3.3.1. First-order conditional estimation was used to estimate all population pharmacokinetic parameters except for the parent absorption lag-time where the first-order estimation method was employed.

4.4.2 Choice of patient covariates

The following covariates were tested: age, weight, body mass index (BMI), albumin, ALT, AST, alkaline phosphatase, time elapsed between meal and rifapentine dose, number of rifampicin doses prior to first rifapentine dose, gender, alcohol abuse, smoking, HIV infection, recreational drug use and if the patient presented as a new or retreatment case. Demographic and covariate data were collected by questionnaire, interview and examination of hospital medical records.

Biochemical laboratory results, in particular albumin, served as markers of disease state. The impact of food on the absorption of rifapentine has been discussed in detail and the interval between food and drug administration was measured and evaluated as a covariate effect. Rifampicin is a potent inducer of hepatic and intestinal enzyme systems. It was assumed that at the time of the first rifapentine dose all patients would be maximally induced. This assumption was tested by evaluating the effect of the number of rifampicin doses patient received before the first study dosing occasion. A previous population study by Aventis (2000)

showed differences in the apparent oral clearance between the two gender groups, although no significant differences where observed in healthy volunteers (Keung *et al.*, 1998). Noncompartmental analysis of our data (chapter 3) showed significant differences between males and females and these differences were studied further to determine if it was a true gender effect or if the difference was related to patient weight. In subjects not infected with tuberculosis, HIV infection has no significant impact on the pharmacokinetics of rifapentine (Keung *et al.*, 1999). The effect of HIV and TB co-infection was examined in our covariate analysis. Smoking, alcohol abuse and recreational drug use were prevalent amongst our study population. The risk that these practices may lead to decreased serum levels is tested in the model. The effect of prior treatment (completed or not) for active TB is also considered as a risk factor for decreased serum levels.

4.4.3 Model development

4.4.3.1 Parent drug model

Single- and multicompartment pharmacokinetic models with linear elimination were fitted to the rifapentine concentration-time data. The models included first-order absorption including/excluding a lag time to determine the basic pharmacokinetic structural model. The need for interindividual variability was evaluated in all basic structural parameters and was modelled as in the case for CL/F:

$$CL/F_i = TV(CL/F) \cdot \exp(\eta_i^{CL/F})$$

where CL/F_i is the clearance value for the i^{th} individual and TV(CL/F) is the clearance in a typical individual. $\eta_i^{CL/F}$ is the inter-subject variability, assumed to be normally distributed around zero and with a variance $\omega^2_{CL/F}$ to distinguish the i^{th} individual's clearance from the population mean as predicted from the regression model. Residual variability incorporated both additive and proportional error terms. Concentration time profiles of patient data displayed a secondary peak in 23 out of 45 patients at 6 hours. This was the first sample taken after lunch

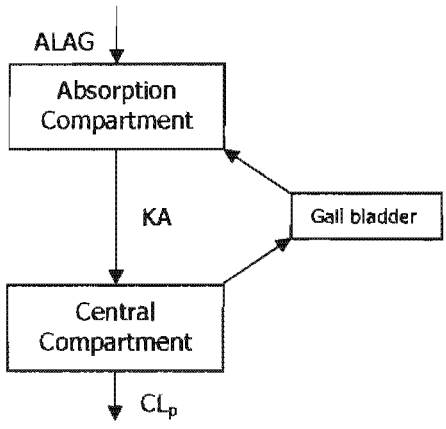


Figure 4.1 Enterohepatic recirculation model

and the peak could be the result of rifapentine being reabsorbed from the small intestine following the release of bile from the gall bladder. An enterohepatic recirculation model (Figure 4.1) was employed to try and characterize this whereby drug continuously entered the hypothetical gall bladder compartment from the central compartment. The gall bladder was emptied into the absorption compartment at times of food intake. This cycle was repeated for the first 24 hours of each dosing occasion. Rifampicin is a known inducer of drug elimination (Acocella G., 1978) and the potential effect of prior administration of rifampicin on rifapentine pharmacokinetics was investigated. As an empirical model for changes in rifapentine clearance due to previous rifampicin administration and a putative induction, the need for separate population CL/F values for the two dose occasions was explored. Furthermore, interoccasional differences in the pharmacokinetic parameters were explored and modelled as in the case for V/F:

$$V/F_i = TV(V/F) \cdot \exp(\eta_i^{V/F} + \kappa_{ik}^{V/F})$$

where $\kappa_i^{V/F}$ is the inter-occasional variability, assumed to be normally distributed around zero and with a variance $n^2_{V/F}$. These provided the structural and statistical models with no covariates *i.e.* primitive parent models.

4.4.3.2 Parent Covariate Analysis

Individual empirical Bayes *posthoc* estimates were generated from the primitive parent model and interindividual differences (*i.e.* the η's) were plotted against the covariates to identify potential relationships as well as the shape of the relationship. Further testing and selection of covariates was achieved using stepwise generalised additive modelling (GAM), implemented in Xpose (Jonsson and Karlsson, 1999). Covariates identified as being important were first assessed in the primitive model by univariate addition and ranked in descending order according to the change in objective function value (ΔOFV). Variables were then tested by stepwise addition to the model. Covariates were included in the model at a significance level of

0.05. When no further covariates could be included at the 5% significance level a backwards deletion was carried out at the 10% significance level. Continuous covariates were centred at the median values and were included in the model as exemplified in the case of body weight as:

$$(CL/F_i) = [TV(CL/F) + \theta_{WTCL/F}\cdot(WT_i - WT_{median})] \cdot \exp(\eta_i^{CL/F} + \kappa_{ik}^{CL/F})$$

Categorical covariates were included in the model as exemplified in the case of HIV status:

IF(HIV.EQ.0)THEN

$(CL/F_i) = [TV(CL/F)] \cdot \exp(\eta_i^{CL/F} + \kappa_{ik}^{CL/F})$

ELSE

$(CL/F_i) = [TV(CL/F)^* \theta_{HIV}] \cdot \exp(\eta_i^{CL/F} + \kappa_{ik}^{CL/F})$

ENDIF

The parent model file is attached as Appendix 2.1.

4.4.3.3 Metabolite Model

The deacetylated metabolite, 25-desacetyl rifapentine, was modelled separately from the parent drug. The individual empirical Bayesian *posthoc* estimates from the final parent drug model, including covariates, were fixed and served as input for the basic structural metabolite model. Single- and multicompartmental models with linear and nonlinear elimination were fitted to the metabolite plasma concentration-time data to describe the kinetic behaviour and disposition of the metabolite (Figure 4.2). Presystemic formation of the metabolite via the first-pass effect was investigated and modelled using a hypothetical metabolite absorption compartment (Kerbusch *et al.*, 2003). Models where the metabolite was assumed to be only formed systemically as well as models including elimination of rifapentine through a second non-hepatic pathway were also

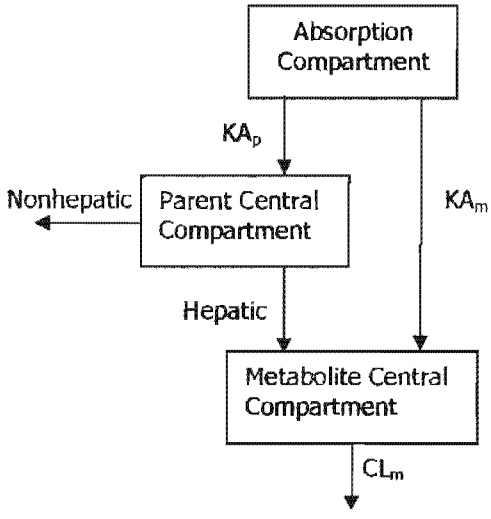


Figure 4.2 Metabolite structural models explored

explored. An increased exposure of 25-desacetyl rifapentine after the second dose compared to the first dose without a significant change in the parent drug kinetics was observed. The different models applied to describe the observed change in exposure included a linear decline in clearance of the metabolite over time, an exponential decline over time, and a clearance saturation model expressed as follows:

$$TVCL_{metabolite} = TVCL_{baseline} + (TVCL_{induced} - TVCL_{baseline}) \cdot exp^{(-Slope \cdot Day)}$$

Exponential models were used to account for the variability between subjects and were initially tested on all basic structural parameters. Residual variability incorporated both additive and proportional error terms.

4.4.3.4 Metabolite Covariate Analysis

The same covariates tested for significance in the parent drug model were included in the metabolite model. These were assessed and included as described for the parent drug.
The metabolite model file is attached as Appendix 2.2.

4.4.4 Model evaluation and qualification

Models were selected by visual inspection of basic goodness-of-fit plots including plots of the observed data *vs.* population (PRED) and individual predictions (IPRED). Plots of individual weighted residuals (IWRES) vs. individual predictions and the distribution of weighted residuals (WRES) over time were assessed for deviations away from zero. Relative standard errors (RSE) of the parameters were also compared to measure parameter precision and the OFV produced by NONMEM was used to discriminate between hierarchical (nested) models. This discrimination was based on a significance level of 0.05 which corresponds to a decrease in OFV of >3.84 (one parameter difference) as the difference in OVF is approximately χ^2-distributed (Sheiner and Beal, 1998; Wählby *et al.*, 2001).

Model qualification for both parent and metabolite were performed by mapping the response surface of the objective function and by bootstrap resampling to confirm parameter stability and sensitivity as well as the robustness of the model. For the former method individual

parameter values were fixed at ± 5, 10, 15, 20, 30, 40 and 60% of the population estimate from the final model and changes in the OFV were plotted against the parameter values. Polynomial equations (4^{th} order) were fitted to the plotted data. Assuming the χ^2-distribution of the OFV the 95% confidence intervals (CI) for the parameter estimate would correspond to a change in OFV of 3.84. The confidence intervals obtained were compared with those based on the standard errors (SE) of the NONMEM estimates and were calculated as point estimate ± (1.96 x SE). Parameter estimates were re-estimated for each of the 2000 bootstrap samples. The mean, SE and 95% CI's were also compared with those of the NONMEM estimates from the final model.

4.5 Results

4.5.1 Parent drug model

A total of 775 rifapentine and 756 25-desacetyl rifapentine concentrations were collected from intensive sampling in 45 patients over the eight day study period. A one-compartment model with first-order absorption and elimination with an absorption lag time included was found to be optimal for further modelling of the parent drug data (Figure 4.3). Interindividual variability (IIV) terms were included on the following structural model parameters: absorption lag time (ALAG), absorption rate constant (KA), volume of distribution of the central compartment (V/F), and clearance from the central compartment (CL/F).

The secondary peaks observed in some individuals in the patient data set could not be adequately characterised by the enterohepatic recirculation model and the model soon became overparameterised. The incorporation of the empirical enzyme induction model did not explain the data any better than the model incorporating interoccasional variability (IOV) in CL/F and the latter model was followed further. Addition of IOV terms to KA and V/F further improved the model and provided the best fit to the data. Different covariates were tested for their ability to improve the model fit, reduce the interindividual variability and/or explain the variability. Graphical analysis and stepwise generalised additive modelling identified weight (WT) as a possible covariate influencing both CL/F and V/F. Furthermore WT, ALT and AST levels were identified as having an influence on KA. The inclusion of the effect of WT on CL/F provided the biggest drop in OFV (ΔOFV = 22.664). In the forward inclusion step only the influence of WT on V/F produced a significant decrease in OFV which negated the need for a

backward deletion process. Inclusion of the two afore mentioned covariate effects improved the relationship between model-predicted and observed concentrations for the population (Figure 4.4a). Individual predictions versus observed values are presented in Figure 4.4b. Individual weighted residuals versus individual predictions and weighted residuals versus time are plotted in Figures 4.4c and 4.4d respectively. The vast majority of points in these two plots lie within 2.5 units of perfect agreement and were evenly distributed around the zero ordinate over the duration of the study. Figure 4.5 shows the plasma concentrations and model predicted values for a representative patient on two dosing occasions. It demonstrates that the model can account for variations in the plasma concentrations between occasions. Parameter estimates from the final model are presented in Table 4.2. Figure 4.7 - 4.9 show the objective function mapping for the parameters estimated from the final model. No local minimum was found in any of the parameters. The 95% confidence intervals of the objective function mapping and those of the final model coincided well (Table 4.3). Results from the bootstrap resampling are presented in Table 4.3 and concur with the NONMEM parameter estimates.

Individual plots of observed, population predicted and individual predicted rifapentine concentration-time data are presented in Appendix 3.1.

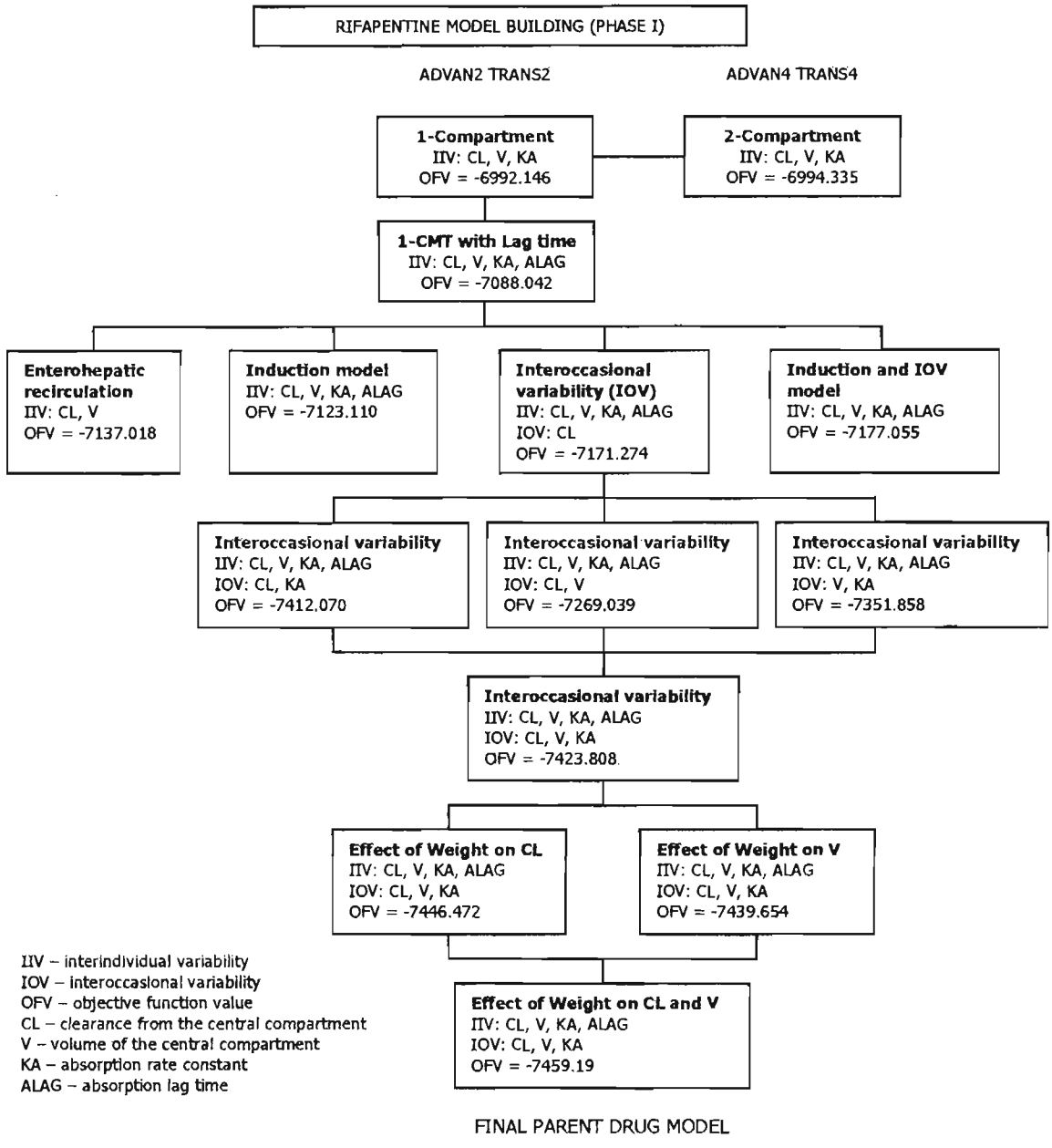


Figure 4.3 Flow diagram detailing the model building of the parent drug model

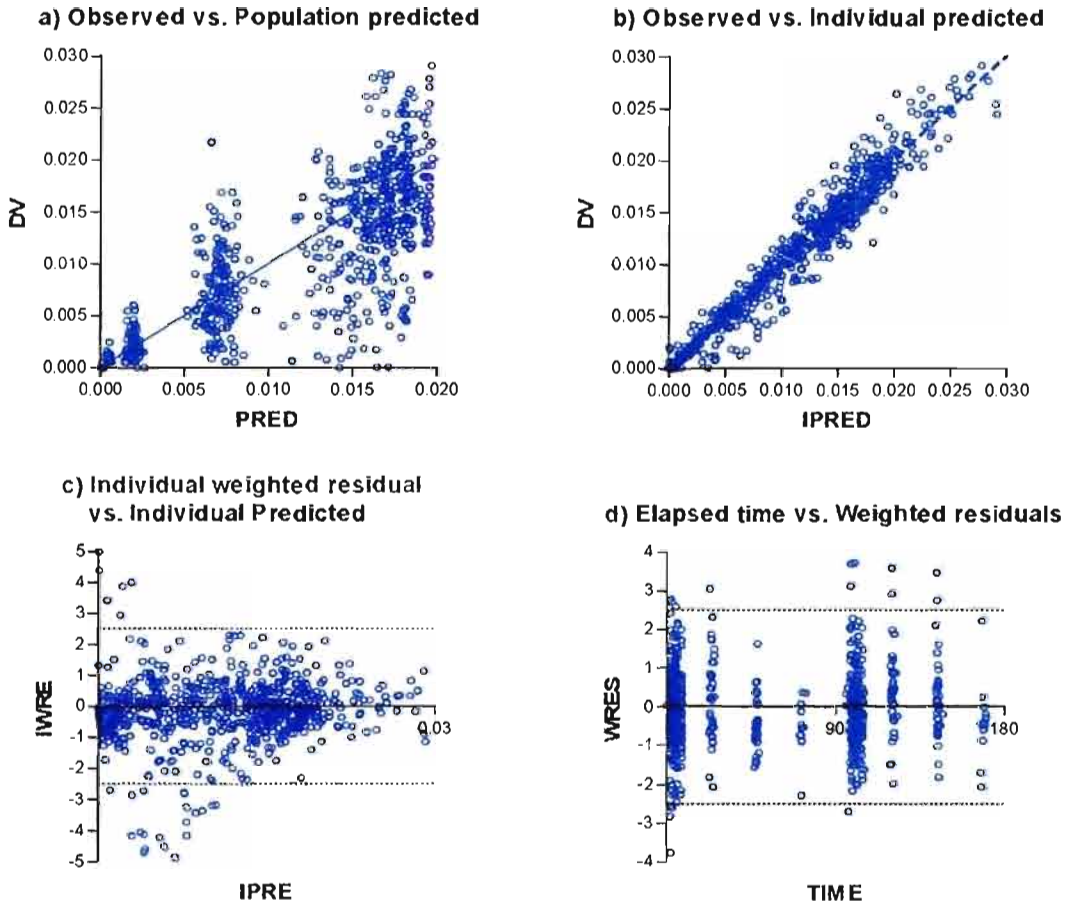


Figure 4.4 Goodness-of-fit plots for the final patient pharmacokinetic model a) observed rifapentine concentrations vs. population predictions b) observed rifapentine concentrations vs. individual predictions c) individual weighted residuals vs. the individual predictions d) weighted residuals vs. time elapsed over the study period. The solid blue line is the line of identity.

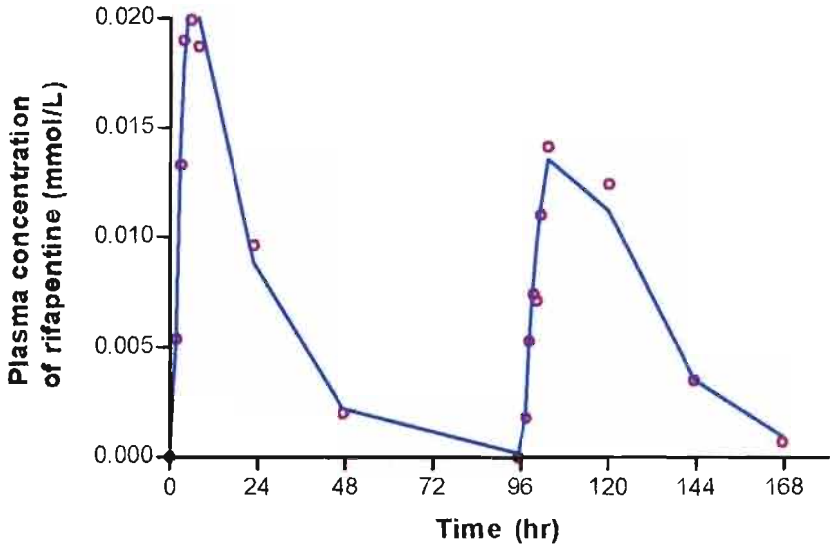


Figure 4.5 Individual predicted (solid blue line) and observed (open red circles) rifapentine molar concentrations in one representative patient given 750 mg of rifapentine orally on study days 1 and 5. One-compartment model, with an absorption lag time and first order absorption and elimination.

Table 4.2 Patient population pharmacokinetic parameters for rifapentine obtained from NONMEM

Parameter	Estimates (RSE*)	Interindividual variation % (RSE*)	Interoccasional variation % (RSE*)
ALAG (hr)	1.45 (0.05)	23 (0.39)	
KA (hr^{-1})	0.641 (0.19)	52 (0.58)	60 (0.27)
CL/F (L/hr)	2.03 (0.05)	22 (0.21)	16 (0.36)
V/F (L)	37.8 (0.04)	16 (0.49)	16 (0.36)
Additive residual error (mmol/L)	3.82×10^{-4} (0.15)		
Proportional residual error	0.144 (0.08)		
Effect of WT on CL† (L/hr.kg)	0.049 (0.19)		
Effect of WT on V‡ (L/kg)	0.691 (0.32)		

* RSE = Relative standard error

† $(CL/F_i) = [TV(CL/F) + \theta_{WTCL/F}.(WT_i - WT_{median})]\exp(\eta_i^{CL/F} + \kappa_{ik}^{CL/F})$

‡ $(V/F_i) = [TV(V/F) + \theta_{WTV/F}.(WT_i - WT_{median})]\exp(\eta_i^{V/F} + \kappa_{ik}^{V/F})$

Table 4.3 Comparison of 95% confidence intervals estimated by standard errors of the NONMEM final estimates, bootstrapping and objective function mapping.

	NONMEM final estimate		
Parameters	**Mean**	**SE**	**95% CI**
ALAG (hr)	1.45	0.08	1.31 – 1.59
KA (hr^{-1})	0.641	0.121	0.404 – 0.878
CL/F (L/hr)	2.03	0.09	1.85 – 2.21
V/F (L)	37.8	1.6	34.7 – 40.9
Additive residual error (mmol/L)	3.82×10^{-4}	5.80×10^{-5}	$2.68 \times 10^{-4} – 4.96 \times 10^{-4}$
Proportional residual error	0.144	0.012	0.121 – 0.167
ω^2 for ALAG	0.0541	0.0211	0.0127 – 0.0955
ω^2 for KA	0.27	0.16	0.00 – 0.58
ω^2 for CL	0.0485	0.0101	0.0287 – 0.0683
ω^2 for V	0.0268	0.0130	0.0013 – 0.0523
π^2 for CL/F and V/F	0.0271	0.0099	0.0077 – 0.0464
π^2 for KA	0.361	0.097	0.172 – 0.550

	Bootstrap resampling		Objective function mapping
Parameters	**Median**	**95% CI**	**95% CI**
ALAG (hr)	1.48	1.26 – 1.60	1.28 – 1.53
KA (hr^{-1})	0.637	0.446 – 0.978	0.506 – 0.832
CL/F (L/hr)	2.03	1.86 – 2.22	1.90 – 2.21
V/F (L)	37.8	34.3 – 44.5	35.3 – 42.0
Additive residual error (mmol/L)	3.74×10^{-4}	$2.49 \times 10^{-4} – 4.90 \times 10^{-4}$	$2.71 \times 10^{-4} – 5.33 \times 10^{-4}$
Proportional residual error	0.144	0.122 – 0.171	0.130 – 0.164
ω^2 for ALAG	0.0473	0.0164 – 0.1525	0.0236 – 0.1163
ω^2 for KA	0.231	0.001 – 0.753	0.044 – 0.502
ω^2 for CL	0.0457	0.0249 – 0.0656	0.0265 – 0.0882
ω^2 for V	0.0257	0.0008 – 0.1569	0.0071 – 0.0537
π^2 for CL/F and V/F	0.0278	0.0091 – 0.0537	0.0168 – 0.0421
π^2 for KA	0.351	0.185 – 0.560	0.228 – 0.611

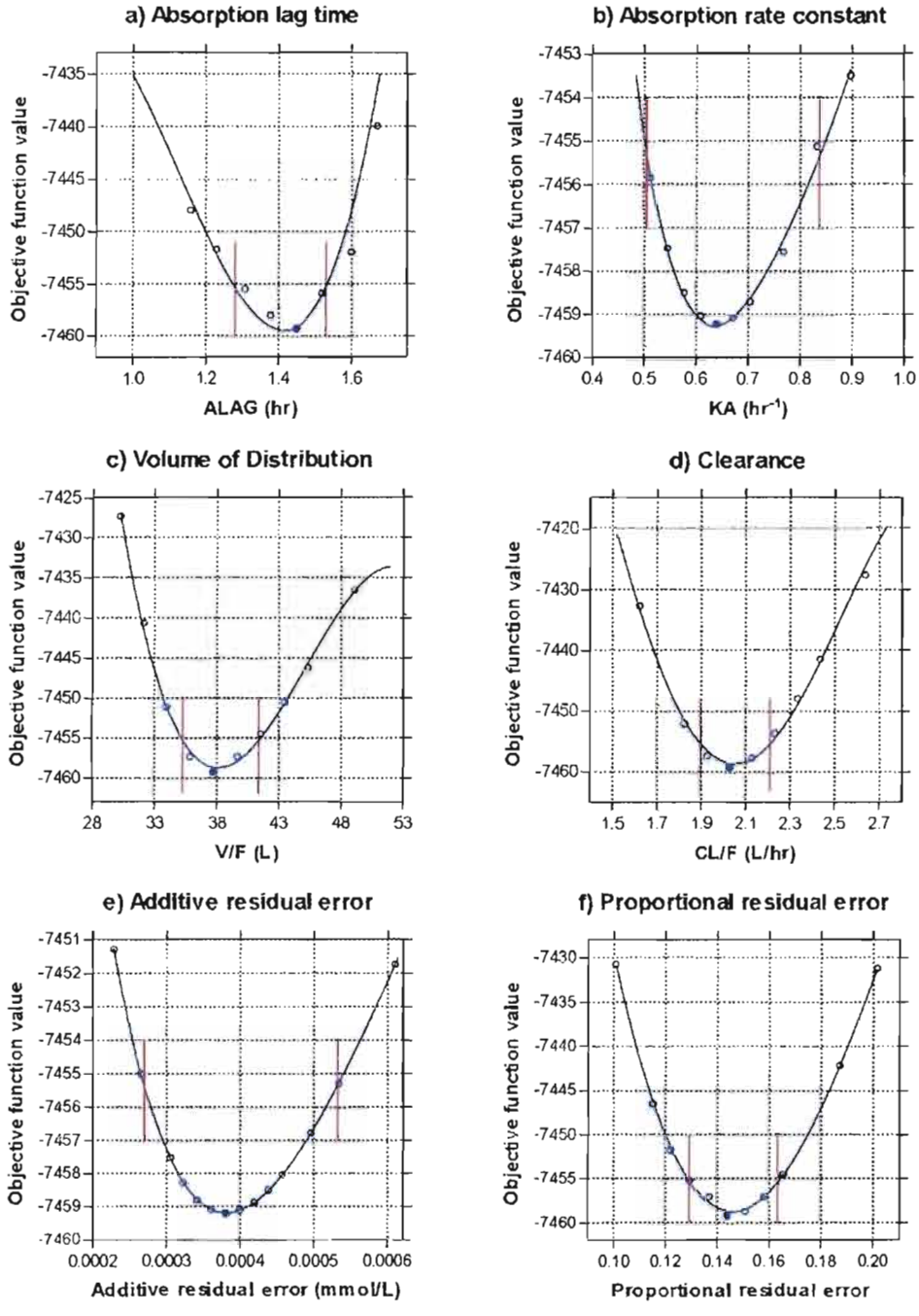


Figure 4.6 Objective function mapping of model parameters: a) absorption lag time b) absorption rate constant c) volume of distribution of the central compartment d) clearance from the central compartment e) additive residual error f) proportional residual error. Pharmacokinetic parameters are fixed to ± 5, 10, 15, 20, 30, and 40% of the population estimate and non-fixed parameters are re-estimated by NONMEM. The fixed parameters (open circles) are plotted against objective function value. The vertical red lines indicate the 95% confidence intervals corresponding to a change in objective function of 3.84.

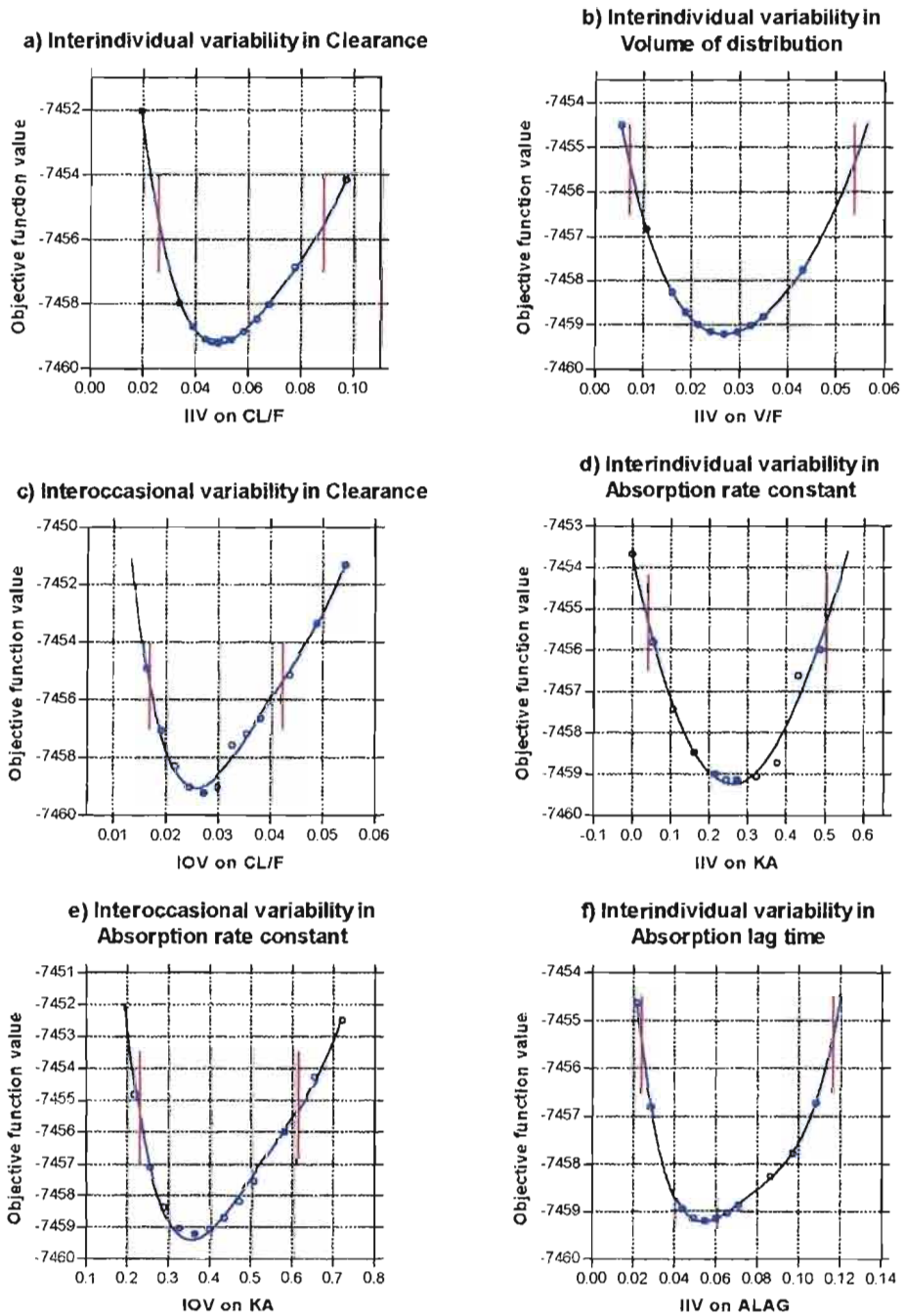


Figure 4.7 Objective function mapping of model parameters: a) interindividual variability on CL/F b) interindividual variability on V/F c) interoccasional variability on CL/F d) interindividual variability on KA e) interoccasional variability on KA f) interindividual variability on ALAG. Pharmacokinetic parameters were fixed to ± 5, 10, 15, 20, 30, and 40% of the population estimate and non-fixed parameters are re-estimated by NONMEM. The fixed parameters (open circles) are plotted against objective function value. The vertical red lines indicate the 95% confidence intervals corresponding to a change in objective function of 3.84.

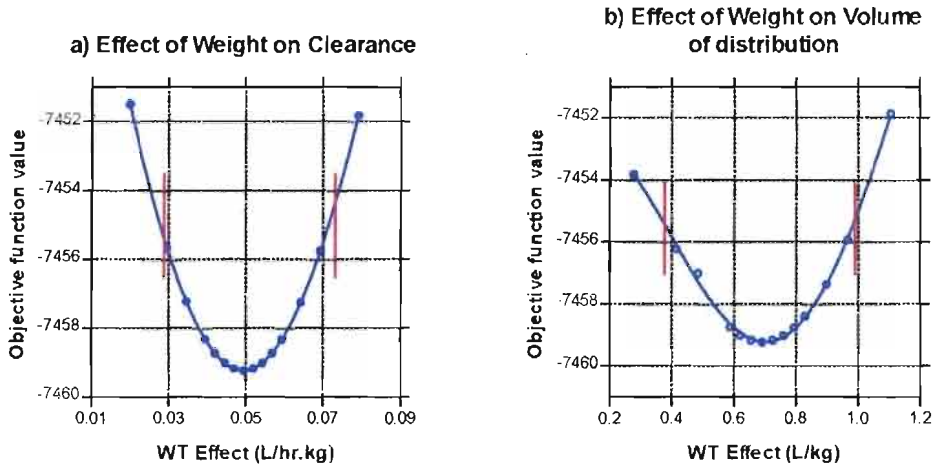


Figure 4.8 Objective function mapping of covariate model parameters: a) effect of weight on oral clearance of the parent compound b) effect of weight on the volume of distribution of the parent compound. Pharmacokinetic parameters were fixed to ± 5, 10, 15, 20, 30, and 40% of the population estimate and non-fixed parameters are re-estimated by NONMEM. The fixed parameters (open circles) are plotted against objective function value. The vertical red lines indicate the 95% confidence intervals corresponding to a change in objective function of 3.84.

4.5.2 Metabolite model

During initial model building the formation of the metabolite via the first-pass effect could not be characterised nor could the distinction be made between hepatic and non-hepatic clearance of the parent drug (Fig 4.2). A one-compartment model with linear elimination was found to be optimal for further modelling of the data. Use of an empirical model to determine the extent of the change in CL_{MET} between occasions improved the model fit (ΔOFV = -248) and showed a significant decline in clearance from occasion 1 to occasion 2. Of the various models tested to describe the shape of the change, the exponential decline over time model (Fig 4.9 Option 2) and the saturated clearance model (Fig 4.9 Option 4) provided equally good fits of the data. The saturation model was chosen based on physiological plausibility and the fact that it provided a maximal induced CL_{MET} value. Model parameters estimated were the metabolite volume of distribution of the central compartment (V_{MET}), the induced metabolite clearance (CL_{IND}) *i.e.* the clearance of the metabolite before study initiation, the baseline metabolite clearance (CL_{BASE}) *i.e.* the clearance at the end of the study period, the slope of the decline in clearance (SLP). Final population pharmacokinetic parameter estimates are presented in Table

4.4 and are accurately estimated with 7/10 parameters having relative standard errors under 12%.

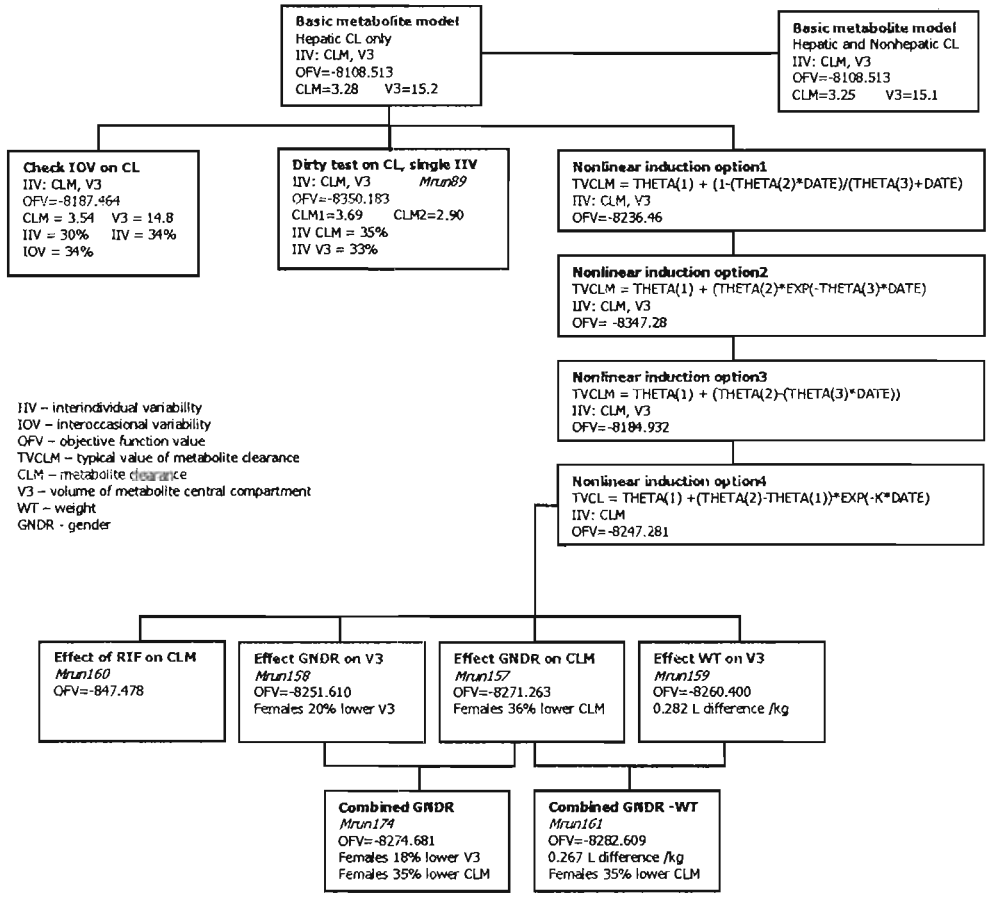
The CL_{MET} on day 1 for a male subject weighing 50 kg was estimated to be 6.74 L/hr. This value declined to 3.56 L/hr on study day 8. Variability between individuals in the parameters describing the pharmacokinetics of the 25-desacetyl rifapentine was 23% for V_{MET} and 36% for CL_{MET}. Inclusion of variability terms in other parameters were not supported by the data.

Basic goodness-of-fit plots of the population and individual predictions versus observed concentrations of the deacetylated metabolite are presented in Fig 4.10. Individual weighted residuals versus individual predictions and weighted residuals versus time are plotted in Figures 4.10c and 4.10d respectively.

During the initial covariate screening gender (GNDR) was found to affect both CL_{MET} and V_{MET} while weight (WT) was found to have a significant effect on V_{MET} only. The number of rifampicin doses prior to study initiation did not have a significant effect on CL_{MET}. During the forward inclusion step the combined effect of GNDR on CL_{MET} and WT on V_{MET} produced the biggest difference in OFV (ΔOFV = -35.33) and were retained in the model (Fig 4.9). Females had a 35% lower CL_{MET} than males and a difference of 0.267 L was observed in V_{MET} for each kilogram an individual's weight deviated, either up or down, from the mean value of 50 kg.

Figure 4.11 shows the plasma concentrations and model predicted values for a representative patient on two dosing occasions. It demonstrates that the model can account for variations in the plasma concentrations between occasions. Figure 4.12 - 4.13 show the objective function mapping for the parameters estimated from the final model. No local minimum was found in any of the parameters. The 95% confidence intervals of the objective function mapping and those of the final model coincided well (Table 4.5). Results from the bootstrap resampling are presented in Table 4.5 and coincide well with the NONMEM parameter estimates.

Individual plots of observed, population predicted and individual predicted 25-desacetyl rifapentine concentration-time data are presented in Appendix 3.2.



Dirty test on CL – separate population CL/F values were estimated for each dosing occasion
DATE – the number of the study day following the first dose administration

Figure 4.9 Flow diagram detailing the model building of the metabolite, 25-desacetyl rifapentine

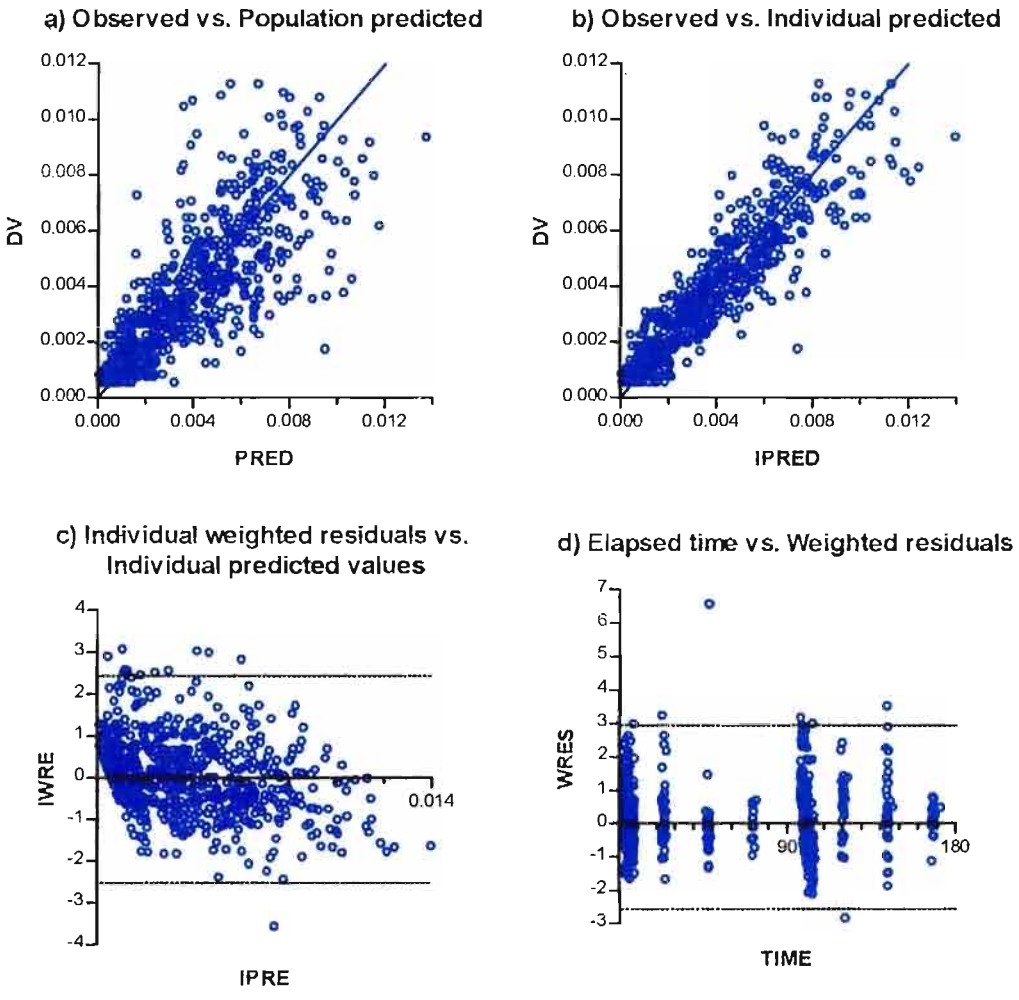


Figure 4.10 a) observed 25-desacetyl rifapentine concentrations vs. the population predictions b) observed 25-desacetyl rifapentine concentrations vs. individual predictions c) individual weighted residuals vs. the individual predictions d) weighted residuals vs. time elapsed over the study period. The solid blue line is the line of identity.

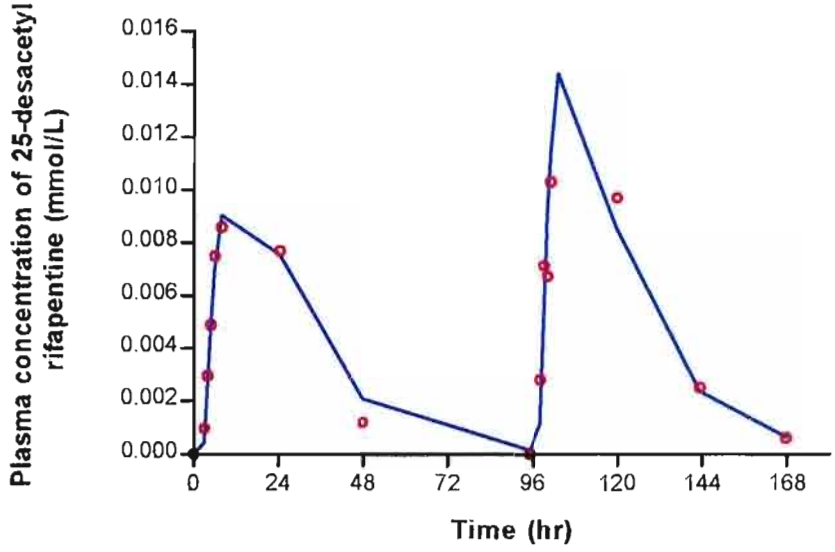


Figure 4.11 Individual predicted (solid blue line) and observed (open red circles) 25-desacetyl rifapentine molar concentrations in one representative patient given 750 mg of rifapentine orally on study days 1 and 5.

Table 4.4 Population pharmacokinetic parameters for 25-desacetyl rifapentine obtained from NONMEM

Parameter	Estimates (RSE*)	Interindividual variation % (RSE*)
V_{MET}/F (L)	11.6 (0.07)	23 (0.39)
Time-specific CL_{MET} (L/hr)¶	-	36 (0.27)
Baseline CL_{BASE} (L/hr)	3.56 (0.08)	
Induced CL_{IND} (L/hr)	21.0 (0.08)	
Slope of induction decline (hr^{-1})	1.7 (0.04)	
Additive residual error (mmol/L)	6.3×10^{-4} (0.09)	
Proportional residual error	0.196 (0.09)	
Effect of Gender on CL† (L/hr.kg)	0.647 (0.12)	
Effect of WT on V‡ (L/kg)	0.267 (0.36)	

* RSE = Relative standard error

¶ $TVCL_{metabolite} = TVCL_{baseline} + (TVCL_{induced} - TVCL_{baseline}) \cdot \exp(-Slope \cdot Day)$

† $(TVCL/F_i) = [TVCL_{baseline} + (TVCL_{induced} - TVCL_{baseline}) \cdot \exp(-Slope \cdot Day)] \cdot \theta_{GNDR}$

‡ $(V/F_i) = [TV(V/F) + \theta_{WTV/F} \cdot (WT_i - WT_{median})] \cdot \exp(\eta_i^{V/F})$

Table 4.5 Comparison of 95% confidence intervals estimated by standard errors of the NONMEM final estimates, bootstrapping and objective function mapping for 25-desacetyl rifapentine.

	NONMEM final estimate		
Parameters	**Mean**	**SE**	**95% CI**
V_{MET}/F (L)	11.6	0.83	10.0 – 13.2
Baseline CL_{BASE} (L/hr)	3.56	0.29	2.98 – 4.13
Induced CL_{IND} (L/hr)	21.0	1.6	13.2 – 24.2
Slope of induction decline (hr^{-1})	1.70	0.16	1.38 – 2.02
Additive residual error (mmol/L)	6.3×10^{-4}	5.6×10^{-5}	$5.2 \times 10^{-4} - 7.4 \times 10^{-4}$
Proportional residual error	0.196	0.017	0.163 – 0.229
Effect of Gender on $CL^{\dagger}$ (L/hr.kg)	0.647	0.078	0.495 – 0.799
Effect of WT on $V^{\ddagger}$ (L/kg)	0.267	0.096	0.046 – 0.079
ω^2 for Time-specific CL	0.131	0.035	0.062 – 0.199
ω^2 for V	0.0646	0.0206	0.0242 – 0.1050

	Bootstrap resampling		Objective function mapping
Parameters	**Median**	**95% CI**	**95% CI**
V_{MET}/F (L)	13.2	10.1 – 13.2	10.6 – 13.2
Baseline CL_{BASE} (L/hr)	3.57	2.91 – 4.15	3.14 – 4.07
Induced CL_{IND} (L/hr)	20.5	13.2 – 45.0	13.2 – 34.1
Slope of induction decline (hr^{-1})	1.67	1.18 – 2.56	1.22 – 2.47
Additive residual error (mmol/L)	6.24×10^{-4}	$5.3 \times 10^{-4} - 7.4 \times 10^{-4}$	$5.6 \times 10^{-4} - 7.1 \times 10^{-4}$
Proportional residual error	0.193	0.162 – 0.225	0.169 – 0.225
Effect of Gender on $CL^{\dagger}$ (L/hr.kg)	0.651	0.527 – 0.844	0.535 – 0.784
Effect of WT on $V^{\ddagger}$ (L/kg)	0.268	0.102 – 0.461	0.119 – 0.417
ω^2 for Time-specific CL	0.123	0.059 – 0.201	0.083 – 0.211
ω^2 for V	0.0585	0.0129 – 0.1510	0.0276 – 0.1229

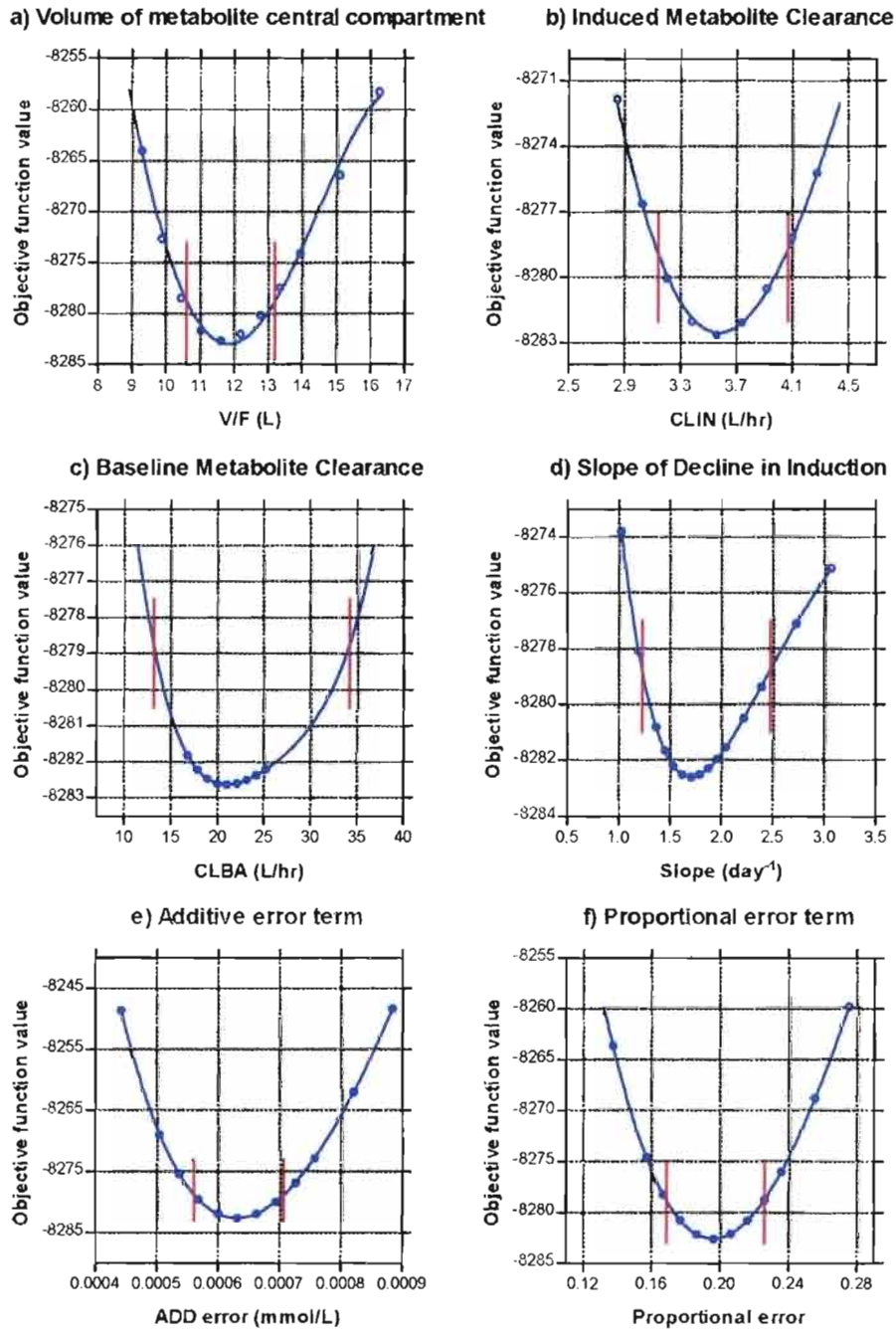


Figure 4.12 Objective function mapping of model parameters: a) volume of distribution of the central compartment b) induced clearance from the central compartment c) baseline clearance from the central compartment d) slope of induction decline e) additive residual error f) proportional residual error. Pharmacokinetic parameters were fixed to ± 5, 10, 15, 20, 30, and 40% of the population estimate and non-fixed parameters are re-estimated by NONMEM. The fixed parameters (open circles) are plotted against objective function value. The vertical red lines indicate the 95% confidence intervals corresponding to a change in objective function of 3.84.

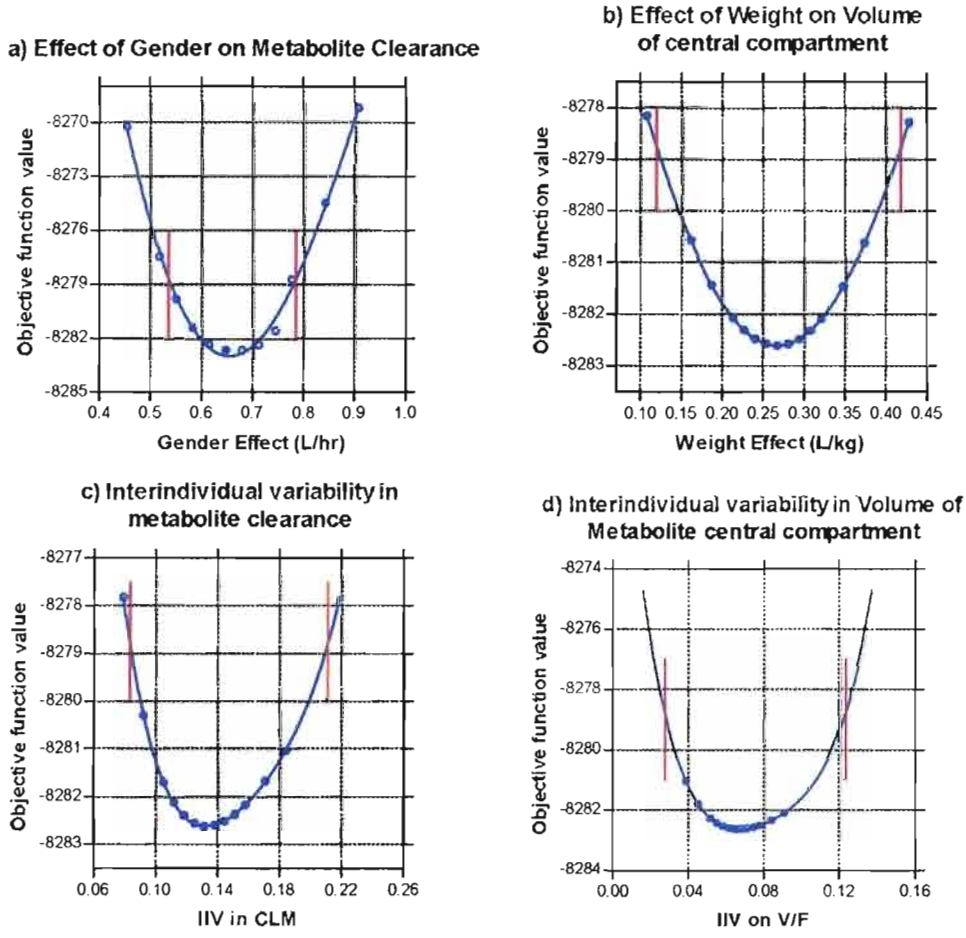


Figure 4.13 Objective function mapping of model parameters: a) effect of gender on clearance of the metabolite b) effect of weight on the volume of distribution of the metabolite c) interindividual variability in time-specific clearance d) interindividual variability in volume of distribution of the metabolite Pharmacokinetic parameters were fixed to ± 5, 10, 15, 20, 30, and 40% of the population estimate and non-fixed parameters are re-estimated by NONMEM. The fixed parameters (open circles) are plotted against objective function value. The vertical red lines indicate the 95% confidence intervals corresponding to a change in objective function of 3.84.

4.6 Discussion

There are only limited reports in the literature describing the pharmacokinetics of rifapentine in patients and the effect of potential covariates. The population pharmacokinetic approach enabled us to describe the compartmental pharmacokinetics of rifapentine and the primary metabolite 25-desacetyl rifapentine as well as characterise the degree of interindividual and interoccasional variability in the parameters of the patient population. Furthermore relationships between the parameters and covariates were established to further explain the variability between individuals.

The pharmacokinetics of rifapentine in the patient population were best described using a one-compartment model with an absorption lag time and first-order absorption and elimination. The final model included weight as the only significant covariate of CL/F and V/F and produced parameter estimates in close agreement with the data analysed by the noncompartmental approach. The variability in CL/F and V/F determined using the standard two-stage approach methodology was found to be between 32% (occasion 1) and 38% (occasion 2) for CL/F and between 29% (occasion 1) and 41% (occasion 2) for V/F. The population pharmacokinetic approach estimates this interindividual variability to be 22% for CL/F and 16% for V/F and the interoccasional variability to be 16% for both parameters. This low level of observed interoccasional variability and the lack of a significant difference in CL/F between occasion one and two supports the findings from the noncompartmental analysis that the prior administration of rifampicin for a period of between four and six weeks does not significantly alter the clearance of the parent drug. It must be emphasized though that the time between rifapentine doses (96 hours) may not be sufficient to capture a true difference if indeed it does exist.

A large degree of variability, both interindividual (52%) and interoccasional (60%), is observed with the absorption rate constant. The effect of concomitant food intake on the extent of absorption has been well described in previous studies (Chan *et al.*, 1994; Keung *et al.*, 1995; Keung *et al.*, 1999). There is, however, a lack of data regarding the effect of the time elapsed between food administration and drug ingestion. Although the minimum time elapsed between food and drug administration in this study was 30 minutes, the extension of this time window beyond 30 minutes was a consequence of logistical issues at the study site. The time elapsed

was however recorded and included as a continuous covariate to try and account of the variability in KA between individuals. The difference, although slight, was not significant. Reordering and inclusion of this elapsed time data as a categorical covariate (*i.e.* category 1: ≤ 40 minutes between food administration and drug ingestion, category 2: 40 – 50 minutes and category 3: ≥ 50 minutes) did not describe the data any better. No further covariate relationship was able to adequately explain or reduce this variability although a possible explanation may lie in the efflux pumps present in the gut wall.

The multi-drug resistant gene 1 (MDR1) product, P-glycoprotein (P-gp) (Loo and Clarke, 1999), functions as an efflux transporter and plays a pivotal role in the absorption and presystemic metabolism of drug agents by actively transporting substances back into the intestinal lumen (Hsing *et al.*, 1992). Large interindividual differences exist in the intestinal expression of P-gp (Lown *et al.*, 1997) and could be the result of genetic polymorphisms (Hoffmeyer *et al.*, 2000) or environmental factors as a number of drugs have been shown to induce P-gp expression (Arceci *et al.*, 1990; Zhao *et al.* 1993; Lown *et al.*, 1996; Seree *et al.*, 1998; Greiner *et al.*, 1999). This includes RIF which up-regulates intestinal expression by activation of the human orphan nuclear pregnane X receptor (PXR) (Gieck *et al.*, 2001). The mechanism of induction is the same as that responsible for the induction of CYP3A4 whereby RIF binds to and activates the PXR. The PXR then forms a heterodimer with the retinoid X receptor (RXR). This heterodimer complex in turn binds to a DNA response element and thus enhances transcription (Lehmann *et al.*, 1998). Full induction after starting daily RIF therapy is reached in approximately one week and returns to baseline two weeks after discontinuing therapy (Lee *et al.*, 1993). The effect of the induction of intestinal P-gp by RIF on the absorption kinetics of RPT was not examined in this study but could account, to a degree, for the large interindividual and interoccasional variability. However, it did not seem to have a negative impact on the extent of absorption as seen in the results from chapter 3.

Results from the noncompartmental approach showed considerable gender differences in the pharmacokinetic parameters, particularly on the second occasion. These differences may be expected because of the different proportions of adipose tissue in men and women combined with the lipophilic nature of the drug. Females in this study generally had a lower bodyweight (47.5 ± 6.1 kg) than their male counterparts (53.5 ± 8.2 kg) and thus smaller organ size and blood flow (Fletcher *et al.*, 1994; Beierle *et al.*, 1999). Results from our population analysis of the parent drug indicate that the differences in weight between individuals correlates better

with the differences in CL/F and V/F than discrete gender differences. This further supports the hypothesis that gender variation does not affect the rifamycin excretory pathways (Keung *et al.*, 1998). Furthermore, it is important to note that co-infection with HIV, prior administration of rifampicin, smoking, alcohol abuse and recreational drug use did not significantly affect the pharmacokinetics of rifapentine.

The pharmacokinetics of 25-desacetyl rifapentine in this study were best described by a one-compartment model with no first-pass metabolism and a clearance value that declined in a nonlinear fashion over time. The increased exposure of the metabolite on the second dosing occasion is thought to be related to a change in biliary excretion rather than to a change in hepatic metabolism of rifapentine as no significant change is observed in the pharmacokinetics of the parent drug over time. This assumption is based on the fact that the prior rifampicin administration (a minimum of 4 weeks daily therapy) is able to increase the capacity of the liver to excrete hydrophobic compounds into the bile. Substrate specificity studies have shown that at least four ATP-dependant transport systems are known to exist on the biliary canalicular side of the hepatocyte (Arias *et al.*, 1993). Of these transporters P-gp has been shown to play an important role in the transport of hydrophobic xenobiotics and is up-regulated in response to exposure of biliary excreted molecules, like RIF, or their metabolites (Gant *et al.*, 1995). This induction of hepatic P-gp by RIF is also caused by activation of the PXR as described above. Unfortunately there is no published data available documenting the effect of RPT on the induction of P-gp but if one takes into account that both rifamycins are potent inducers of CYP3A4 (Acocella G., 1978; Kenny and Strates, 1981; Aventis, 2000) and the fact that CYP3A4 and MDR1 are co-induced (Scheutz *et al.*, 1996) the it is not unreasonable to assume that RPT also possesses the ability to induce P-gp. However, the induction potential of RPT is less than RIF because of the intermittent dosing (Keung *et al.*, 1999) which would result in a net loss of P-gp induction over this study period. This loss of induction would therefore account for the occasional decline CL_M and the increased exposure of the metabolite on the second dosing occasion.

Furthermore gender was found to have a significant impact on the CL_{MET} with females demonstrating a 35% lower value than their male counterparts. The weight differences between the two gender groups could not account for the lower value which could point to an underlying genetic variation. A previous study by Schuetz *et al.* (1995) found that women

displayed only one-third to one-half the hepatic P-gp levels of men. Evidence from this study seems to support these findings and could account for the disparity between the genders.

The results of the model validation for both parent drug and metabolite confirmed that the parameter estimates were consistent and stable because the 95% confidence intervals calculated by the objective function mapping and the bootstrap resampling were in close agreement (Table 4.3 and 4.5).

In conclusion, a population pharmacokinetic model for rifapentine and its deacetylated metabolite was developed that incorporated the effect of weight on distribution and clearance parameters of the parent compound and the effect of gender on the clearance of he metabolite. Parameter estimates were in close agreement with those from the noncompartmental analysis and were consistent between individuals and between occasions.

Chapter 5

Research Summary, Conclusions and Perspectives

In terms of morbidity and mortality, tuberculosis is one of the world's most dangerous bacteriological diseases and seriously impacts on health and economic welfare in sub-Saharan Africa (Global Tuberculosis Control, WHO Report 2003). This has been recognised by the global health community and the acceleration of discovery and development of a new antimycobacterial agent has been initiated. Despite this, the earliest a new drug will be available is 2010 and until then the use of the therapeutic agents we have at our disposal needs to be optimised.

Rifapentine holds an advantage over rifampicin with its extended half-life and intermittent dosing. This however is negated by the poor treatment outcomes when rifapentine is dosed together with isoniazid during the continuation phase of therapy (Aventis Pharmaceuticals, 2000). The possible reasons for this are two-fold: firstly, rifapentine is highly protein bound (Reith *et al.*, 1998) which only allows a small unbound fraction to penetrate into the site of infection. Secondly, a suitable companion drug for rifapentine is lacking. The short half-life of isoniazid combined with the relatively long half-life of rifapentine essentially results in monotherapy for at least five of seven days during continuation phase therapy. This could account for the rifamycin monoresistance that emerged during the North American efficacy study (Vernon et al., 1999). Although the outlook for further development of rifapentine is uncertain at this stage, there is a need for studies that are able to shed more light on why rifapentine has not performed as well as was initially expected.

Our study was one of the first worldwide to effectively describe the pharmacokinetics of rifapentine in a representative tuberculosis patient population. Our objectives were as follows:

1. Obtain full pharmacokinetic profiles of patients receiving rifapentine on two occasions
2. Develop and validate a high pressure liquid chromatography assay for the estimation of plasma concentrations of rifapentine and 25-desacetyl rifapentine
3. Describe plasma levels of rifapentine and the desacetyl metabolite using traditional pharmacokinetic analysis
4. Develop a multi-compartmental model to describe the pharmacokinetics of rifapentine
5. Develop a multi-compartmental model to describe the pharmacokinetics of 25-desacetyl rifapentine

6. Characterise the degree of interindividual variability in rifapentine and 25-desacetyl rifapentine serum levels present in a patient population
7. Compare the degree of variability in patient levels to the variability observed in healthy volunteers for both the parent drug and metabolite
8. Identify potential factors influencing variation and quantify and describe their role

All eight of the stated objectives were met. Pharmacokinetic samples were collected intensively from 45 patients on two occasions in just over four months. A new assay for the simple and rapid detection of rifapentine and 25-desacetyl rifapentine was developed and validated and proved to be less labour and time intensive than previous published methods. The development of a compartmental model describing the pharmacokinetics of rifapentine and the primary metabolite allowed us to characterise the degree of interindividual, interoccasional and residual variability present in our patient population as well as identify factors responsible for this variability. Once the clinical, laboratory and pharmacokinetic elements of the project were completed we were in a position to answer our research questions.

1. What are the peak serum drug levels of rifapentine in a South African tuberculosis patient population?

The median peak serum levels observed in our patient population were 15.19 mg/L and 15.56 mg/L on occasions one and two respectively. A recent study published in February of this year by the Tuberculosis Trials Consortium (TBTC) (Weiner *et al.*, 2004) examined the pharmacokinetics in 35 patients receiving various doses (600 mg, 900 mg and 1200 mg) of rifapentine in conjunction with isoniazid during the continuation phase of therapy. Results from their study showed mean C_{max} levels ranging from 12.2 mg/L for the 600 mg dose to 14.6 mg/L for the 900 mg dose and up to 18.6 mg/L for the 1200 mg dose. Elimination half-life was not significantly different across the dosage groups and was also similar to the value found in our patient population. Rifapentine $AUC_{0-\infty}$ from the TBTC study was significantly associated with plasma albumin concentration, gender and race. Results from our study were not linked to either dose or race. This finding was not unexpected as the dose patients in our study received was stratified according to weight and resulted in a narrow dose/kg range ($\approx$ 15 mg/kg). Based on the median weight in the TBTC study (65 kg) the dose/kg range possibly extended from 10 mg/kg to 20 mg/kg and could account for the differences observed in the three dosage groups. Gender differences were observed in both studies although there is disagreement with respect to the association with plasma

albumin concentrations. This could point to a limitation in our study as the majority of our patients demonstrated albumin levels below the normal range, and secondly these concentrations were all within a narrow range. This lack of range could have prevented the elucidation of a difference, if indeed it does exist.

A further important finding of the TBTC study was that 54% of patients had unbound rifapentine and 25-desacetyl rifapentine concentrations detected for more than 36 hours after the clearance of concurrently administered isoniazid. The amount of free drug was not measured but rather calculated as 2% and 6.9% of total plasma rifapentine and metabolite concentrations respectively. This, however, is based on the assumption that the amount of free drug is consistent over the measured time period. Because rifapentine is extensively bound to serum albumin (Reith *et al.*, 1998) the possibility does exist that the serum protein becomes saturated at higher concentrations of parent drug and metabolite in tuberculosis patients, who, typically have lower albumin levels. This hypothesis requires further examination but would provide valuable insight because if it were true then rifapentine and 25-desacetyl rifapentine concentrations above a critical threshold would allow the possibility of additional unbound drug being able to circulate and penetrate into the site of infection. Even a 2% increase would double the amount of free drug available. Furthermore, it would shed light on the question if indeed the high protein binding is partly responsible for the limited therapeutic success of rifapentine.

2. *What is the degree of variability in serum drug levels between individuals and between occasions?*

Variability in rifapentine pharmacokinetic parameters both between individuals on a single occasion and in a single individual between dosing occasions was found to be low. Interindividual variability in rifapentine C_{max} and AUC calculated using the two-stage approach was less than 35%. Interindividual variability in CL/F and V/F was close to 23% and the variability in these parameters between occasions was 16%. These values compare favourably to those observed in healthy volunteer studies with rifapentine where data was collected under more standardised conditions. When comparing these results to other studies involving rifampicin we see much higher coefficients of variation. These typically range from 30% (Peloquin *et al.*, 1997; Peloquin *et al.*, 1999) to 60% (Pargal and Rani, 2001) in healthy volunteers and could yet be higher in tuberculosis patients. Although this variability in

rifampicin pharmacokinetic metrics has not yet been explicitly defined in a tuberculosis patient population the extensive hepatic, intestinal and autoinductive properties of rifampicin could provide an explanation for the disparity between itself and rifapentine.

3. What are the factors that contribute to the variability?

One of the main aims of this project was to describe and quantify the role of covariate factors that affect the pharmacokinetics of rifapentine. Previous studies found that significant gender differences exist (Aventis Pharmaceuticals, 2000; Peloquin *et al.*, 2004). Results from our data analysis found that the weight difference between males and females is responsible for the discrepancy with the decreased CL/F in females correlating to a decreased V/F. Furthermore gender differences were also found to be significant with respect to the clearance of the metabolite. This could be linked to the lower levels of P-glycoprotein found in females (Schuetz et al., 1995) which results in a reduced biliary excretion.

It is also interesting to note that HIV co-infection was not correlated to decreased serum levels. One has to question our relatively small numbers (14 HIV positive versus 22 HIV negative) and whether the analysis was adequately powered to detect a difference. A study enrolling a larger number of patients is required to determine if indeed a difference does exist. Including subjects at various stages of HIV disease would further strengthen a study of this nature and would hopefully shed some light on why HIV positive subjects are at an increased risk of developing rifamycin monoresistance (Vernon *et al.*, 1999) when receiving rifapentine during the continuation phase of therapy.

In conclusion this thesis showed for the first time that in South African pulmonary tuberculosis patients rifapentine is well absorbed and shows limited variability, both between individuals and between occasions. These findings combined with the other advantages rifapentine holds over rifampicin suggest that once an optimal dose and companion drug are established, rifapentine may become the rifamycin of choice for the treatment of pulmonary tuberculosis.

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Appendix 1

Clinical Protocol, Information leaflet and Consent form

Appendix 1.1 Protocol Outline

Title

Investigation of interindividual variation in rifapentine plasma levels in patients at DP Marais Hospital.

Aim

Our aim is to describe the pharmacokinetics of two consecutive doses of rifapentine equivalent to 15 mg/kg in pulmonary tuberculosis patients during the intensive phase of treatment and investigate whether the values of the parameters obtained are reproducible between individuals and between occasions.

Objectives

- Obtain a full plasma pharmacokinetic profile of patients receiving rifapentine on two occasions
- Characterise the degree of interindividual variability in rifapentine serum levels present in a clinic patient population
- Compare the degree of variability in rifapentine levels to the variability observed in healthy volunteers
- Identify potential factors influencing variation
- Quantify and describe the role of determining factors

Sample size

60 volunteers, 18 years and older

Medication/dosage

INN:	Rifapentine
Trade Name:	Priftin®
Dosage form:	150 mg film coated tablet
Dose:	28-35kg 450 mg

	36-45kg	600 mg
	46-55kg	750 mg
	>56kg	900 mg
Batch:	All of the same batch to be supplied by Aventis	
Route:	Oral	
Duration of treatment:	single dose	

Safety data

1. Clinical examination at screening
2. Vital functions

   Blood pressure, heart rate and temperature at screening and before each drug administration
3. Recording of adverse events: throughout the study
4. Laboratory tests
   - AST, ALT, AP, Total bilirubin:
   - Haematology, full blood count and differential: on first study day
   - Urine screen for drug abuse
   - HIV test

Assay method

Concentrations of rifapentine and the active metabolite, 5-desacetyl rifapentine, in plasma and urine, by HPLC methods

Study durations and dates

Clinical part of study April 2003 to August 2003

Study design

A prospective cohort pharmacokinetic study will be carried out by the Tuberculosis Unit of the Department of Pharmacology, University of Cape Town.

Our method requires that subjects who have been on a multiple drug regimen including rifampicin have a single 600 mg rifampicin dose replaced with a 15 mg/kg dose of rifapentine

on the day of the study. The patients will then receive their usual TB treatment minus rifampicin for the next three days. On day four they would be required to repeat the procedure and allow for sampling on a second occasion.

Patients who have been receiving daily antimycobacterial treatment containing rifampicin for a period of not less than 4 weeks and not more than 6 weeks will be recruited from DP Marais Hospital. This allows the patients to be suitably induced and relatively stable. The patients will be sampled in their wards on the respective study days.

A group of patients will be recruited over a three-week period. The study protocol will be discussed with them one week after they have been diagnosed with pulmonary tuberculosis and have started taking their antimycobacterial therapy.

Table 3. Proposed method of recruitment of patients over a three-week period.

Week							
1	2	3	4	5	6	7	8
Diagnosis	Recruitment Diagnosis	Recruitment Diagnosis	Recruitment			Sampling	Sampling

Selection of subjects

Because data regarding the variability between patients receiving rifapentine is not available to us, an estimation of sample size was made using the variability observed in patients receiving rifampicin. Using a pilot study of 12 patients it was estimated that 30% of patients would have variable serum levels of rifampicin across different sampling occasions. Therefore, to achieve a 90% confidence interval, it was estimated that 60 patients would be required.

A group of 60 patients will be recruited over a twenty-week period. The study protocol will be discussed with them one week after they have been diagnosed with pulmonary tuberculosis and have started taking their antimycobacterial therapy. After 15 patients have been sampled, an interim analysis will be carried out. The preliminary information on variability will make it possible to anticipate certain flaws in study design as well as informative ones. It will also allow us to optimise design features for accurate and precise estimation of population pharmacokinetic parameters and final sample size.

Inclusion criteria

Patients meeting all of the following criteria will be considered for admission to the study:

- Patients diagnosed with pulmonary tuberculosis
- Patients that have been receiving antimycobacterial therapy (including rifampicin) for not less than 4 weeks and no more than 6 weeks
- Between 18 and 65 years of age
- Patients have given their written informed consent to participate in the study

Informed consent must be obtained for all subjects before enrolment in the study.

Exclusion criteria

- History of hypersensitivity to any rifamycin antibiotics
- Known resistance to rifampicin before study initiation
- Contraindication to multiple blood sampling, e.g. mental confusion or poor venous access
- Blood donation of more than 500ml during the previous month
- Mental capacity is limited to the extent that the subject cannot provide legal consent or information regarding the side effects or tolerance of the study drug
- Subject unlikely to comply with protocol, e.g. uncooperative attitude, inability to return for follow-up visits, and unlikely to complete the study

Any waiver of these inclusion and exclusion criteria must be approved by the investigator on a case-by-case basis prior to enrolling the subject. This must be documented by the investigator.

No subject will be allowed to enrol in this study more than once.

Study Treatments

Details of study treatments

Dosage form

INN: Rifapentine

Trade Name: Priftin®

Dosage form:	150mg film coated tablet	
Dose:	28-35kg	450 mg
	36-45kg	600 mg
	46-55kg	750 mg
	>56kg	900 mg
Batch:	All of the same batch to be supplied by Aventis	
Route:	Oral	
Duration of treatment:	Single dose	

At each of the two treatment periods each subject will receive a single dose of Priftin® after ingestion of a low fat, high fluid content breakfast (soup based).

Dosage schedule

Each subject will receive two treatments. After an overnight fast, subjects will eat a standardised meal on the day of drug administration. A single dose of Priftin® will be swallowed with 100ml of water 30 minutes later.

Details of the exact dose and time of medication will be documented in the case report form.

Treatment assignment

The study medication will be administered only to subjects included in this study following the procedures set out in this protocol.

After fulfilling the inclusion and exclusion criteria, subjects will be numbered consecutively from 1 to 60. Each subject must be given only the medication carrying his/her number. Subjects withdrawn from the study retain their subject number. New and replacement subjects will always be assigned a new subject number.

Packaging, and labelling

The study medication will be labelled and packed in the Pharmacology Division of the University of Cape Town at Groote Schuur Hospital.

For each subject, the packaging will be labelled as follows:

M00002707 – GL24

RPT Dose

Subject number

Treatment period i.e. visit 1 or 2

Two sets of replacement treatments for each weight bracket will be prepared, except that the labels will mention neither subject nor visit number. The investigators will supervise administration of the study medication.

Supplies and accountability

The investigator will keep an inventory and acknowledge receipt of all shipments of study medication. The study medication will be stored in accordance with the manufacturer's instructions. The investigator will also keep records of the quantities of study medication dispensed and used by each subject. At the conclusion of the study all unused study medication will be returned to Aventis.

Prior and concomitant illnesses and treatment

Prior and concomitant illnesses

This study will be performed in patients diagnosed with pulmonary tuberculosis. Illnesses first occurring or detected during the study are to be regarded as adverse events and must be documented as such in the case report form.

Prior and concomitant treatments

Any treatment that is given in addition to the study treatment during the study is regarded as a concomitant treatment and must be documented in the case report form. If the administration of any concomitant treatment becomes necessary, it must be reported in the case report form and in the subject's medical records. As far as possible, no changes (dose, frequency) should be made to the concomitant treatment during the study.

Study Procedures and Schedule

Overview of data collection

During screening; consent, medical history and a physical examination will be performed. The results will be documented in the case record form supplied. Information will be collected as described in this protocol. The subject will remain anonymous and will only be referred to by a number and initials in any data collected.

Prestudy screening

The following tests and examinations will be carried out in each subject during the 4 weeks before the study:

- Physical examination and medical history, including age, sex, and race
- Height and weight
- Blood pressure and heart rate
- Oral body temperature

Study days

The following tests and examinations will be carried out on the first study day:

- Haematological status (haemoglobin, haematocrit, RBC, WBC, differential count and platelet count)
- Blood chemistry (AST. ALT, AP, total bilirubin, total protein, albumin)
- Urinalysis (screening for possible drug abuse)
- HIV test

Pre-test counselling which includes information regarding the HI virus/AIDS, ways of spreading, risk factors and consequences will be provided verbally by a trained counsellor on the day of the first sampling occasion. Patients will be informed as to their HIV status at the end of the second sampling occasion (72-hour blood) by a trained professional. For individuals testing positive, follow-up will be made available at the HIV Clinic.

On the morning of the evaluation day subjects who qualify to enter the study will have the study schedule explained to them by the investigator. The subjects will be receive a standardised breakfast at 07h30. Subjects will consume the whole breakfast and then receive

the test drug at 08h00. Subjects will consume a standard lunch at 13h00. Contents of all snacks and meals will be recorded in the study report.

Blood samples for the determination of the plasma concentration-time profiles of rifapentine and its primary metabolite 25-desacetyl rifapentine will be collected by venipuncture into lithium-heparin coated tubes at the following times:

1) 60 to 15 minutes prior to drug administration (10 ml)
2) 2.0 hours (10 ml)
3) 3.0 hours (10 ml)
4) 4.0 hours (10 ml)
5) 5.0 hours (10 ml)
6) 6.0 hours (10 ml)
7) 8.0 hours (10 ml)
8) 24.0 hours (10 ml)
9) 48.0 hours (10 ml)
10) 72.0 hours (10 ml)

Methods of evaluation

The blood samples will be taken in lithium-heparin coated tubes and centrifuged at 2500 rpm for 10 minutes within 1 hour. Between blood sampling and centrifugation, tubes containing blood will be kept out of direct light on crushed ice. Plasma samples will be transferred into two dry polypropylene tubes and stored at –80°C out of light until assay.

The label on each tube will state: M00002707-GL24, Subject number, Sampling time and date.

The determination of rifapentine and 25-desacetyl rifapentine will be done by a validated HPLC method. The assays will be performed by the Division of Pharmacology at the University of Cape Town.

Haematology, blood chemistry, and urinalysis will be carried out according to standard operating procedures by the validated laboratory of the University of Cape Town.

Adverse events

Definitions

Adverse events spontaneously reported will be recorded throughout the study.

The term adverse event covers any sign, symptom, syndrome, or illness that appears or worsens in a subject during the period of observation in the clinical study and that may impair the well being of the subject. The term also covers laboratory findings or results of other diagnostic procedures that are considered to be clinically relevant.

The adverse event may be:

- A new illness
- Worsening of a sign or symptom of the condition under treatment or of a concomitant illness
- An effect of the study medication
- Unrelated to participation in the clinical study
- A combination of one or more of these factors

Thus no causal relationship with the study medication is implied by the use of the term "adverse event".

Adverse events fall into the categories nonserious and serious.

Serious adverse event

A serious adverse event is any adverse event that at any dose of the study medication or at any time during the period of observation:

- Results in death
- Is immediately life threatening
- Requires or prolongs hospitalisation
- Results in significant disability and incapacity
- Occurs with overdose
- Involves cancer
- Involves congenital anomaly
- Is medically important

- Requires medical intervention to prevent permanent impairment or damage

"Medically important events" are events that may not be immediately life threatening or result in death or hospitalisation, but may jeopardise the patient or require intervention to prevent one of the other outcomes listed in the definition above. "Events requiring medical intervention to prevent permanent impairment or damage" are events where the investigator believes that medical or surgical intervention is necessary to preclude permanent impairment of a body function or to prevent permanent damage to a body structure.

As there is an overlap between the terms "medically important event" and "events requiring medical intervention to prevent permanent impairment or damage", it is left to the discretion of the investigator to select the more applicable of the two criteria when completing the "Serious adverse events" form.

Period of observation

The period of observation extends from the time the subject gives informed consent until 72 hours after the last dose of medication has been administered.

Documentation and reporting of adverse events by investigator

All adverse events that occur after the subject has signed the informed consent form must be documented in the case report form in accordance with the "Instructions for the completion of adverse event reports in clinical studies" in the investigator-training manual. The adverse events must also be recorded in the subjects' medical records.

The following approach will be taken to documentation:

- All serious adverse events must be documented on "Serious adverse event forms"
- Nonserious adverse events that occur after the first dose of study medication must be documented on adverse events forms.

For each event occurring after the first dose of study medication, the investigator will classify whether the event is to be considered treatment emergent and whether there is a reasonable possibility that the event was associated with the use of the study medication.

An adverse event that occurs during the study after the first dose of study medication will be considered to as treatment emergent if (1) it was not present at the time of the first administered dose of study medication and is not a chronic condition that is part of the patients medical history, or (2) it was present at the time of the first dose of study medication or as a part of the patients medical history, but its intensity (severity or frequency) has worsened after the first dose of study medication.

Adverse events that fulfil the criteria for seriousness have to be reported to Aventis immediately. Adverse events that do not fulfil the criteria for immediate reporting will be supplied to Aventis in the same way as all other case report form pages.

All subjects who experience adverse events – whether considered to be associated with the study drug or not – must be monitored to determine the outcome. The clinical course of the adverse event will be followed up – even after the end of the observation period – until a satisfactory explanation is found or the investigator considers it is medically justifiable to terminate follow-up. Should the adverse event result in death, a full pathologists report should be supplied if possible.

Immediate reporting by investigator to sponsor

Serious adverse events must be documented on "Serious adverse event" forms and supplied to Aventis within 24 hours or on the following working day. The sponsor will ensure that all legal reporting requirements are met.

The initial report must be as complete as possible, including details of the current illness and (serious) adverse event and an assessment of the causal relationship between the event and the study medication. Copies of the case report form pages containing the following information must be sent with the "Serious adverse event" form:

- Demography
- Medical and surgical history
- Previous and concomitant medication
- Study medication administration record

The forms documenting all non-serious adverse events that have occurred up to the time of occurrence of a serious adverse event, even if still incomplete, must also be sent with the "Serious adverse event" form.

Information not available at the time of the initial report must be documented on a follow-up "Serious adverse event" form that carries the number(s) of the initial report(s).

Withdrawal of subjects

Subjects may be withdrawn from study medication for the following reasons:

- At their own request or at the request of their legally authorised representative without prejudice to him or her for doing so
- If, in the investigators opinion, continuation in the study would be detrimental to the subject's well-being
- At the specific request of Aventis

Subjects must be withdrawn from study medication under the following circumstances:

- Severe adverse events believed to be drug related

In all cases the reason for withdrawal must be recorded in the case report form and in the subject's medical records. The subject must be followed up to establish whether the reason was an adverse event, and, if so, this must be reported in accordance with the procedures set out in *Section 8.*

Volunteers who are withdrawn or who withdraw will be replaced to maintain the final sample size of 60 subjects.

Emergency Procedures

During and following a subject's participation in the trial, the investigator should ensure that adequate medical care is provided to a subject for any adverse events, including clinically significant laboratory values, related to the trial. The investigator should inform a subject when medical care is needed for intercurrent illness of which the investigator becomes aware.

Statistical procedures

Analysis variables

On each series of the plasma concentrations the following kinetic parameters will be calculated:

- Plasma peak concentration (Cmax)
- Time to plasma peak concentration (Tmax)
- Apparent half-life ($t_{1/2}$)

A noncompartmental or population approach will be used for data analysis. Serum concentration-time curves will be plotted for all patients at all occasions in order to determine the maximal observed serum concentration (C_{max}) and the time to reach this concentration (T_{max}). The terminal half-life will be calculated according to the following formula:

$t_{1/2} = \ln 2/z = 0.693/z$ where z is the terminal rate constant

Pharmacokinetic parameters will be calculated using the WinNonLin software package. The measurement of the effect of the below-mentioned covariates on the patient pharmacokinetic profiles will be made using NONlinear Mixed-Effect Modelling (NONMEM) software package.

Covariates

Interindividual variability in patient pharmacokinetic (PK) and pharmacodynamic (PD) parameters is the result of a number of fixed and random effects. The fixed effects draw a correlation between covariates and PK-PD parameters. The interindividual random effects quantify the residual unexplained variability.

The task of finding an adequate population model is two-fold:

1) finding covariates that significantly influence the PK-PD parameters
2) determining the shape of the relationship between covariates and parameters

The following covariates will be investigated. Body mass index, sex, ethnic group, use of either tobacco, alcohol or cannabis. Concomitant use of other drugs or traditional medicines and diet. Haematological and other serum markers including urea, creatinine, total protein, albumin, total bilirubin, ALT, AST, alkaline phosphatase, haemoglobin, red cell count, haematocrit, RDW,

white cell count and platelet count. . The effect of HIV and TB co-infection on rifapentine pharmacokinetics in patients will be investigated.

Ethical and legal issues

Good clinical practice

The procedures set out in this study protocol, pertaining to the conduct, evaluation, and documentation of this study, are designed to ensure that the investigators abide by the principles of the good clinical practice (GCP) guidelines of the European Community. The study will be carried out in keeping with the South African legal requirements and the Ethical principles laid down in the Declaration of Helsinki, Hong Kong Amendment 1989.

Delegation of investigator responsibilities

It is the responsibility of the principle investigator to inform the trial assistants about the protocol as well as any amendments that may have been made. They should also be adequately informed about their duties and functions within the scope of the trial.

A list should be set up by the investigator stating the responsibilities of each co-investigator and other appropriately qualified persons to whom trial-related duties have been assigned.

Subject information and informed consent

A subject may not be admitted to the clinical study unless he/she has been fully informed and understands the nature, scope and possible consequences of participating in the study.

An informed consent document will be handed out to each subject and will contain information regarding the study that is easily understandable and in their language of preference. After the subject has read or had the informed consent document read to them, they must consent to participate both verbally and in writing. The subject's consent must be confirmed at the time of consent by the personally dated signature or thumbprint of the subject or the subject's legally authorized representative and by the personally dated signature of the person conducting the informed consent discussions.

A copy of the signed consent document will be given to the subject, while the investigator will hold the original.

The investigator will not undertake any measures specifically required for the clinical study until full informed consent has been obtained.

Confidentiality

Subject names will not be supplied to Aventis. Only the subject code (subject number and initials) will be recorded in the case record form, and if the subject's name appears on other documentation it must be deleted before the document is supplied to Aventis. The subjects will be told that representatives from Aventis or regulatory authorities may inspect their medical records to verify the information collected, but that their personal details will not be divulged. Only the investigator will maintain a personal identification list corresponding to subject names to enable records to be identified.

Subject remuneration

Subjects will be remunerated for inconvenience as a direct result of the study. *Bona fide* withdrawal will result in an adjusted *pro rata* remuneration. Protocol violation (that is, wilful violation on the patient's behalf) may be terms for subject withdrawal and *pro rata* remuneration will be adjusted if not forfeited completely.

HIV pre- and post-test counselling

All subjects are required to give informed consent to participate in the study which will include screening for HIV. Pre-test counselling which includes information regarding the HI virus/AIDS, ways of spreading, risk factors and consequences will be provided verbally by a trained councillor on the day of the first sampling occasion. Patients will be informed as to their HIV status at the end of the second sampling occasion (72-hour blood) by a trained professional, who will provide post-test counselling regardless of the outcome of the test. For individuals testing positive, follow-up will be made available at the Groote Schuur Hospital HIV Clinic.

Protocol amendments

No protocol amendments will be carried out without the written consent of both the relevant regulatory authority and the University of Cape Town's Ethics committee. Once the study has commenced amendments will only be made in exceptional cases.

Approval of the study protocol

Before the start of the study, the study protocol, informed consent document, and any other appropriate documents will be submitted to the Medicines Control Council (MCC) and the Ethics Committee (EC) of the University of Cape Town.

Aventis will only provide study medication to the investigator once approval has been obtained from the MCC and the EC, and all legal and ethical requirements have been met. Formal approval should mention the study title, study code, study site, date of approval and any other documents reviewed.

It is the responsibility of the investigator to keep a record of all communication with the relevant regulatory authorities.

Premature closure of the study

Aventis and the investigator reserve the right to close the study at any time after mutual consultation between the two parties. If an unacceptable number of patients experience serious adverse effects the study will be discontinued.

Should the study be discontinued prematurely all materials (study medication, etc.) supplied by the company will be returned as if the study had been completed.

Liability and insurance

Subjects will be covered by an insurance policy from the time of consent to the end of the observation period. This policy will cover, in its terms and provisions, the investigator's legal liability for injuries caused to participating persons and arising out of research performed strictly in accordance with the scientific protocol as well as with applicable law and professional standards.

Study Monitoring and Auditing

Monitoring and auditing procedures will be followed, in order to comply with GCP guidelines. The study will be monitored by Assoc. Prof. Peter Smith, Division of Pharmacology, University of Cape Town. Monitoring will include checking of the case record forms for completeness and clarity, cross checking with source documents as well as ensuring the study is conducted according the procedures laid down in the protocol.

Documentation and use of study findings

Documentation of study findings

A case report form will be provided for each subject.

All protocol-required information collected during the study must be entered by the investigator, or designated sub-investigator, in the case report form. The case report pages should be completed as soon as possible after the information is collected. An explanation should be given for all missing data.

The completed case report form must be reviewed and signed by the investigator or a designated sub-investigator.

The following records will be retained by the investigator for 2 years after the completion or termination of the study:

- Signed informed consent documents for all subjects
- Subject identification code list
- Records of communications with the Ethics Committee
- Records of communications with the Medicines Control Council
- A list of investigators and assistants with their delegated duties and signatures
- Copies of case report forms and of documentation of corrections for all subjects
- An inventory of drugs received and dispensed
- Record of any body fluids or tissue samples retained
- Source documents and laboratory result records

Use of study findings

All information concerning the product as well as any matter concerning the operation of Aventis, such as clinical indications for the drug, its formula, methods of manufacture and other scientific data relating to it, that have been provided by Aventis and are unpublished, are confidential and must remain the remain the sole property of Aventis. The investigator will agree to use the information only for the purposes of carrying out this study and for no other purpose unless prior written permission is obtained from Aventis.

The findings of the study may be published in a scientific journal or presented at a scientific meeting, provided the manuscript for publication or presentation is submitted to Aventis and the investigator allows Aventis 30 days in which to review and comment on the manuscript.

Appendix 1.2 Subject Information Form

Study title

Investigation of interindividual variation in rifapentine plasma levels in patients at DP Marais Hospital.

Why are we doing the study?

Rifapentine is a tablet that has been made to treat patients with tuberculosis. After you take the tablet the drug goes into the blood. Because all people are different the amount of drug in your blood will be different to the other patients. What we want to see is how much drug you have in your blood and compare it to other patients as well as to healthy people who don't have tuberculosis and see how different it is.

When and where will we be doing the study?

The study will last eight days at DP Marais Hospital from the first time you take the rifapentine tablet. You must not eat breakfast on the study days. We will give you breakfast at the hospital. Before breakfast a needle will be put into your arm and will be left in for 8 hours while blood is being taken. After breakfast you will have to take your isoniazid (INH), pyrazinamide (PZA), and ethambutol (ETH) tablets **but not the rifampicin tablet**. You will take rifapentine in the place of the rifampicin tablets. Blood will be taken after 2, 4, 5, 6, 7, 8, 24, 48 and 72 hours. The needle will be removed from your arm after the 8hour blood sample is drawn that afternoon. For the next three mornings one blood sample will be taken and the same procedure will be repeated on the fourth day. **You must carry on taking isoniazid (INH), pyrazinamide (PZA) and ethambutol (ETH) tablets during that week. Only three days after the last study day at the hospital must you start taking your rifampicin (RIF) again.**

Three days later the same thing will be done again. You will be given breakfast again and blood will be taken the same way as before. For the next three mornings one blood sample will be taken.

You must carry on taking isoniazid (INH), pyrazinamide (PZA) and ethambutol (ETH) tablets during the week after your last visit. Only three days after your last study day at the hospital must you start taking your rifampicin (RIF) again.

Taking part in the study

You are allowed to stop being in the study at any time. Nobody will be angry and nothing will be happen to you if you stop. If you want to stop you don't have to tell us why. You just have to say "no." If you carry on with the study you must listen to the doctors and do as they ask. The study will not make you get better faster but it will help the community because it will help us to give them better medicine.

Subject remuneration

You will be paid money for the time you spend in the study. This will be paid at the end. If you deliberately go against what the investigator asks you to do according to the trial, you may be withdrawn from the study and will only be receive money for the time you spent on the study. You will not have to pay for any of the medicines you will get on the trial. We will also pay for your transport costs and meals (if any) while you are on the study.

According to the law, during the study you will be covered by insurance. Compensation for any injury caused by taking part in this study will be in accordance with the guidelines of the Association of the British Pharmaceutical Industry. The University of Cape Town will compensate you without you having to prove that it is at fault. This applies in such cases where it is likely that such injury results from giving the study drug and any other procedure carried out in accordance with the protocol for the study. The University of Cape Town will not compensate you where such injury results from any procedure carried out which is not in accordance with the protocol for the study. Your right at law to claim compensation for injury where you can prove negligence is not affected

Confidentiality

Any personal information we collect from you will be kept secret. The only people that will be allowed to see your information will be Grant Langdon, Dr. Helen McIlleron and Justin Wilkins. Once we have collected the information on the forms we will not be able to link your name with the information.

HIV testing

We want to test you for HIV and AIDS. This is to see whether people with HIV/AIDS have more or less drug in their blood. If you do not want to be tested you don't have to and nothing will happen to you if you are not tested. You can still participate on the study if you have not been tested. A health professional will discuss HIV/AIDS and the risks with you before the test. You

will be given the results of your HIV-test after the last blood sample is taken. If you test positive follow-up care will be made available at the Groote Schuur Hospital HIV/AIDS Clinic. This does not mean that you will get antiretroviral drugs if you test positive.

Side effects and risks of rifapentine

Please tell the investigators if you start to feel sick or have any unusual symptoms. Rifapentine can have the following side effects: skin irritation, rash, loss of appetite, nausea, vomiting, abdominal pain and headaches are relatively common (1/10 to 1/100). Uncommonly rifapentine may lead to liver damage, damage to blood cells and platelets. Rifapentine may cause body fluids to turn orange. Contact lenses must not be worn for 4 days after taking rifapentine.

Because rifapentine is new medicine it may be dangerous to take other medicines while you are on the trial. Please speak to your doctor before you take any other medicines to make sure it is safe.

Ethics Committee

The University of Cape Town has a special committee to check that the study is legal. They have looked at the study and found it to be legal and fair. If you have any complaints you can phone them or write a letter to them.

Research Ethics Committee
Faculty of Medicine
Anzio Road
Observatory
7925
Telephone: (021)–406 6492

Appendix 1.3 Deelnemer Inligting

Studie titel

Vergelyking van variasie in plasma vlakke rifapentine en rifampisien in tuberkulosis pasiente van DP Marais Hospitaal

Hoekom ons die study doen

Rifapentien is 'n pil wat vervaardig is om tuberculosis pasiente te behandel. Nadat die pil ingeneem is, word dit in die bloedstroom opgeneem. Aangesien alle mense verskil, sal die hoeveelheid medikasie in die bloedstroom by almal verskillend wees. Ons probeer vastel hoe die hoeveelheid medikasie van u bloed verskil van ander pasiente en diegene wat gesond is.

Waar en wanneer word die studie gedoen

Die studie sal vir agt dae by die DP Marais Hospitaal plaasvind. U moet nie ontbyt by die hospital eet nie.Ons sal vir u onybyt gee. Voor ontbyt word 'n naald in die arm gesit en dit word vir 8 uur ingehou terwyl bloedmonsters getrek word. Na ontbyt sal u isoniazid (INH), pyrazinamide (PZA) en ethambutol (ETH) tablette moet sluk, **maar nie jou rifampisien tablette nie**. U sal die rifapentine neem in plaas van die rifampisien. Bloedmonsters sal dan na 2, 4, 5, 6, 7, 8, 24, 48, en 72 uur geneem word. Die naald sal na die 8 uur bloedmonster uitgetrek word. Vir die volgende drie oggende sal bloed geterk word.

U moet aanhou om die isoniazid (INH), pyrazinamide (PZA), en ethambutol (ETH) medikasie te neem gedurende die week.

Drie dae later word die prosedure herhaal. U sal weer ontbyt by ons kry en bloed sal op die selfde manier getrek word. Vir die volgende drie oggende sal bloed geterk word.

U moet aanhou om die isoniazid (INH), pyrazinamide (PZA), en ethambutol (ETH) medikasie te neem gedurende die week. U moet die rifampisien weer begin neem 3dae na die studie voltooid is.

Deelname aan die studie

U mag enige tyd aan die studie onttrek. Niemand sal ontsteld daaroor wees nie en niks sal met u gebeur as u ophou nie. Indien u wil onttrek uit die studie hoef u ons nie 'n rede te gee nie. U

hoef net "nee" te sê. Indien u voortgaan met die studie moet u na die navorsers luister en doen wat hulle vra. Die studie sal u nie vinniger gesond maak nie, maar dit sal tot die voordeel van die gemeenskap wees, want daardeur kan ons vir u 'n beter medikasie ontwikkel.

Deelnemer vergoeding

U word vir u tyd in die studie vergoed. Dit sal betaal word aan die einde van die studie. As u aspiris nie luister na die navorser se instruksies in verband met die studie sal u van die studie ontrek wees en geen vergoeding kry nie. Jy hoef nie vir enige van die medisyne te betaal nie. Ons sal ook vir u vervoer en voedsel koste betaal.

Volgens die wet word u gedurende die studie deur versekering gedek. Kompensasie vir enige besering wat u mag opdoen gedurende die studie sal in ooreenstemming met die riglyne gestel duer die Association of the British Pharmaceutical Industry wees. Die Kaapstad Universiteit sal u vergoed sonder dat u hoef te bewys dat dit ons skuld is. Dit word toegepas waar dit lyk asof die besering 'n gevolg is van die studie medisyne of enige prosedure wat uitgevoer word in ooreenstemming met die protokol. Die Kaapstad Universiteit sal u nie vergoed waar u 'n besring opdoen as gevolg van enige prosedure wat nie uitgevoer word in ooreenstemming met die protokol. U reg om kompesasie te eis war u kan bewys u besering as gevolg van nalatigheid word nie geaffekteer nie.

Vertroulikheid

Enige vertroulike informasie wat u ons gee, word geheim gehou. Die enigste persone wat die informasie sal sien is Grant Langdon, Dr. Helen McIlleron en Justin Wilkins. Enige informasie sal nie deur ons met die name op die vorms vergelyk word nie.

MIV toetsing

Ons will u toets vir MIV/HIV op die eerste dag. Dit is om te vasstel of mense met HIV meer of minder medikasie in hul bloedstroom het. As u nie getoets wil word nie, hoef u nie te en niks sal aan u gebeur nie. U kan stil deelneem aan die studie as u wil. Raadgewing wat inligting insluit oor die MIV virus/VIGS, manier waarop dit versprei word, risiko faktore en gevolge sal deur 'n opgeleide raadgewer aan jou gegee word. Pasiënte sal aan die einde van die neem van die tweede sessie ingelig word oor hulle MIV status deur 'n opgeleide persoon. Opvolg sal beskikbaar wees by Groote Schuur Hospitaal vir persone wat positief toets. Dit beteken nie dat u antiretroviral medisyne sal kry as u positief toets nie.

Newe-effekte en waarskuwings

U moet asseblief vir die navoreser se as u siek of enige ander simptome voel. Rifapentine kandie volgende newe-effekte he: vel irritasie, veluitslag, aptytverlies, naarheid, vomering, maag pyne en hoofpyn (1/10 tot 1/100). Rifapentine mag ook lei na lewerbeskadiging, beskadiging van bloed selle en plaatjies. Rifapentine mag ook ligaamlike vloeistowwe oranje kleur. Kontaklense moet nie vir 4 dae na u rifapentine geneem het gedra word nie.

Omdat rifapentine 'n nuwe pil is, sal dit miskien gevaarlik wees om ander medikasie te neem terwyl u aan die studie deelneem. Vra eers u dokter of dit veilig is as u ander medikasie wil saam met die rifapentine wil neem.

Etiese Komitee

Die Universiteit Kaapstad het 'n spesiale komitee om te verseker dat die studie wettig en regverdig is. Hulle het die studie ondersoek en het besluit dat dit regverdig is. As u enige klagtes het kan u hulle bel of 'n brief skryf.

Research Ethics Committee
Faculty of Medicine Anzio Road
Observatory
7925
Telefoon: (021)–406 6492

Appendix 1.4 Iphetshana Lenkcukacha

Isihloko Soluphando

Uqhathaniso lokwahlukana kwamaqondo eRifapentine kunye ne Rifampicin egazini lezigulana zesifo sephepha (TB) kwisibhedlela sase-DP Marais

Yintoni imbangi yokuba senze oluphando?

IRifapentine yipilisi eyenzelwe ukunyanga iTB. Emva kokuba uyisele ingena egazini. Njengoko abantu behlukile, namaqondo eRifapentine asegazini labantu ngabantu ayohluka. Sifuna ukubona ukuba amaqondo eliyeza ahluka kangakanani phakathi kwezigulana zeTB kwakunye nakubantu abangaguliyo.

Uphando luyakwenzelwa phi, nini?

Uphando luyakuthabatha iintsuku ezisibhozo ukusuka kusuku lokusela ipilisi kwisibhedlela I-DP Marais. Kufunek aungatyi isidlo sakusasa ngosuku lophando. Ukutya uyakufumana esibhedlela. Phambi kwesidlo sakusasa, inaliti iyakufakwa kumthambo osengalweni yakho. Iyakuhlala ke apho kangangeyure ezisibhozo ukwenzela ukuba sikwazi ukutsala igazi. Emva kwesidlo eso uzakuthabatha iipilisi i-Isoniazid (INH), i-Pyrazinamide (PZA) neEthambutol (ETH) **kodwa akuyi kuthabatha I-Rifampicin**. Kanti ke endaweni yeRifampicin uyakuthabatha ipilisi yeRifapentine. Igazi elincinane liyakutsalwa kwiyure yesibini, yesine, yesihlanu, yesithandathu, yesixhenxe, yesibhozo, eyamashumi amabini anesine, ayamashumi amane anesibhozo, kunye neyamashumi asixhenxe anesibini. Inaliti iyakususwa engalweni yakho emva kweeyure ezisibhozo. Ekuseni kwiintsuku ezintathu ezilandelayo kuyakufuneka utsalwe igazi kwakhona. Oku kuyakuphindwa ngosuku lwesibhozo. **Kubalulekile ukuba emva kolutyelelo esibhedlele njengesiqhelo usele iIsoniazid, iPyrazinamide kunye ne Ethambutol. Kanti ke uyakubuyela kwiRifampicin emva kwentsuku ezintathu.**

Emva kweentsuku ezintathu uyakuphinda kwenziwe oluphando njengesiqhelo. Uyakunikwa isidlo sakusasa esibhedlele ukuze usele amayeza negazi likutsalwe njengaphambili.

Kubalulekile ukuba emva kolutyelelo esibhedlele njengesiqhelo usele iIsoniazid, iPyrazinamide kunye ne Ethambutol. Kanti ke uyakubuyela kwiRifampicin emva kwentsuku ezintathu.

Ukuthabatha inxaxheba koluphando

Uvumelekile ukuba uphume koluphando nanini na ufuna. Akukho mntu uyakuba nasikhwasilima ngalonto kwaye akukho mntu uyakukunqanda. Akukho nembangi yakunika izizathu zoko. Ufanele nje uthi "hayi". Kodwa ke xa uqhubekeka noluphando kuyakufuneka umamele imiyalelo kagqirha ozakuba ephethe. Oluphando aluyi kukunceda uphile ngokukhawuleza kodwa luyakunceda uluntu jikelele ngokuba sikwazi ukunika amayeza abhetele.

Ukuhlawulwa komphandwa

Uyakuthi ke unikwe imali ngexesha olichithe apha koluphando. Uyakuthi ke kodwa uyifumane ekupheleni kophando. Ukuba uthe wenza okunxaxhileyo emgaqweni wabaphandi ngelilixa lophando, ungakhutshwa ngoko nangoko. Kanti ke uyakuthi uhlawulelwe elolixa ubulichithe kuphando kuphela. Awuyi kuwahlawulela amayeza owafumanayo ngelixesha lophando. Siyakuzihlawula iindleko zokhenketho lakho kunye nezidlo (ukuba zikhona) othe wazisebenzisa ngelixa lophando. Ngokomthetho, ngelilixa lophando uyekukhuselwa yimali ebekelwa bucala (Insurance).

Ukubonelelwa kwiingozi ezinokuthi zenzeke kuwe ngokuthabatha lwayeza iyakwenziwa ngokwemigaqo ebekwe liqumrhu le British Pharmaceutical Industry. I University of Cape Town iyakukubonelela ungakhe uphande ngalomba kusini ukubonisa ukuba iyatyholeka. Uku kuquka iimeko apho leyo ngozi yenzaka ngokuba usele lamayeza okanye ngenxa yesenzo esimalunga nemigaqo yoluphando. Amalungelo akho ngokusemthethweni ukufuna ubonelelo ngesehlo onokubonisa ukuba sibangwe kukungakhathali kumphandi ahlala engaphazamisekanga.

Iimfihlo

Zonke inkcukacha esizithabathe kuwe ziyakuba yimfihlo. Kuphela nguGrant Langdon, nogqirha Helen McIlleron kunye noJustin Wilkins abayakuvumeleka bazibone. Emva kukuba zifakwe kumaphetshana okubhalisa, aziyikuphinda zikwazi ukunxunyulaniswa naliphi na igama lomntu, njengoko niyakunikwa iinombolo.

Uhlolelo lukaGAWULAYO (HIV)

Sifuneka sikujonge intsholongwane kunye nesifo sikagawulayo (HIV kunye ne-AIDS). Oku kukujonga ukuba abantu abanesifo okanye intsholongwane kagawulayo banamaqondo angaphezulu okanye asezantsi amayeza egazini. Ukuba awufuni kujongelwa esisifo, angala, akukho nto iyakwenzeka kuwe xa ukungajongwanga. Usenakho ukuthabatha inxaxheba koluphando nokuba awujongwanga. Ugqirha uyakunixoxela ngeHIV/AIDS phambi kohlolo.

Iziphumo ziyakufumaneka ekupheleni kophando ngosuku lokugqibela. Ukukhathalelwa kwabo abafunyanwe benalo eligciwane luyakufumaneka kwikliniki ye HIV/AIDs yase-Groote Schuur. Oku akuthethi ukuba uyakufumana amachiza esifo sikagawulayo.xa ufunyanwe unaso.

Iingxaki ezinokuthi zivele xa usebenzisa iRifapentine

Nceda ixelele abaphandi ngoko nongoko xa uthe waziva ungemnandanga okanye uneempawu ezingaqhelekanga ngethuba lophando. Irifapentine ingeza neengxaki eziloluhlobo: Ukurhawuzelela kolusu, amaqhakuva okutyabuka, ukungafuni kutya, isicefucefu okanye ukubanaar, ukugabha, intlungu zamazantsi kunye nentloko ebuhlungu {Kanti ke ezi ngxaki zibakho kwisinye eshimini (1/10) ukuya kwisinye ekhulwini (1/100) labantu}. Iingxaki ezingaxhaphakanga zezi: ukonakala kwesibindi, ukonakala kwecells zegazi kanti nakumajoni omzimba. Irifapentine ingazijika incindi zomzimba ezifana nomchamo zibe orange. Iizibuko zecontact lenses zezingantlitywa iintsuku zibene emva kokusela iRifapentine.

Kuba iRifapentine iyipilisi entsha, ingayingozi ukusela amanye amayeza alo naluphina uhlobo ngaphandle kwala achazwe ngasentla. Thetha nogqirha phambi kokuba usele neliphina iyeza ngaphandle kwala uzakuwasela ngelilixa lophando.

Ikomiti evume oluphando

Ikomiti yesisi kolo semfundo ephakamileyo saseKapa ejongene nophando kubantu nezilwanyana ivumile ukuba oluphando lolusemthethweni kwaye alugxobhagxobhi lungelo lamntu.

Idilesi yabo yile:
Research Ethics Committee
Faculty of Medicine
Anzio Road
Observatory 7925
Umnxeba: (021) 406 6492

Appendix 1.5 Permission to take part in a clinical study

Study Leaders: Grant Langdon, Dr. Helen McIlleron, Justin Wilkins

I have read the information sheet	Yes ☐	No ☐
I have been able to ask questions and talked about the study	Yes ☐	No ☐
I understand the following:		
Personal information will be written down	Yes ☐	No ☐
On two days 7 blood samples will be taken from me and then one more the next morning	Yes ☐	No ☐
Each blood sample will be about one spoonful, so altogether I will be giving 8 spoons of blood in a day.	Yes ☐	No ☐
I can leave whenever I want without telling anyone why and nothing will happen to me	Yes ☐	No ☐
I have answered all the questions truthfully	Yes ☐	No ☐
I agree to join in the study	Yes ☐	No ☐

NAME OF SUBJECT	SIGNATURE	DATE
NAME OF INVESTIGATOR	SIGNATURE	DATE
NAME OF TRANSLATOR	SIGNATURE	DATE

I agree to have an HIV antibody test Yes ☐ No ☐

NAME OF SUBJECT	SIGNATURE	DATE

Appendix 1.6 Toestemming om deel te neem aan n' kliniese studie

Navorsers: Grant Langdon, Dr. Helen McIlleron, Justin Wilkins

Ek het die inligtinf stuk gelees	Ya ☐	Nee ☐
Ek kon vrae vra en oor die studie praat	Ya ☐	Nee ☐
Ek verstaan die volgende:		
Persoonlike inligting sal neergeskryf word	Ya ☐	Nee ☐
Gedurende die dag sal 7 bloedmonsters getrek word en nog een die volgende oggend vir drie dae	Ya ☐	Nee ☐
Elke bloedmonster sal omtrent een lepelvol wees, so altesame sal 8 lepels bloed getrek word	Ya ☐	Nee ☐
Ek mag enige tyd onttrek sonder om 'n rede te gee en niks sal met my gebeur nie	Ya ☐	Nee ☐
Ek het die vrae eerlik beantwoord	Ya ☐	Nee ☐
Ek gee my toestemming om deel te neem	Ya ☐	Nee ☐

NAME OF SUBJECT	SIGNATURE	DATE
NAME OF INVESTIGATOR	SIGNATURE	DATE
NAME OF TRANSLATOR	SIGNATURE	DATE

Ek gee my toestemming om vir MIV getoets te word Ya ☐ Nee ☐

NAME OF SUBJECT	SIGNATURE	DATE

Appendix 1.7 Isivumelwano sokuthabatha inxaxheba kuphando lwezamayeza esifo sephepha (TB)

Abaphandi abaphambili: Grant Langdon, Gqirha Helen McIlleron no Justin Wilkins

Ndilifundile iphetshana elinenkcukacha ngoluphando	Ewe ☐	Hayi ☐
Ndilinikiwe ixesha lokubuza nelukuthetha	Ewe ☐	Hayi ☐
Ndiyakuqondo oku kulandelayo:		
Kuyakushicilelwa inkcukacha ngam kwakunye nezonyango lwam	Ewe ☐	Hayi ☐
Igazi liyakuthatyathwa amatyeli asixhenxe kanti elinye ngentsasa elandelayo	Ewe ☐	Hayi ☐
Isixa ngasinye segazi siyakulingana necephe	Ewe ☐	Hayi ☐
Ndinelungelo lokuphuma nangaliphi ixesha koluphando ndinganikanga nkcaza	Ewe ☐	Hayi ☐
Ndiphendule yonke imibuzo ngenene nangenyaniso	Ewe ☐	Hayi ☐
Ndiyavuma ukuthabatha inxaxheba kulophando	Ewe ☐	Hayi ☐

_____	_____	_____
IGAMA LUMGULI	UMSAYINO	UMHLA
_____	_____	_____
IGAMA LOMPHANDI	UMSAYINO	UMHLA
_____	_____	_____
IGAMA LOMGUQULELI	UMSAYINO	UMHLA

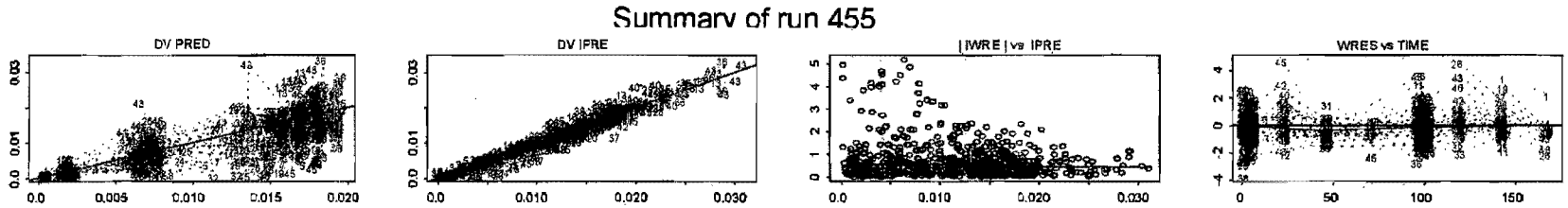
Ndiyavuma ukuhlolelwa isigulo sikagawulayo (HIV) Ewe ☐ Hayi ☐

_____	_____	_____
IGAMA LOMPHANDWA	UMSAYINO	UMHLA

Appendix 2

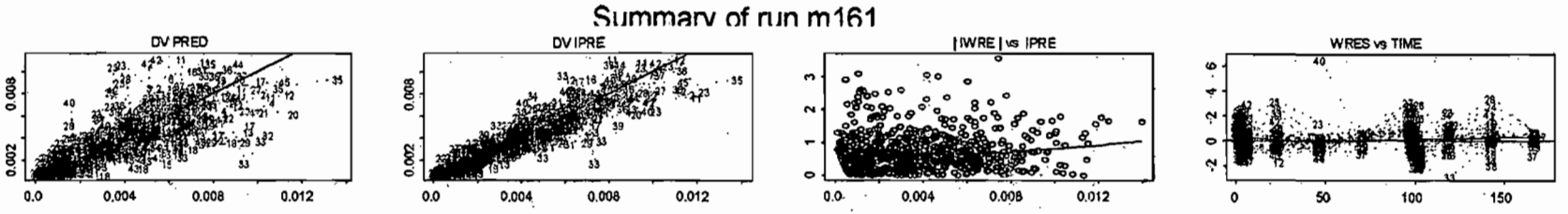
NONMEM Model Files

Appendix 2.1 Final parent drug model file




```
MINIMIZATION SUCCESSFUL
NO. OF FUNCTION EVALUATIONS USED:  358
NO. OF SIG. DIGITS IN FINAL EST.:  3.1
ETABAR IS THE ARITHMETIC MEAN OF THE ETA-ESTIMATES,
AND THE P-VALUE IS GIVEN FOR THE NULL HYPOTHESIS THAT THE TRUE MEAN IS 0.
ETABAR: -0.38E-02 -0.19E-01  0.17E-01 -0.31E-01 -0.24E-01  0.54E-02 -0.16E+00 -0.16E+00 -0.47E-01  0.00E+00
P VAL.  0.89E+00  0.31E-90  0.75E-01  0.11E+00  0.10E+00  0.72E+00  0.76E-02  0.18E-01  0.50E+00  0.12E+01

Objective: -7459.2

TH1         0.664          0.19          TH9         0.691          0.32          OM1:1       0.52          0.58
TH2         37.8           0.041         OM2:1       0.22           0.21          OM3:3       0.6           0.27
TH3         2.03           0.045         OM2:2       0.16           0.49          OM9:9       0.6           0
TH4         1.45           0.051         OM3:3       0.16           0.36          OM10:10     0.23          0.19
TH5         0.000382       0.15          OM4:4       0.16           0             S1:1:1      1             NA
TH6         0.144          0.082         OM5:5       0.16           0
TH7         0.0434         0.19          OM6:6       0.16           0

$PROB Effect of WT on CL and V,IOV on CL,V and KA
;; mod 3PT plasma data
;; 1-comp model with Lag Time on absorption
;; Interoccasional variability on CL,V and KA
;; FOCE on CL, V, KA and FO on ALAG
;; ADD+PROP error model
$DATA /home/glang/Hybrid/data4CC.prn IGNORE=@
$INPUT ID DROP=M3 AMT DATE TIME OCC CMT EVID DROP=M(EDV DV MDV AGE WT
 DROP=SMI ALB ALT AST DROP=AD1 DROP=AD2 RDOS ONER RACE ALCH SMK HIV
 DROP=DRUG DROP=NWRT
$SUBROUTINE ADVAN2 TRANS2
;;$MODEL COMP = ABSN   ;RPT abs comp comp 1
      COMP = CENT   ;RPT central comp comp 2
$PK
   CALL FL=-2
    TVKA = THETA(1)
     TVV = THETA(2)*THETA(8)**(WT-50)
    TVCL = THETA(3)*THETA(7)**(WT-50)
  TALAG1 = THETA(4)
;; Interoccasional variability on CL
    BSVCL = ETA(1)          ; between subject variability
    IF(OCC.EQ.1)THEN
    BOVCL = ETA(3)          ; between occasion variability
    ENDIF
    IF(OCC.EQ.2)THEN
    BOVCL = ETA(4)          ; between occasion variability
    ENDIF
      CL = TVCL*EXP(BSVCL+BOVCL) ; clearance from the central compartment
;; Interoccasional variability on V
    BSVV = ETA(2)           ; between subject variability
    IF(OCC.EQ.1)THEN
    BOVV = ETA(5)           ; between occasion variability
    ENDIF
    IF(OCC.EQ.2)THEN
    BOVV = ETA(6)           ; between occasion variability
    ENDIF
      V = TVV*EXP(BSVV+BOVV)  ; volume of the central compartment
;; Interoccasional variability on KA
    BSVKA = ETA(7)          ; between subject variability
    IF(OCC.EQ.1)THEN
    BOVKA = ETA(8)          ; between occasion variability
    ENDIF
    IF(OCC.EQ.2)THEN
    BOVKA = ETA(9)          ; between occasion variability
    ENDIF
     KA = TVKA*EXP(BSVKA+BOVKA) ; absorption constant
   ALAG1 = TALAG1*EXP(ETA(10))  ; lag time into absorption compartment
   OCCA = OCC
     S2 = V
    K10 = CL/V
$ERROR
    AA1 = A(1)
    AA2 = A(2)
   IPRED = 0.000
    IF (F.GT.0) IPRED = F
    IRES = DV-IPRED
  W = SQRT(THETA(5)**2+IPRED**2*THETA(6)**2)
 IWRES = IRES/W
    Y    = IPRED + W*EPS(1)
$THETA (0,0.6,6)          ;1 KA
$THETA (0,48,200)         ;2 V
$THETA (0,2.5,6)          ;3 CL
$THETA (0,1.5,3)          ;4 ALAG
$THETA (0,0.002,1)        ;5 ADD error
$THETA (0,0.2,1)          ;6 PROP error
$THETA (-0.05,.2)         ;7 Effect of WT on CL
$THETA (0,1,5)            ;8 Effect of WT on V
$OMEGA 0.08               ;1 IIV on CL
$OMEGA 0.05               ;2 IIV on V
$OMEGA BLOCK(1)0.05       ;3 IOV on CL OCC1
$OMEGA BLOCK(1)SAME       ;4 IOV on CL OCC2
$OMEGA BLOCK(1)SAME       ;5 IOV on V OCC1
$OMEGA BLOCK(1)SAME       ;6 IOV on V OCC2
$OMEGA 0.5                ;7 IIV on KA
$OMEGA BLOCK(1)0.5        ;8 IOV on KA
$OMEGA BLOCK(1)SAME       ;9 IOV on KA
$OMEGA 0.08               ;10 IIV on ALAG
$SIGMA 1 FIX
$ESTIMATION NOABORT SIGDIG=3 MAXEVALS=9999 POSTHOC PRINT=5
METHOD=HYBRID ZERO=-100 MSF=msf455
$COV PRINT=E ; to get SE's (uncertainty in parameters)
$TAB ID TIME KA K10 CL V ALAG1 ETA1 ETA2 ETA3 ETA4 ETA5
 ETA6 ETA7 ETA8 ETA9 ETA10
ONEHEADER NOPRINT FILE=sdtab455
$TAB ID TIME AA1 AA2 IPRED IWRES
ONEHEADER NOPRINT FILE=patab455
$TAB ID AGE WT ALB ALT AST RDOS
ONEHEADER NOPRINT FILE=catab455
$TAB ID ONER RACE ALCH SMK HIV
ONEHEADER NOPRINT FILE=catab455
```

Appendix 2.1 Final metabolite drug model file



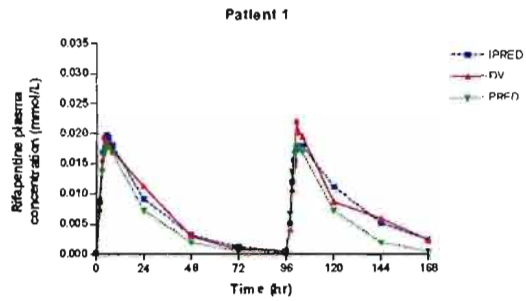

```
MINIMIZATION SUCCESSFUL
NO. OF FUNCTION EVALUATIONS USED: 413
NO. OF SIG. DIGITS IN FINAL EST.:  5.6
ETABAR IS THE ARITHMETIC MEAN OF THE ETA-ESTIMATES,
AND THE P-VALUE IS GIVEN FOR THE NULL HYPOTHESIS THAT THE TRUE MEAN IS 0.
ETABAR:  0.21E-01 -0.15E-01
PVAL.:  0.68E+00  0.81E+00

Objective:  -9282.609

TH1          11.6          0.072         TH6          0.196         0.086         SI1:1         1             NA
TH2          2.56          0.083         TH7          0.647         0.12
TH3          21            0.077         TH8          0.267         0.36
TH4          1.7           0.095         OM1:1        0.36          0.27
TH5          0.00063       0.089         OM2:2        0.25          0.32
```

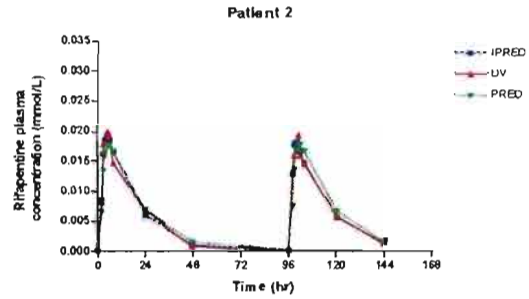
```
$PROB Induction option 4(DATE),GNDR on CLM,WT on V3
;; PK analysis of Metabolite patient data
;; Parent 1-comp model with Lag Time on absorption
;; Metabolite 1-comp model,no first pass metabolism,hepatic clearance only
;; FOCE
;; ADD+PROP error model
$DATA /home/pjm/Metabolite/mista150.prn IGNORE=@
$INPUT ID AMT DATE TIME OCC CMT EVID IKA ICL IV IALA DV MDV AGE WT DROP=BMI
 DROP=ALB DROP=ALT DROP=AST DROP=AD1 DROP=AD2 RDSE GNDR DROP=RACE DROP=ALCH
 DROP=SMK HIV DRUG DROP=NWRI
$SUBROUTINE ADVANS TOL=5
$MODEL COMP=ABSN        ;Parent abs comp comp 1
   COMP=CENT            ;Parent central comp comp 2
   COMP=(PERI, DEFDOSE) ;Metabolite central comp comp 3
$PK
    F2=0
    F3=0
;; Parent drug
   ALAG1=IALA           ;individual posthoc lag time
    KA=IKA              ;individual posthoc absorption constant
    V2=IV               ;posthoc volume of parent central comp 2
    CL=ICL              ;posthoc clearance of parent from central comp 2
;; Metabolite
   TVV3=THETA(1)*THETA(8)**(WT-56)
   TVCLN=THETA(2)
   TVCLBA=THETA(3)
   TVSLP=THETA(4)
   DECLN=TVCLN+(TVCLBA-TVCLN)*EXP(-TVSLP*DATE)
   IF(GNDR.EQ.1)THEN
   TVCLM=DECLN
   ELSE
   TVCLM=DECLN*THETA(7)
   ENDIF
   CLM=TVCLM*EXP(ETA(1))
   V3=TVV3*EXP(ETA(2))    ;volume of metabolite central comp3
   CLBA=TVCLBA
   CLN=TVCLN
   SLP=TVSLP
   S2=V2
   S3=V3
   K23=CLP/V2
   K30=CLM/V3
   OCCA=OCC
$DES
  DADT(1)=-KA*A(1)
  DADT(2)=KA*A(1)-K23*A(2)
  DADT(3)=K23*A(2)-K30*A(3)
$ERROR
   AA1=A(1)
   AA2=A(2)
   AA3=A(3)
  IPRED=0.0001
  IF(F.GT.0) IPRED=F
  IRES=DV-IPRED
 W=SQRT(THETA(5)**2+IPRED**2*THETA(6)**2)
 IWRES=IRES/W
 Y=IPRED+W*EPS(1)
$THETA (0,15,50)      ;1 V3
$THETA (0,3,10)       ;2 CLN
$THETA (0,23,70)      ;3 CLBA
$THETA (0,2,10)       ;4 SLP
$THETA (0.0005,1)     ;5 ADD error
$THETA (0,0.3,1)      ;6 PROP error
$THETA (0,0.7,7)      ;7 GNDR on CLM
$THETA (0,1.5)        ;8 WT on V3
$OMEGA 2.35           ;1 IIV on CLM
$OMEGA 2.35           ;2 IIV on V3
$SIGMA 1 FIX
$ESTIMATION NOABORT SIGDIG=6 MAXEVALS=9999 POSTHOC PRINT=5 METHOD=1
$COV PRINT=E ; to get SE's (uncertainty in parameters)
$TAB ID TIME OCCA IPRED IWRES AA1 AA2 AA3
 ONEHEADER NOPRINT FILE=mdltbm161
$TAB ID TIME K23 CLM V2 V3 CLBA CLN SLP ETA1 ETA2
 ONEHEADER NOPRINT FILE=patabm161
$TAB ID AGE WT RDSE
 ONEHEADER NOPRINT FILE=cotabm161
$TAB ID GNDR HIV DRUG
 ONEHEADER NOPRINT FILE=catabm161
```

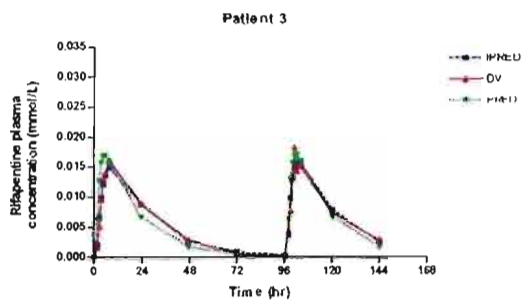
Appendix 3

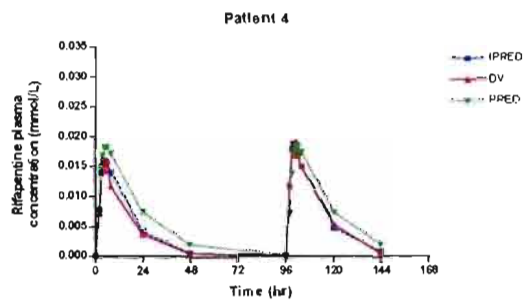
Patient Plasma-concentration Time Profiles

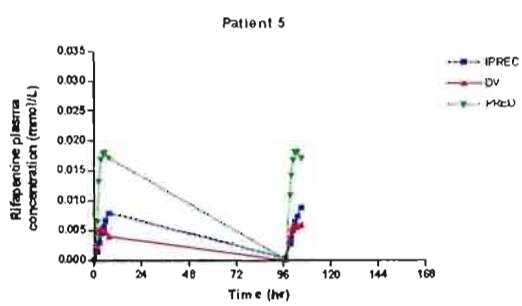
Appendix 3.1 Individual plots of observed, population predicted and individual predicted rifapentine concentration-time data

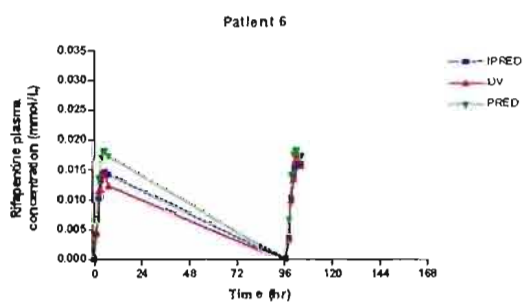


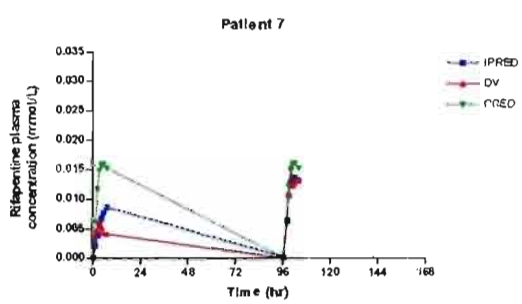


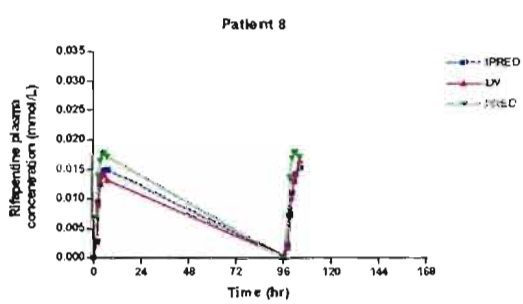


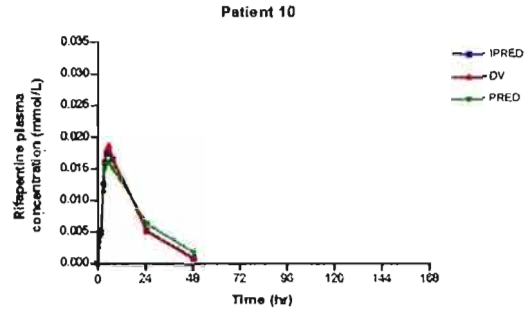


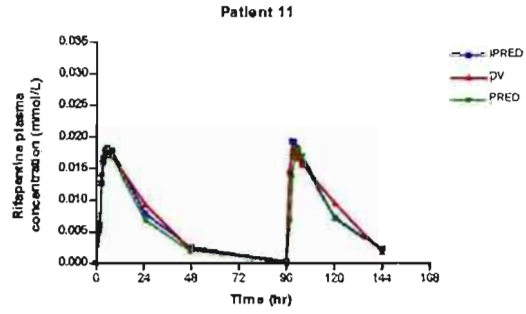


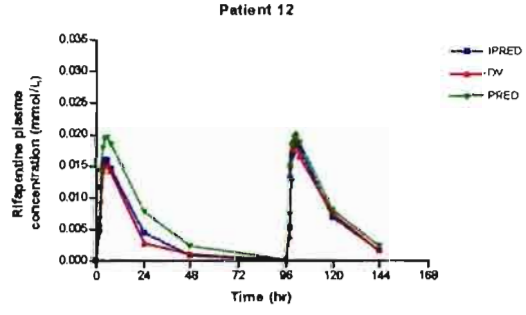


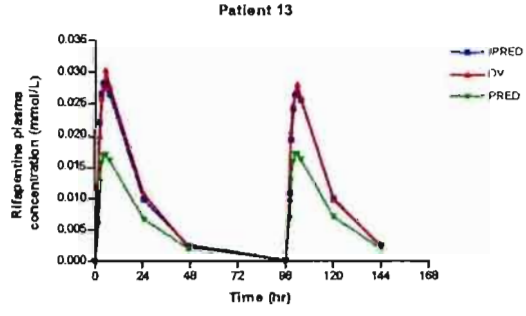


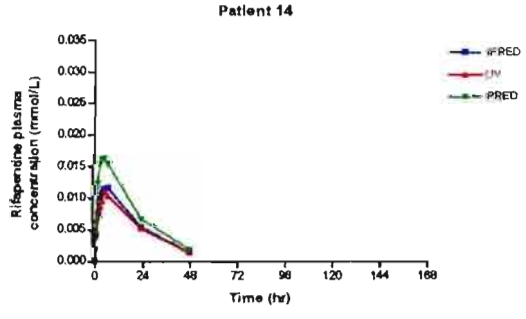


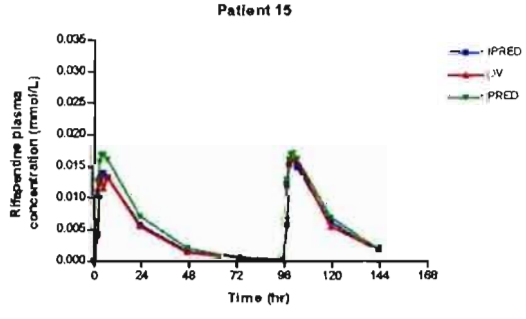

Patient 10
Rifapentine plasma concentration (mmol/L)
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
0
24
48
72
96
120
144
168
Time (hr)
IPRED
DV
PRED

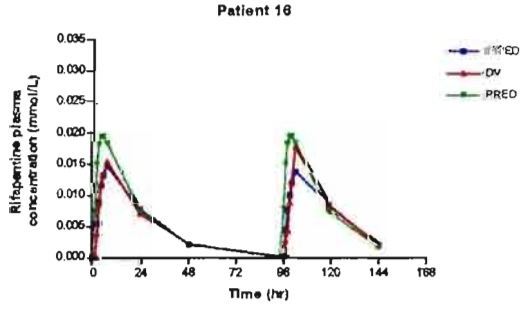

Patient 11
Rifapentine plasma concentration (mmol/L)
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
0
24
48
72
96
120
144
168
Time (hr)
IPRED
DV
PRED

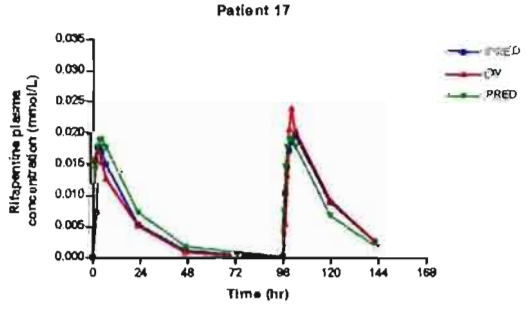

Patient 12
Rifapentine plasma concentration (mmol/L)
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
0
24
48
72
96
120
144
168
Time (hr)
IPRED
DV
PRED

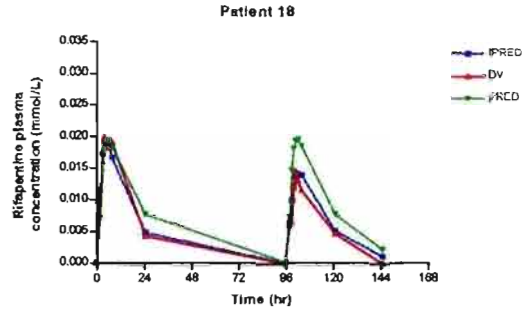

Patient 13
Rifapentine plasma concentration (mmol/L)
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
0
24
48
72
96
120
144
168
Time (hr)
IPRED
DV
PRED

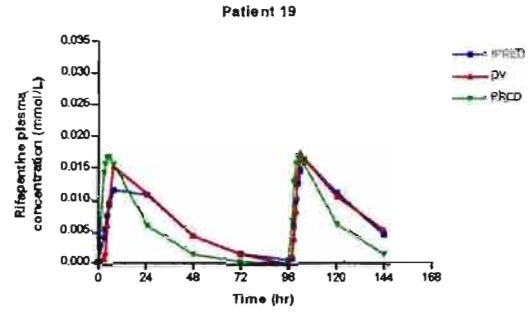

Patient 14
Rifapentine plasma concentration (mmol/L)
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
0
24
48
72
96
120
144
168
Time (hr)
IPRED
DV
PRED

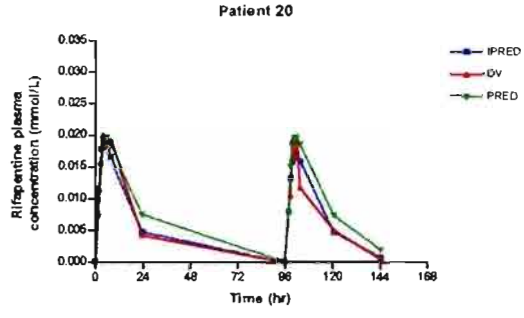

Patient 15
Rifapentine plasma concentration (mmol/L)
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
0
24
48
72
96
120
144
168
Time (hr)
IPRED
DV
PRED

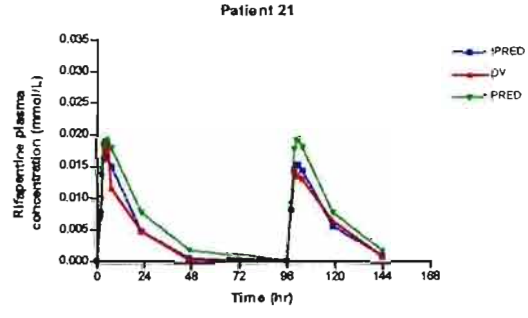

Patient 16
Rifapentine plasma concentration (mmol/L)
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
0
24
48
72
96
120
144
168
Time (hr)
IPRED
DV
PRED

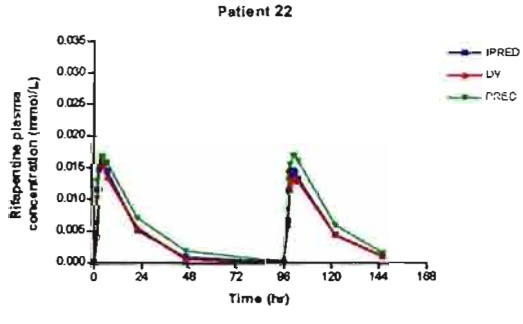

Patient 17
Rifapentine plasma concentration (mmol/L)
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
0
24
48
72
96
120
144
168
Time (hr)
IPRED
DV
PRED

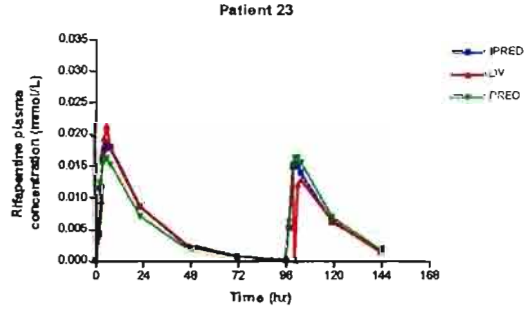

Patient 18
Rifapentine plasma concentration (mmol/L)
Time (hr)
IPRED
DV
PRED

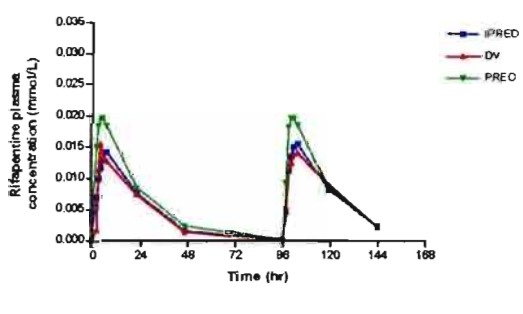

Patient 19
Rifapentine plasma concentration (mmol/L)
Time (hr)
IPRED
DV
PRED

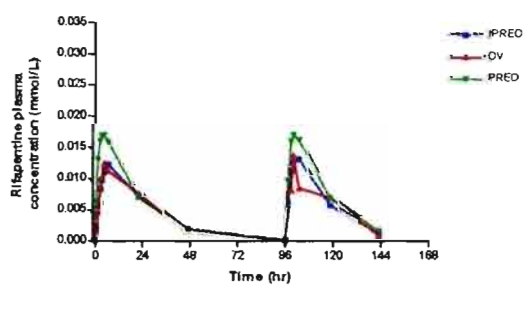

Patient 20
Rifapentine plasma concentration (mmol/L)
Time (hr)
IPRED
DV
PRED

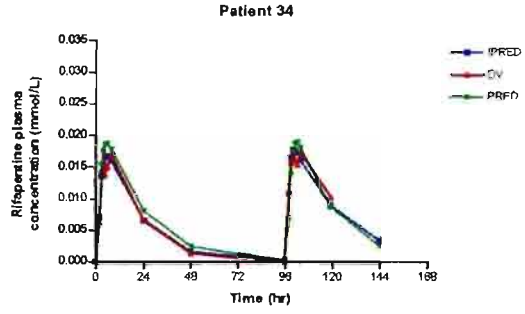

Patient 21
Rifapentine plasma concentration (mmol/L)
Time (hr)
IPRED
DV
PRED

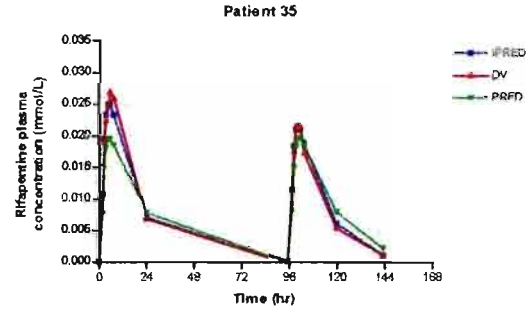

Patient 22
Rifapentine plasma concentration (mmol/L)
Time (hr)
IPRED
DV
PRED

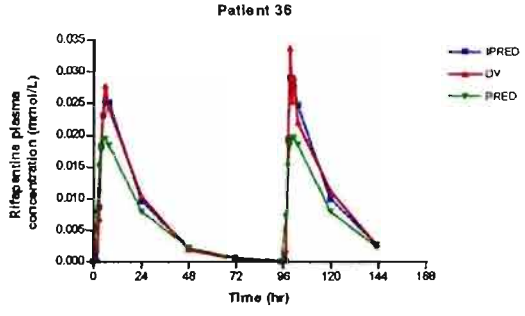

Patient 23
Rifapentine plasma concentration (mmol/L)
Time (hr)
IPRED
DV
PRED

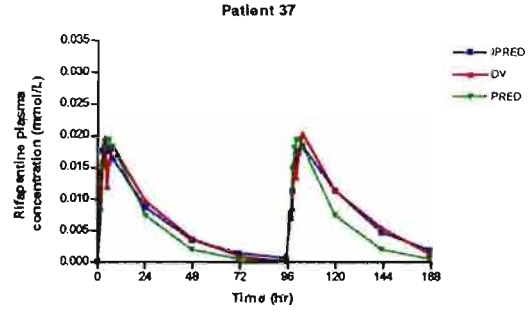

Patient 24
Rifapentine plasma concentration (mmol/L)
Time (hr)
IPRED
DV
PRED

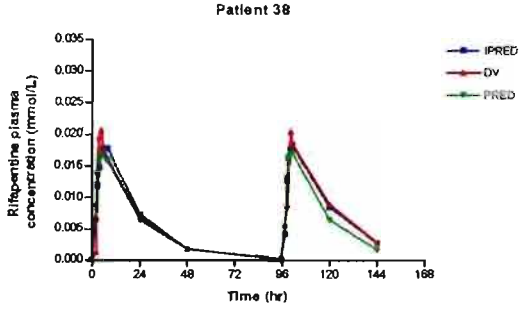

Patient 25
Rifapentine plasma concentration (mmol/L)
Time (hr)
IPRED
DV
PRED

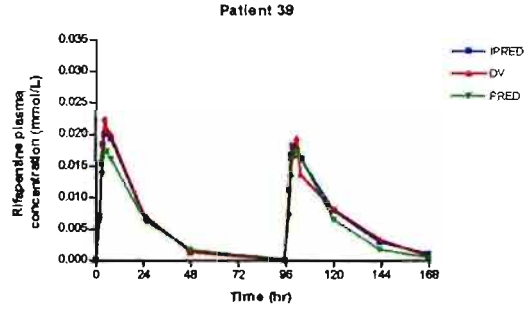

Patient 34
Rifapentine plasma concentration (mmol/L)
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
0
24
48
72
96
120
144
168
Time (hr)
IPRED
DV
PRED

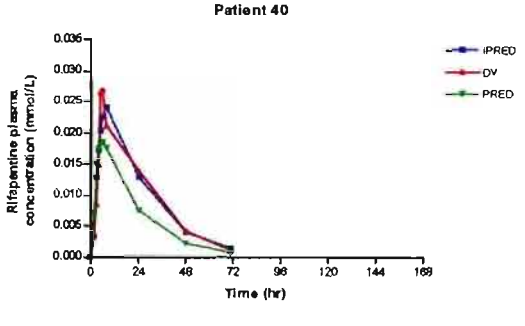

Patient 35
Rifapentine plasma concentration (mmol/L)
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
0
24
48
72
96
120
144
168
Time (hr)
IPRED
DV
PRED

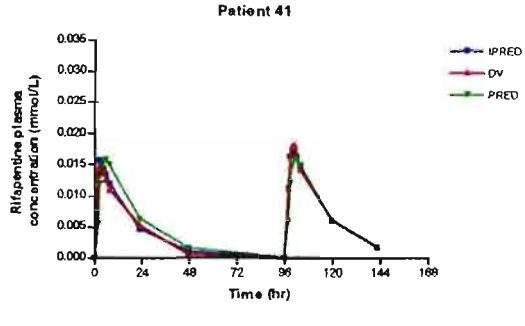

Patient 36
Rifapentine plasma concentration (mmol/L)
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
0
24
48
72
96
120
144
168
Time (hr)
IPRED
DV
PRED

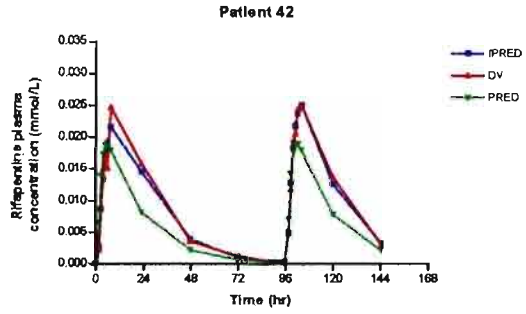

Patient 37
Rifapentine plasma concentration (mmol/L)
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
0
24
48
72
96
120
144
168
Time (hr)
IPRED
DV
PRED

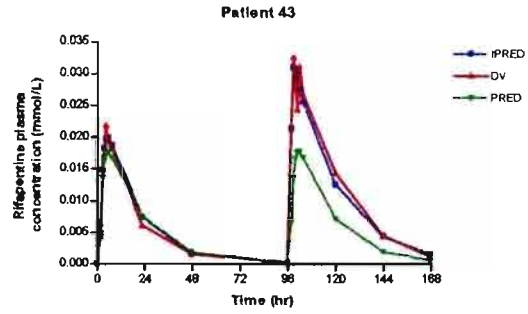

Patient 38
Rifapentine plasma concentration (mmol/L)
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
0
24
48
72
96
120
144
168
Time (hr)
IPRED
DV
PRED

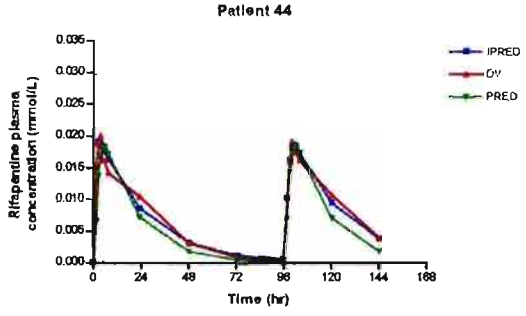

Patient 39
Rifapentine plasma concentration (mmol/L)
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
0
24
48
72
96
120
144
168
Time (hr)
IPRED
DV
PRED

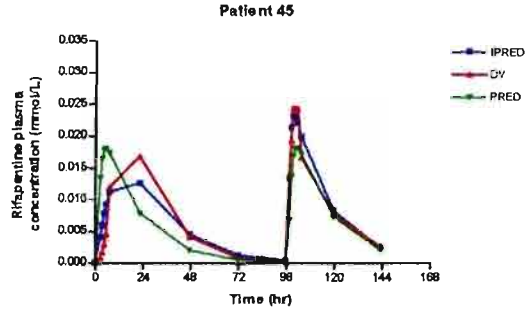

Patient 40
Rifapentine plasma concentration (mmol/L)
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
0
24
48
72
96
120
144
168
Time (hr)
IPRED
DV
PRED

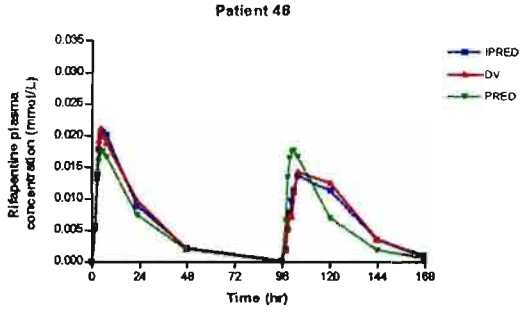

Patient 41
Rifapentine plasma concentration (mmol/L)
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
0
24
48
72
96
120
144
168
Time (hr)
IPRED
DV
PRED


Patient 42
Rifapentine plasma concentration (mmol/L)
0.035
0.030
0.025
0.020
0.016
0.010
0.005
0.000
0
24
48
72
96
120
144
168
Time (hr)
IPRED
DV
PRED

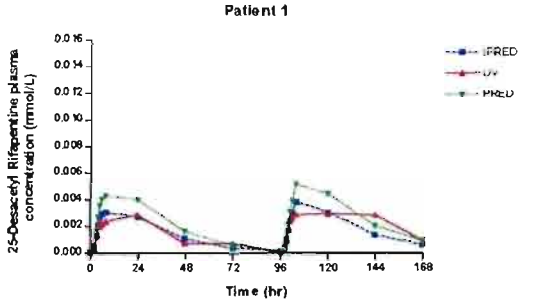

Patient 43
Rifapentine plasma concentration (mmol/L)
0.035
0.030
0.025
0.020
0.016
0.010
0.005
0.000
0
24
48
72
96
120
144
168
Time (hr)
IPRED
DV
PRED

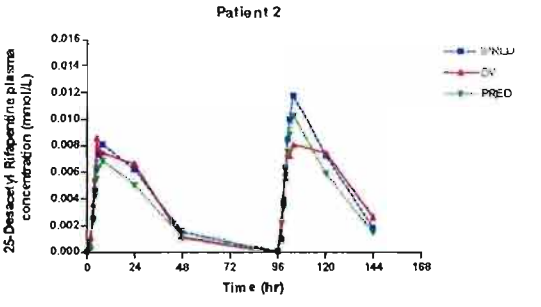

Patient 44
Rifapentine plasma concentration (mmol/L)
0.035
0.030
0.025
0.020
0.016
0.010
0.005
0.000
0
24
48
72
96
120
144
168
Time (hr)
IPRED
DV
PRED

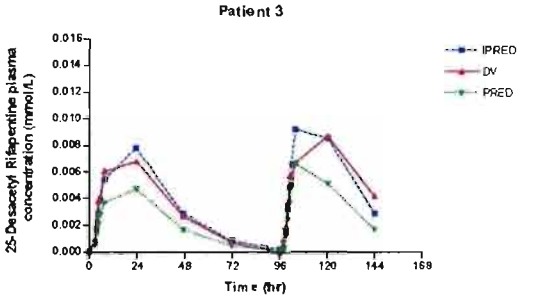

Patient 45
Rifapentine plasma concentration (mmol/L)
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
0
24
48
72
96
120
144
168
Time (hr)
IPRED
DV
PRED

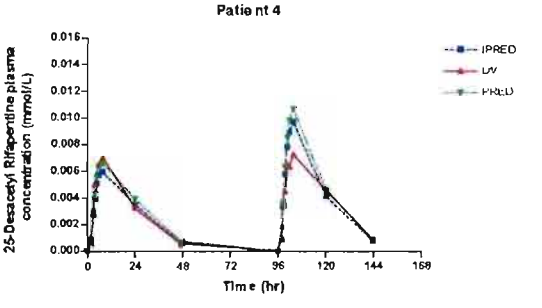

Patient 46
Rifapentine plasma concentration (mmol/L)
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
0
24
48
72
96
120
144
168
Time (hr)
IPRED
DV
PRED

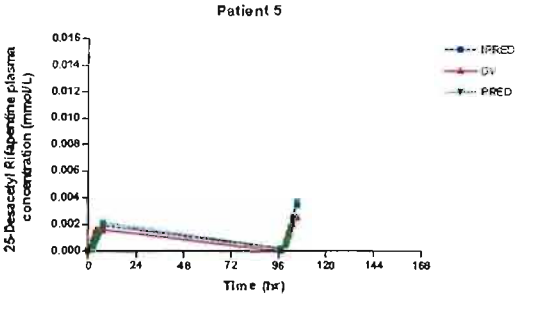
Appendix 3.2 Individual plots of observed, population predicted and individual predicted 25-desacetyl rifapentine concentration-time data

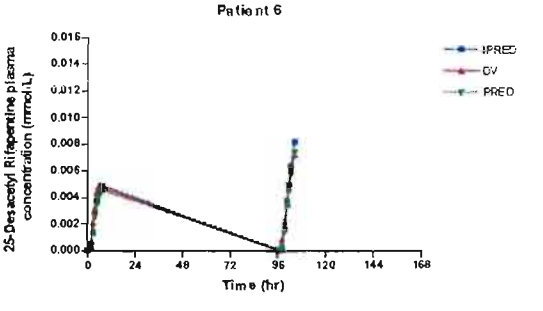


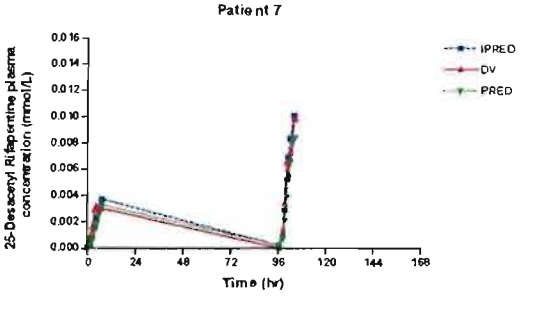


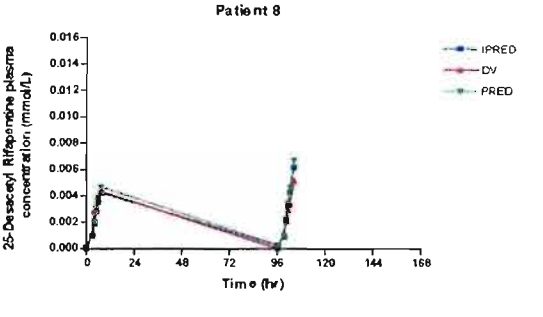


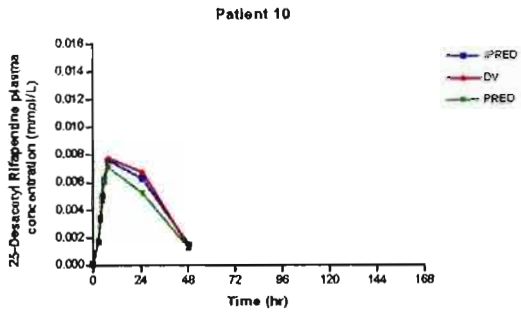


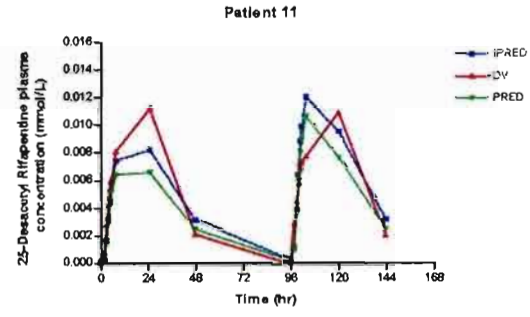


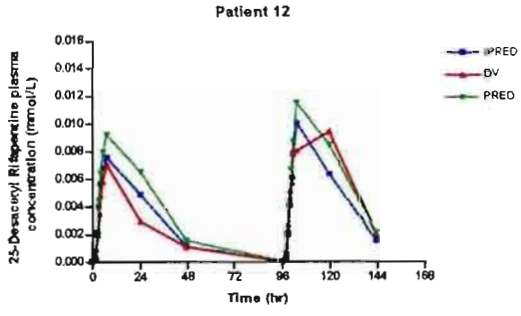


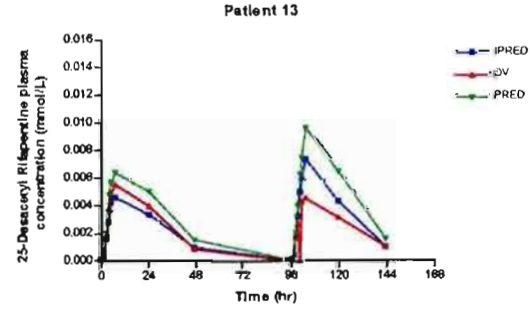


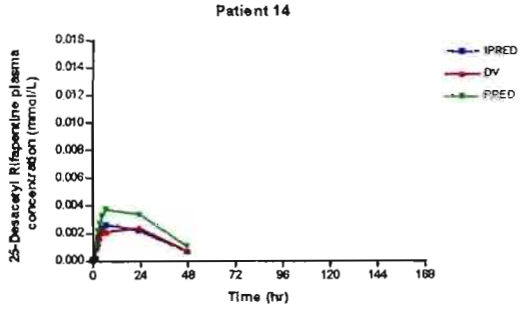


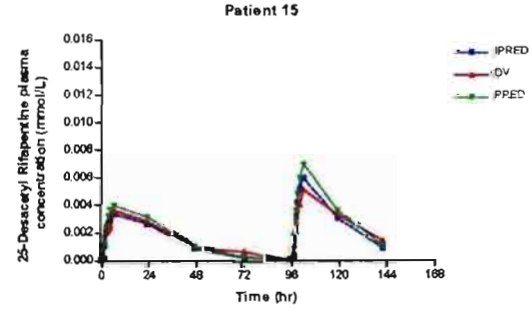

Patient 10
25-Desacetyl Rifapentine plasma concentration (mmol/L)
0.016
0.014
0.012
0.010
0.008
0.006
0.004
0.002
0.000
0
24
48
72
96
120
144
168
Time (hr)
IPRED
DV
PRED

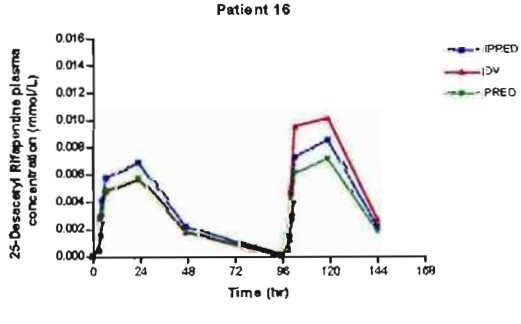

Patient 11
25-Desacetyl Rifapentine plasma concentration (mmol/L)
0.016
0.014
0.012
0.010
0.008
0.006
0.004
0.002
0.000
0
24
48
72
96
120
144
168
Time (hr)
IPRED
DV
PRED

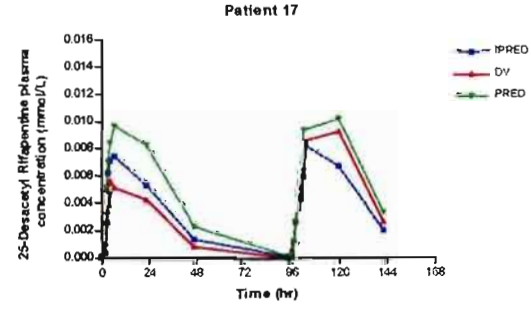

Patient 12
25-Desacetyl Rifapentine plasma concentration (mmol/L)
0.016
0.014
0.012
0.010
0.008
0.006
0.004
0.002
0.000
0
24
48
72
96
120
144
168
Time (hr)
IPRED
DV
PRED

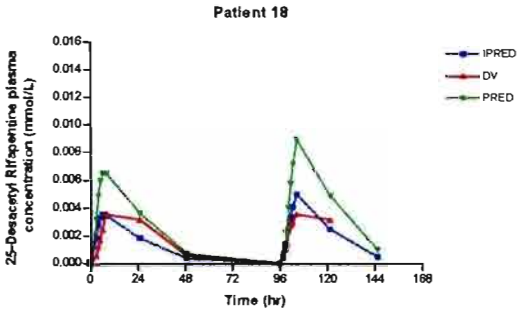

Patient 13
25-Desacetyl Rifapentine plasma concentration (mmol/L)
0.016
0.014
0.012
0.010
0.008
0.006
0.004
0.002
0.000
0
24
48
72
96
120
144
168
Time (hr)
IPRED
DV
PRED

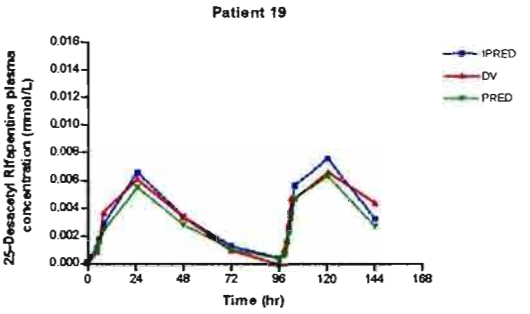

Patient 14
25-Desacetyl Rifapentine plasma concentration (mmol/L)
0.016
0.014
0.012
0.010
0.008
0.006
0.004
0.002
0.000
0
24
48
72
96
120
144
168
Time (hr)
IPRED
DV
PRED

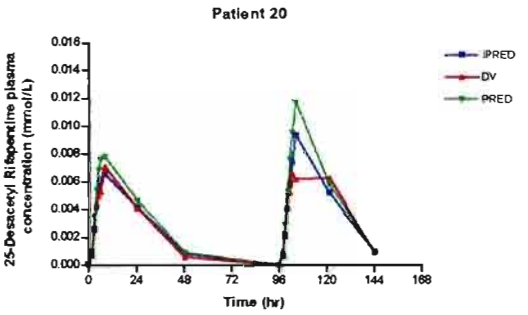

Patient 15
25-Desacetyl Rifapentine plasma concentration (mmol/L)
0.016
0.014
0.012
0.010
0.008
0.006
0.004
0.002
0.000
0
24
48
72
96
120
144
168
Time (hr)
IPRED
DV
PRED

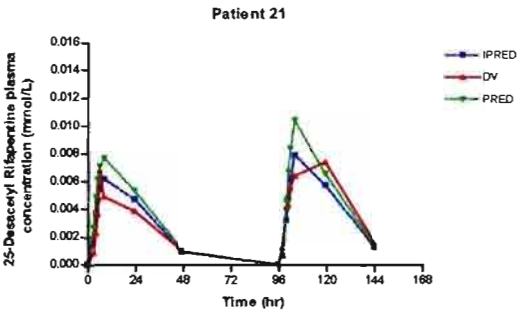

Patient 16
25-Desacetyl Rifapentine plasma concentration (mmol/L)
0.016
0.014
0.012
0.010
0.008
0.006
0.004
0.002
0.000
0
24
48
72
96
120
144
168
Time (hr)
IPRED
DV
PRED

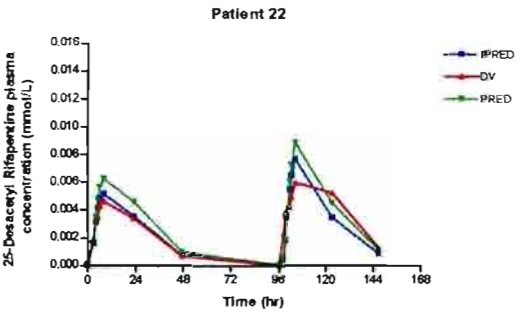

Patient 17
25-Desacetyl Rifapentine plasma concentration (mmol/L)
0.016
0.014
0.012
0.010
0.008
0.006
0.004
0.002
0.000
0
24
48
72
96
120
144
168
Time (hr)
IPRED
DV
PRED

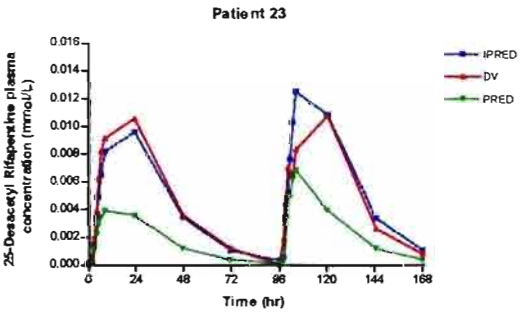

Patient 18
25-Desacetyl Rifapentine plasma concentration (mmol/L)
Time (hr)
IPRED
DV
PRED

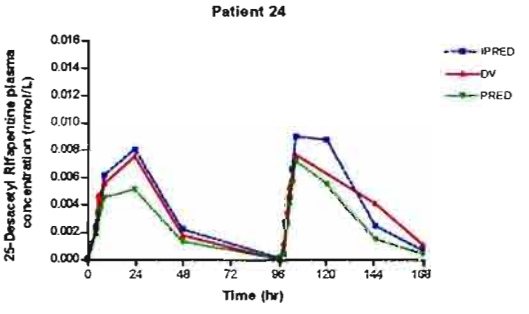

Patient 19
25-Desacetyl Rifapentine plasma concentration (mmol/L)
Time (hr)
IPRED
DV
PRED

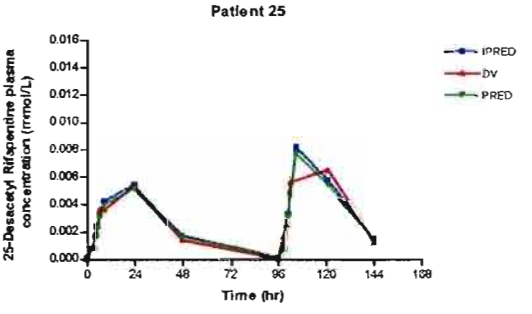

Patient 20
25-Desacetyl Rifapentine plasma concentration (mmol/L)
Time (hr)
IPRED
DV
PRED


Patient 21
25-Desacetyl Rifapentine plasma concentration (mmol/L)
Time (hr)
IPRED
DV
PRED

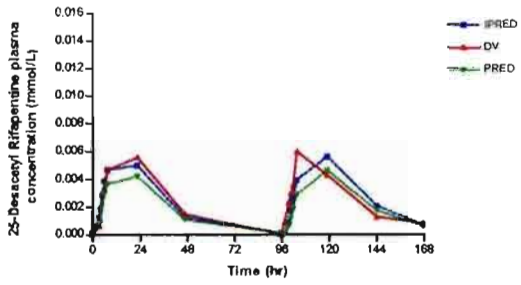

Patient 22
25-Desacetyl Rifapentine plasma concentration (mmol/L)
Time (hr)
IPRED
DV
PRED


Patient 23
25-Desacetyl Rifapentine plasma concentration (mmol/L)
Time (hr)
IPRED
DV
PRED

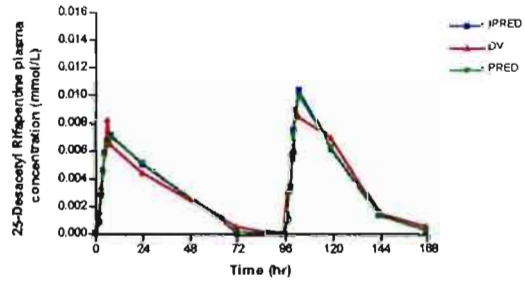

Patient 24
25-Desacetyl Rifapentine plasma concentration (mmol/L)
Time (hr)
IPRED
DV
PRED


Patient 25
25-Desacetyl Rifapentine plasma concentration (mmol/L)
Time (hr)
IPRED
DV
PRED

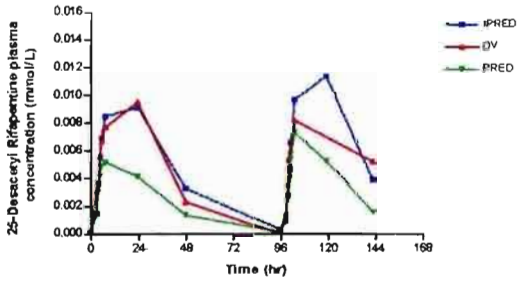
Patient 26



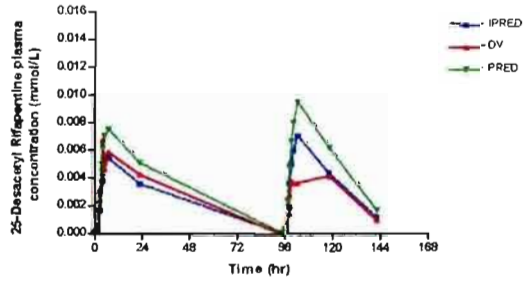
Patient 27



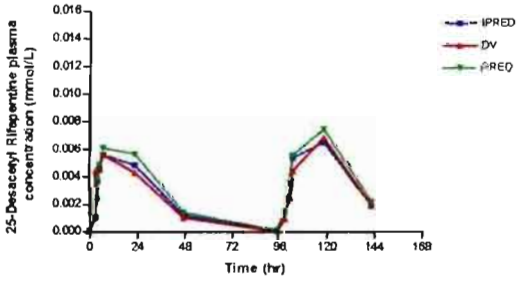
Patient 28



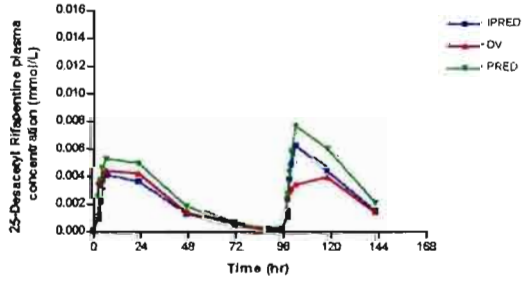
Patient 29



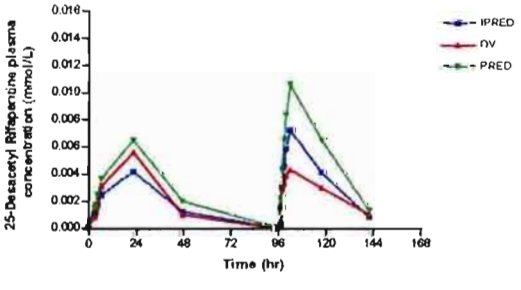
Patient 30



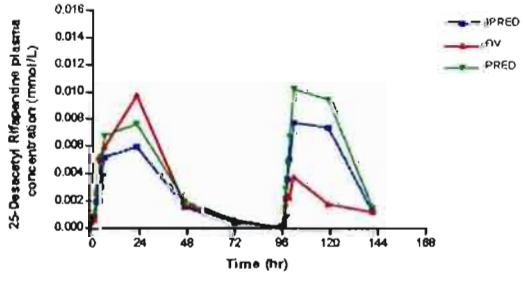
Patient 31

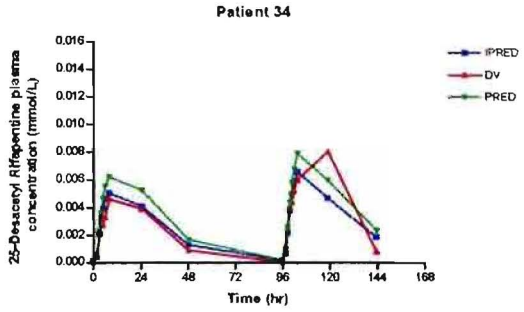


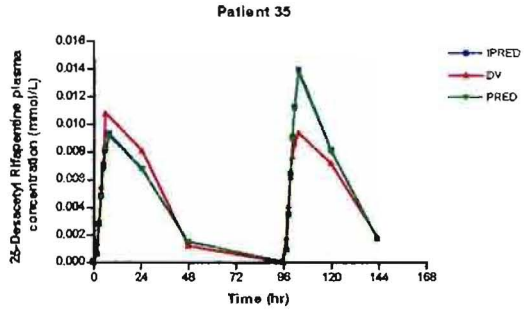
Patient 32

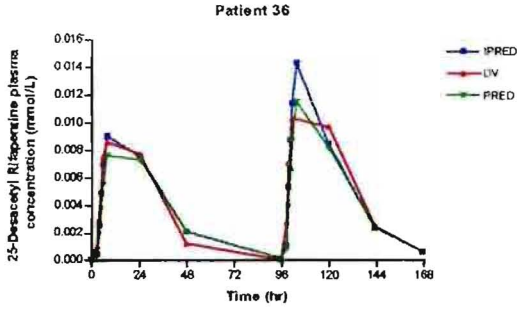


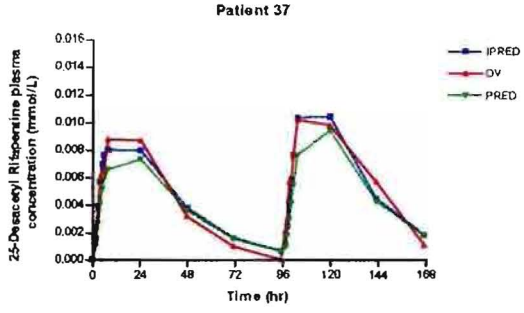
Patient 33

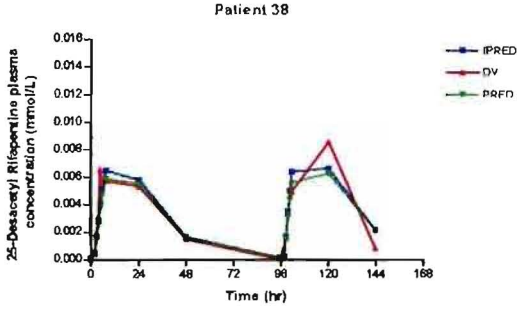


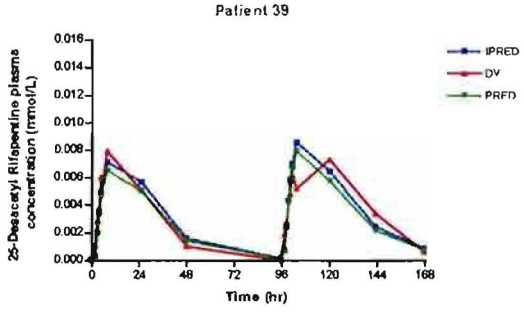

Patient 34
25-Desacetyl Rifapentine plasma concentration (mmol/L)
Time (hr)
IPRED
DV
PRED

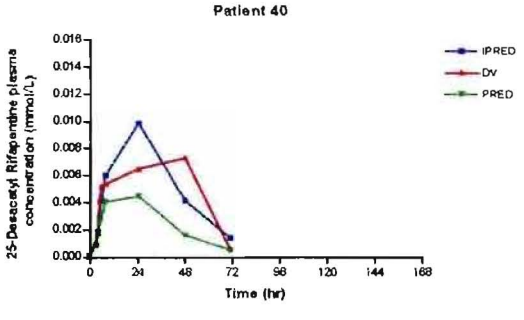

Patient 35
25-Desacetyl Rifapentine plasma concentration (mmol/L)
Time (hr)
IPRED
DV
PRED

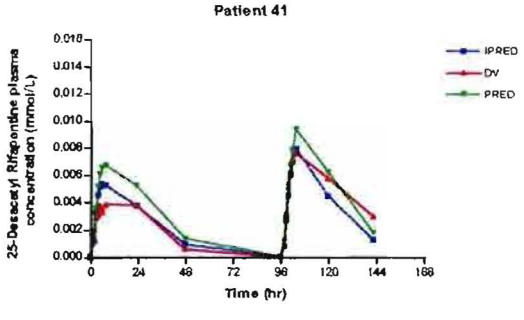

Patient 36
25-Desacetyl Rifapentine plasma concentration (mmol/L)
Time (hr)
IPRED
DV
PRED

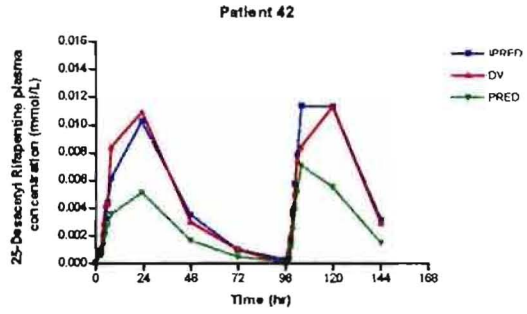

Patient 37
25-Desacetyl Rifapentine plasma concentration (mmol/L)
Time (hr)
IPRED
DV
PRED

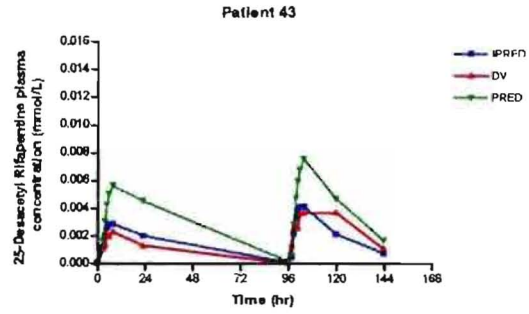

Patient 38
25-Desacetyl Rifapentine plasma concentration (mmol/L)
Time (hr)
IPRED
DV
PRED

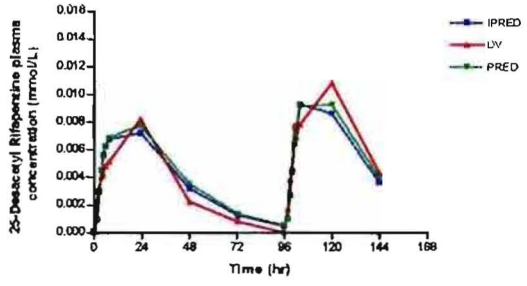

Patient 39
25-Desacetyl Rifapentine plasma concentration (mmol/L)
Time (hr)
IPRED
DV
PRED

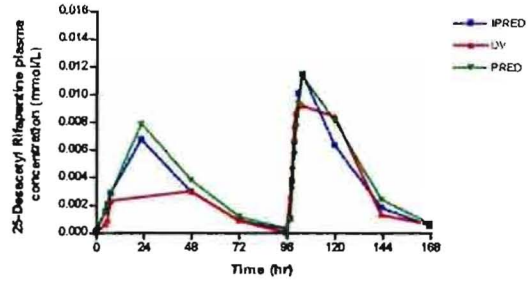

Patient 40
25-Desacetyl Rifapentine plasma concentration (mmol/L)
Time (hr)
IPRED
DV
PRED

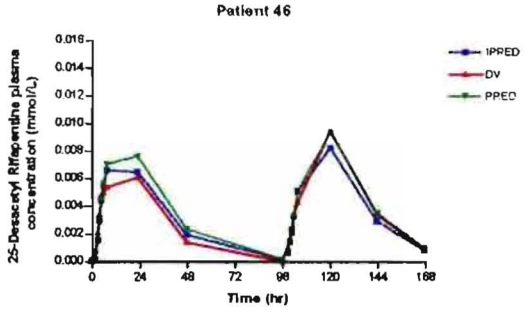

Patient 41
25-Desacetyl Rifapentine plasma concentration (mmol/L)
Time (hr)
IPRED
DV
PRED


Patient 42
25-Desacetyl Rifapentine plasma concentration (mmol/L)
0.016
0.014
0.012
0.010
0.008
0.006
0.004
0.002
0.000
0
24
48
72
96
120
144
168
Time (hr)
IPRED
DV
PRED


Patient 43
25-Desacetyl Rifapentine plasma concentration (mmol/L)
0.016
0.014
0.012
0.010
0.008
0.006
0.004
0.002
0.000
0
24
48
72
96
120
144
168
Time (hr)
IPRED
DV
PRED


Patient 44
25-Desacetyl Rifapentine plasma concentration (mmol/L)
0.016
0.014
0.012
0.010
0.008
0.006
0.004
0.002
0.000
0
24
48
72
96
120
144
168
Time (hr)
IPRED
DV
PRED


Patient 45
25-Desacetyl Rifapentine plasma concentration (mmol/L)
0.016
0.014
0.012
0.010
0.008
0.006
0.004
0.002
0.000
0
24
48
72
96
120
144
168
Time (hr)
IPRED
DV
PRED


Patient 46
25-Desacetyl Rifapentine plasma concentration (mmol/L)
0.016
0.014
0.012
0.010
0.008
0.006
0.004
0.002
0.000
0
24
48
72
96
120
144
168
Time (hr)
IPRED
DV
PRED

Appendix 4

Raw demographic, haematological and chemical pathology data

Appendix 4.1 Individual demographic patient data

Number	Age	Dose	Weight	Height	BMI	Race	Sex	HIV Status	Smoker					Alcohol			Cannabis	Mandrax	New/ Retreatment
									Quit	Light (<15)	Medium (15-30)	Heavy (>30)	None	Light (<1lt)	Medium (1-3lt)	Heavy (>3lt)			
1	56	750	50.5	1.70	17.47	C	M	U	X				X						New
2	37	900	61.0	1.72	20.62	B	M	U		X					X				Retreatment
3	36	750	53.5	1.65	19.65	C	M	N				X				X			Retreatment
4	42	750	49.0	1.76	15.82	C	M	N			X		X						Retreatment
5	30	750	50.0	1.67	17.93	B	M	P		X					X		X		Retreatment
6	47	750	49.5	1.68	17.54	C	M	N		X				X			X		Retreatment
7	46	600	45.0	1.62	17.15	C	F	P					X						New
8	52	600	40.0	1.62	15.24	B	F	P		X				X					Retreatment
9	34		45.0	1.51	19.74	C	F	P		X					X				
10	46	600	45.0	1.49	20.27	C	F	P	X				X						New
11	20	750	50.0	1.59	19.78	C	F	N		X					X				Retreatment
12	29	600	46.0	1.51	20.17	C	F	N			X			X					Retreatment
13	41	600	42.0	1.60	16.41	C	F	P			X					X			Retreatment
14	26	600	44.0	1.65	16.16	C	M	P			X					X	X		new
15	18	600	42.0	1.62	16.00	C	M	U				X				X		X	Retreatment
16	23	900	56.0	1.61	21.60	C	M	N								X			New
17	19	900	57.0	1.62	21.72	B	F	P		X			Missing						Retreatment
18	27	750	46.5	1.67	16.67	C	M	P		X			X				X		new
19	28	900	65.0	1.72	21.97	C	M	N		X						X	X		Retreatment
20	47	900	55.5	1.65	20.39	C	M	N		X			Missing				X		new
21	38	900	56.5	1.64	21.01	C	M	U			X				X				Retreatment
22	43	750	54.0	1.60	21.09	C	M	U			X			X					Retreatment
23	34	600	43.5	1.63	16.37	C	M	N			X				X		X		new
24	51	750	46.0	1.68	16.30	C	M	P		X				X					new
25	30	750	53.0	1.78	16.73	B	M	P		X			X						new
26	37	600	44.0	1.57	17.85	C	M	N				X				X	X		Retreatment
27	33	900	72.0	1.82	21.74	C	M	N				X				X	X		Retreatment
28	24	750	50.0	1.70	17.30	C	M	N			X				X				new
29	31	900	64.0	1.63	24.09	B	M	U	X				X						Retreatment
30	46	900	56.0	1.62	21.34	B	M	N					X						Retreatment
31	55	900	56.0	1.67	20.08	C	M	U	X				X					X	Retreatment
32	46	900	64.0	1.77	20.43	B	M	N							X				new
33	27	750	49.0	1.60	19.14	C	F	N						X					Retreatment
34	24	600	38.0	1.51	16.67	C	F	U		X				X			X		new
35	42	750	46.0	1.53	19.65	C	F	P		X					X				Retreatment
36	38	750	46.0	1.50	20.44	C	F	U				X			X				Retreatment
37	29	900	56.0	1.59	22.15	C	F	N							X				Retreatment
38	35	900	64.5	1.83	19.26	C	M	N		X			X						new
39	32	900	63.0	1.72	21.30	C	M	P				X	X				X		Retreatment
40	52	750	49.0	1.61	18.90	C	M	N				X	X						Retreatment
41	29	900	69.5	1.72	23.49	C	M	N			X								Retreatment
42	33	750	48.0	1.69	16.81	C	M	N			X								new
43	40	750	51.0	1.61	19.68	C	F	N											Retreatment
44	27	900	60.0	1.65	22.04	B	F	P											Retreatment
45	26	750	50.0	1.68	17.72	B	F	P		0			X						new
46	35	750	52.0	1.67	18.65	C	F	N		X				X					Retreatment

Appendix 4.2 Individual heamatological patient data

Number	Haemoglobin g/dl 13.3-17.3	Red Cell Count x10e12/l 4.5-5.9	Haematocrit ratio 0.37-0.53	RDW units 7.4-13.6	MCV fl 80-95	MCH pg 27.5-30.5	MCHC % 32-36	White Cell Count x10e9/l 4.0-11.0	Platelets x10e9/l 150-450
1	11.5	4.15	0.35	16.5	85	27.8	32.7	7	319
2	14.1	4.34	0.42	14	97	32.5	33.6	6	255
3	12.1	4.36	0.37	16	84	27.7	33.1	10.6	354
4	12.1	3.58	0.36	16.2	101	33.8	33.5	7.5	296
5	12.8	4.2	0.38	15.8	91	30.5	33.6	7.6	308
6	12.4	3.86	0.38	15.2	97	32.1	33	2	386
7	17.6	5.38	0.58	18.9	107	32.7	30.5	6.5	109
8	8.2	2.38	0.26	21.2	111	34.5	31	3	521
9									
10	8.3	2.77	0.27	19.9	99	30.1	30.5	2.5	363
11	11.1	3.98	0.33	20.4	82	27.9	33.9	5.9	387
12	11.2	3.86	0.35	18.3	91	29	31.8	3.2	284
13	10.9	3.47	0.34	18.6	99	31.5	31.9	6.4	431
14	9.8	3.36	0.3	25.5	89	29.3	33	4	256
15	10.8	4.2	0.33	15.7	79	25.7	32.8	8.8	429
16	9.6	3.62	0.31	19.1	85	26.7	31.4	9.2	636
17	10.6	3.66	0.32	15.6	87	28.8	33.3	5	263
18	9.8	3.35	0.3	26.5	90	29.1	32.6	2.7	190
19	16.1	5.13	0.48	14.7	95	31.5	33.3	8.1	317
20	11.1	4.19	0.36	14.6	85	26.6	31.1	10.4	350
21	11.3	3.71	0.34	15.2	93	30.4	32.7	6	399
22	13	4.66	0.4	14.4	91	29.8	32.6	6.8	361
23	9.3	3.23	0.29	15.3	90	28.8	32	13.4	649
24	7.9	2.59	0.26	18.7	100	30.3	30.3	13.1	892
25	8.9	2.82	0.29	18.3	101	31.7	31.3	2.9	248
26	6.9	2.87	0.23	19.5	82	24	29.4	7.9	447
27	14.1	4.6	0.44	15.1	95	30.7	32.2	9.6	388
28	12.7	4.54	0.39	15.8	85	28	33	11.9	403
29	13.3	4.5	0.41	14.6	90	29.5	32.7	5.8	505
30	13.2	4.33	0.39	14.4	91	30.4	33.5	7.6	260
31	11.6	3.75	0.35	13.5	94	30.9	32.9	10.1	524
32	10.3	3.58	0.3	14.6	85	28.7	33.8	12.5	391
33	9.9	3.63	0.31	15.6	85	27.3	31.9	9.4	682
34	12.2	4.98	0.37	17.4	75	24.5	32.6	14.3	625
35	11.8	4.13	0.35	16.7	84	28.7	34.2	4.7	277
36	10.9	3.55	0.32	19.3	91	30.6	33.6	10.8	484
37	10.2	3.62	0.31	16.5	85	28.1	33.2	7.2	405
38	12.9	5.1	0.4	17.8	79	25.3	32.2	11.5	570
39	11.7	4.47	0.36	17	81	26.3	32.4	10.1	365
40	9.9	3.41	0.29	16.2	87	29	33.6	6.8	334
41	13.5	4.69	0.41	14.4	87	28.9	33.2	10.4	343
42	11.5	3.84	0.34	17.3	89	30	33.7	13.4	684
43	11.7	4.52	0.38	16	84	25.9	30.7	15.1	565
44	11.1	3.71	0.34	14.8	91	29.8	32.8	15	540
45	8.3	3.32	0.26	22.7	79	25.1	31.8	1.5	271
46	12	4.69	0.38	15.8	82	25.6	31.3	9.2	468

Appendix 4.3 Individual chemical phathology patient data

Number	Creatinine umol/l 60-115	Total Protein g/l 60-80	Albumin g/l 35-50	Total bilirubin umol/l 1-17	ALT units/l 1-41	AST units/l 1-38	Alk Phos units/l 39-117
1	75	84	40	4	20	23	73
2	85	79	40	7	22	24	78
3	77	80	35	6	12	19	117
4	69	73	42	7	18	25	94
5		75	23	4	49	110	
6		72	28	4	15	34	
7	128	92	32	4	15	22	61
8		82	18	5	25	67	
9							
10	71	68	21	7	23	41	347
11	73	77	35	5	15	18	59
12	61	79	36	9	20	20	54
13	55	80	29	6	31	45	174
14	57	78	24	6	37	40	208
15	59	70	31	7	12	18	76
16	69	79	34	5	12	21	68
17	89	104	35	4	25	27	60
18	69	66	27	2	31	30	106
19	86	83	39	4	23	26	77
20	87	77	32	3	13	11	85
21	80	80	35	6	13	19	52
22	79	79	37	4	14	22	97
23	64	75	25	9	15	23	148
24	122	73	26	3	17	30	
25	88	76	28	4	38	44	104
26	66	62	24	3	8	15	54
27	96	74	37	11	20	24	83
28	75	82	33	7	25	21	81
29	102	82	36	8	34	28	86
30	94	75	38	7	23	31	63
31	78	75	35	6	15	20	99
32	63	90	35	7	7	15	71
33	59	79	31	7	8	13	52
34	70	86	37	4	21	31	99
35	69	90	39	11	24	32	58
36	67	78	38	6	19	27	59
37	73	75	28	5	22	25	89
38	78	82	37	5	15	18	80
39	76	89	37	6	14	22	53
40	79	75	37	6	10	26	80
41	94	71	35	7	30	42	99
42	58	74	27	5	19	19	126
43	67	74	31	6	17	17	69
44	58	73	26	6	32	32	127
45	64	84	28	5	31	48	1143
46	67	68	31	6	13	14	73