Pharmacokinetic and Pharmacodynamic Characteristics of Isoniazid and Rifampicin in Patients with Multidrug-Resistant Tuberculosis

by

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Submitted for the degree of

DOCTOR OF PHILOSOPHY

in the

Department of Pharmacology
Faculty of Health Sciences
University of Durban Westville
Durban, South Africa
1998

dedicated to

mom and dad

towards the realisation of a dream

DECLARATION

This document describes original work by the author and has not been submitted in any form to any other University. Where use was made of the work of others it has been duly acknowledged in the text.

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ACKNOWLEDGEMENTS

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GLOSSARY OF TERMS AND ABBREVIATIONS

ITEM	DEFINITION
η	inter-individual variability
θ	Theta – a regression term depicting a covariate effect on a pharmacokinetic parameter
ε	intra-individual (residual) variability
Φ	A general parameter model
ADD	Additive error model
AFB	Acid fast bacilli
ALB	Serum albumin
ALKP	Alkaline phosphatase
ALT	Alanine transaminase
ANOVA	Analysis of variance
AST	Aspartate transaminase
AUC	Area under the serum concentration versus time curve
AUC>MIC	AUC above the MIC
BILI	Total bilirubin
cAUC	Dose and mass corrected AUC
cCmax	Corrected (for dose and mass) Cmax
CCV	Constant coefficient of variation error model
CI	95% confidence interval
CL	Apparent clearance
Cmax	Maximum serum drug concentration
Cmax>MIC	Cmax above the MIC
CV	Coefficient of variation
D1	Duration of the zero order absorption process
DOBF	Difference in the OBF between 2 NONMEM runs
DOT	Directly observed therapy
F	Bioavailability
GAM	Generalised additive model
GGT	Gamma glutamyl transpeptidase
GLOB	Serum globulin
HIV	Human immunodeficiency virus
HPLC	High performance liquid chromatography
INH	Isoniazid
KA	Absorption rate constant
KE	Elimination rate constant
KGV	King George V Hospital
LFT	Liver function test
LQ	Limit of assay quantitation
MDR-TB	Multi-drug Resistant Tuberculosis
MIC	Minimum inhibitory concentration
NONMEM	Nonlinear mixed effects modelling
OBF	Minimum value of the objective function
PD	Pharmacodynamics
PK	Pharmacokinetics
Q	Intercompartmental clearance
RFA	Rifampicin

ITEM	DEFINITION
RNA	Ribonucleic acid
RSE	Relative standard error
t>MIC	Time the serum concentrations remain above the MIC
$t\frac{1}{2}$	Half life
TB	Tuberculosis
TLAG	Absorption lag time
tmax	Time to Cmax
V	Apparent volume of distribution
V2	Apparent volume of distribution of the central compartment
V3	Apparent volume of distribution of the peripheral compartment
WHO	World Health Organisation
WRES	Weighted residual

SUMMARY of CHAPTER 2 – Literature Review

OBJECTIVE

To review the history, epidemiology, clinical and microbiological manifestations of tuberculosis (TB) with emphasis on drug-resistance.
To review the pharmacotherapy of TB with particular emphasis on pharmacokinetic and pharmacodynamic considerations and the drugs: isoniazid and rifampicin.

DATA SOURCES

The current medical literature, including primary and secondary references. References were identified using electronic retrieval systems such as Medline and the Iowa Drug Information Systems as well as published abstracts from scientific meetings.

STUDY SELECTION

While data relating to the primary research questions were targeted, some background information was included so as to contextualise the study within the South African context. Data supporting and disputing the specific study objectives are presented.

DATA SYNTHESIS

There is a global increase in the incidence of TB despite the availability of a sound scientific strategy for its control. The WHO has declared the TB crisis in South Africa to be the worst in the world. The human immunodeficiency virus (HIV) epidemic is a major contributor to this "global emergency". An increase in drug-resistant TB has also been noted globally although the exact extent of the problem in South Africa is not known.

Malabsorption of the anti-TB drugs in HIV+ and acquired immunodeficiency syndrome (AIDS) patients has been noted and has sound mechanistic explanations. However some studies have failed to confirm early case reports of malabsorption. If proved correct this has serious public health implications particularly in areas with a high prevalence of HIV infection. Apart from treatment failure in the individual patient, absorption of a single drug in a TB drug regimen equates to monotherapy and has been associated with the acquisition of drug resistance.

The available drugs against TB are being depleted as a result of drug resistance and there appears to be very few, if any, promising agents on the horizon. The importance of studying available agents to better understand their use is therefore emphasised.

SUMMARY of CHAPTER 3 - Temporal Trends in *Mycobacterium Tuberculosis* Drug Resistance in Kwazulu-Natal, South Africa : 1983 To 1995

OBJECTIVE

To determine temporal trends in *Mycobacterium tuberculosis* drug resistance in KwaZulu-Natal, South Africa between 1983 and 1995.

DESIGN

Routine drug susceptibility data from the central provincial mycobacteriology laboratory in KwaZulu-Natal, South Africa for the 13 year period from 1983 to 1995 were analysed. The laboratory used the 1% proportional method in Lowenstein-Jensen medium for susceptibility testing throughout the study period.

RESULTS

A total of 21 704 sputum samples from which M. tuberculosis was cultured, were subjected to susceptibility testing. Multi-drug resistance (MDR-TB), defined as combined resistance to isoniazid (INH) and rifampicin (RFA) was 2.2% in 1983 and 3.7% in 1995 (p = 0.01). Examination of the temporal trends in MDR-TB revealed a downward trend from 2.6% in 1984 to 0.9% in 1987 (chi square for linear trend 9.34 ; p = 0.002). The prevalence did not change much between 1987 and 1990 followed by an upward trend from 1.1% in 1990 to 3.7% in 1995 (chi square for linear trend 30.56; p < 0.001). Resistance to INH alone was 6.7% in 1983 and 7.0% in 1995 (p = not significant) and for RFA alone 8.1% and 7.2% (p = not significant), respectively.. All other drugs tested showed a statistically significant decrease in drug resistance between 1983 and 1995 viz. streptomycin (13.7% and 7.7%; p < 0.001), ethionamide (9.3% and 4.5%; p < 0.001), thiacetazone (7.8% and 3.2%; p < 0.001) and ethambutol (3.4% and 2.1%; p = 0.01).

CONCLUSIONS

Following an initial downward trend, a significant increase in the prevalence of MDR-TB was noted. It is particularly disconcerting that there was a reversal in a previous downward trend in MDR-TB. While these data cannot simply be extrapolated to all patients with TB due to the selective basis on which susceptibility tests were requested, they nevertheless provide valid temporal trends.

SUMMARY OF CHAPTERS 4 to 7 - Pharmacokinetic and Pharmacodynamic Characteristics of Isoniazid and Rifampicin in Patients with Multi-drug-Resistant Tuberculosis

OBJECTIVE

To investigate if there is an association between the pharmacokinetic parameters of isoniazid (INH) and rifampicin (RFA) and multi-drug resistant tuberculosis (MDR-TB) in HIV positive (HIV+) and HIV negative (HIV-) patients.
To describe the pharmacokinetic and pharmacodynamic characteristics of INH and RFA in MDR-TB and drug-sensitive TB patients stratified according to HIV status.

DESIGN

Prospective case-control pharmacokinetic study.

SETTING

King George V Hospital, a large specialist referral TB in-patient treatment facility in Durban, KwaZulu-Natal, South Africa

PATIENTS

A total of 138 adult pulmonary tuberculosis patients: 62 MDR-TB (21 HIV+ and 41 HIV-) and 74 drug-sensitive TB (37 HIV+ and 36 HIV-; 1 not classified). A further 2 patients (1 HIV+ and 1 HIV-) could not be classified according to drug susceptibility status.

METHODS

Single daily doses of INH (300 or 400 mg) and RFA (450 or 600 mg) were administered under supervision for 2-5 days prior to the study. Any other drug treatment prescribed for TB or concomitant complaints was noted but not discontinued. Thereafter 6 blood samples were drawn over 2 dosing intervals at 0, 1, 2, 4, 8 and 12 hours after dose administration. Clinical, socio-demographic, radiological (extent and severity of lung involvement), clinical chemistry (liver function tests) and microbiological (drug susceptibility and minimum inhibitory concentration (MIC)) data were collected. Serum drug concentrations were determined using a validated high performance liquid chromatography (HPLC) assay.
Pharmacokinetic data analysis was conducted according to the population approach using the NONMEM program as well as with non-compartmental methods.

RESULTS

Results from the non-compartmental analysis were similar to those obtained from the population approach. Upon initiation of treatment, the average 54-kg patient had a CL/F for RFA of 7.7 L/hr. After continuous daily treatment, maximal enzyme auto-

induction was reached at approximately 10 days at which time the CL/F was 15.6 L/hr. The mean population V/F for RFA was 26.5 L at initiation of treatment and 42.1 L after 10 days of therapy. The inter-individual variability (% coefficient of variation [CV] for RFA was 39% for CL/F and 26% for V/F. Residual variability was described with a proportional component of 39% and an additive component of 0.05 μg/ml.

The proportion of INH fast acetylators in the population was found to be in the majority (85%). The mean population CL/F was 13.0 L/hr for fast acetylators and 4.7 L/hr for slow acetylators. The V/F for INH was 50.0 L. The inter-individual variability in INH CL/F was 32% for slow acetylators and 41% for fast acetylators. There was also a 41 % variability in V/F. Residual variability was described with a proportional component of 28% and an additive component of 0.02 μg/ml.

The pharmacokinetic parameters for both INH and RFA obtained in this study compare well with that reported in the literature.

Population pharmacodynamic parameters (maximum serum concentration [Cmax]:MIC ratios, time above the MIC and area under the curve [AUC] above the MIC) for INH and RFA were described and represent potential benchmarks for future prospective clinical evaluation.

CONCLUSIONS

There was no association between the pharmacokinetic parameters of INH and RFA and MDR-TB. Neither was there any association between HIV status or degree of immune compromise as determined using HIV viral loads and the pharmacokinetic or pharmacodynamic parameters of INH and RFA.

Chapter 1

INTRODUCTION

The World Health Organisation has declared tuberculosis (TB) to be a global public health emergency with an estimated one-third of the world's population being infected with *Mycobacterium tuberculosis*. It is estimated that the number of active TB carriers is about 20 million with more than 3 million deaths annually *(WHO, 1994)*.

The incidence of TB has been increasing globally in recent years, partially due to the human immunodeficiency virus (HIV) epidemic. More ominous, however, has been the increasing occurrence of strains of *Mycobacterium tuberculosis* that are resistant to currently used chemotherapy *(Edlin et al. 1992; Iseman, 1993; Bloch et al. 1994; Neville et al. 1994)*. If this resistance includes resistance to isoniazid (INH) and rifampicin (RFA) in combination, then the deadly, almost incurable, "third epidemic" of multi-drug resistant tuberculosis (MDR-TB) emerges *(Kochi et al. 1993; Neville et al. 1994)*.

Several of the "hotspots" for MDR-TB identified by the WHO exist in sub-Saharan Africa *(Cohn et al. 1997; Macready, 1997)*. This is not surprising given the poor TB cure rates in many of these countries. However, it is particularly disconcerting that the TB situation in South Africa should have been labelled *"... the most serious in the world"* despite the relative affluence of this country. This invidious conclusion was the result of a 1996 review by the WHO and the South African Department of Health *(WHO, 1996)*. They suggested that the formula for this "crisis" was the high TB case rates, the emergence of MDR-TB and the growing HIV epidemic.

There is a paucity of information on the exact extent of the MDR-TB problem in South Africa. In a study of newly admitted Black TB patients in South African hospitals, researchers from the Medical Research Council demonstrated a marked decrease in primary and acquired drug resistance over the period 1965 to 1988 *(Weyer and Kleeberg, 1992)*. There has been no investigation into the trends in drug resistance thereafter.

KwaZulu-Natal, one of South Africa's 9 provinces, is reported as having the highest prevalence rate of HIV infection (27 %) compared to the other provinces which have prevalence rates that range from 6 % in the Western Cape to 23 % in Mpumalanga province *(Department of Health, 1998)*. The province ranks fifth in terms of the estimated incidence of TB *(WHO, 1996)*. There is currently no published data on the prevalence of MDR-TB in the province.

The first aim of this study was to determine the temporal trends in *M. tuberculosis* drug resistance using routine drug susceptibility data from the central provincial Mycobacteriology Laboratory in KwaZulu-Natal, South Africa. This is addressed as a distinct study in Chapter 3, which provides the setting for the second major component of this thesis.

Several authors have shown that the greatest predictor for drug-resistant TB is a history of prior treatment for TB *(Iseman, 1993; Frieden et al. 1993; Cole and Telenti, 1995).* While re-infection with drug-resistant strains has been reported *(Iseman, 1993)*, there is a strong suggestion of an inadequately treated initial infection usually due to poor treatment adherence. The observation, however, that tubercle bacilli can survive in cells and tissues of the patient despite the adequate and regular administration of drugs *(Grange, 1990)* leads one to query reasons other than poor compliance as an explanation for treatment relapses and drug resistance. Case reports of patients with acquired immunodeficiency syndrome (AIDS) have shown malabsorption of the anti-TB drugs *(Berning et al. 1992; Peloquin et al. 1993; Patel et al. 1995).* This is of grave concern and has serious implications for TB control programmes, particularly in areas with a high HIV seroprevalence. Abnormal pharmacokinetics in the individual patient receiving a multi-drug combination chemotherapy regimen may result in monotherapy if only one drug is absorbed - an untenable situation in TB treatment.

Sahai et al (1997) prospectively evaluated the role of HIV infection on the absorption of anti-TB drugs in volunteer groups of symptomatic and asymptomatic HIV positive patients (with no TB) and found reduced total drug exposure to RFA and pyrazinamide. In a similar prospective evaluation in AIDS patients with concurrent TB infection, 2 . studies *(Choudhri et al. 1997; Taylor and Smith, 1998)* were unable to reproduce these findings of reduced absorption.

While several patients in the case reports *(Peloquin et al. 1993; Patel et al. 1995)* were infected with MDR-TB, no study has prospectively investigated the association between anti-TB drug pharmacokinetics and drug resistance. Such an association has been suggested *by (Bradford et al. 1996)* in their observation of the changing epidemiology of acquired drug resistance in San Francisco.

Therefore the second major aim of this study was to investigate if there is an association between drug pharmacokinetic parameters and MDR-TB in HIV positive (HIV+) and HIV negative (HIV-) patients. Pharmacokinetics were investigated using non-compartmental analysis techniques as well as a compartmental model based population approach. The latter approach utilised Non-linear Mixed Effects Modelling as implemented in the NONMEM program *(Boeckmann et al. 1994).* The NONMEM approach provided the opportunity to investigate the influence of a wide variety of concurrent clinical scenarios such as drug and disease interactions on the pharmacokinetics of INH and RFA. This was facilitated by a quasi-experimental design in which attempts were made to minimise disruptions to the normal hospital ward programme and to study the drugs under conditions of routine use.

Finally, the pharmacokinetic parameters in the individual patient were used to predict the time course of INH and RFA concentrations. This was used to predict the ratio of the maximum drug concentration (Cmax) to the minimum inhibitory concentration (MIC), the time serum concentrations remained above the MIC (t>MIC) and the area under the serum concentration versus time curve (AUC) above the MIC. In this way, the pharmacodynamic parameters for INH and RFA were described in MDR-TB and drug-sensitive TB patients.

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Chapter 2

LITERATURE REVIEW

Summary

OBJECTIVE

To review the history, epidemiology, clinical and microbiological manifestations of tuberculosis (TB) with emphasis on drug-resistance.
To review the pharmacotherapy of TB with particular emphasis on pharmacokinetic and pharmacodynamic considerations and the drugs: isoniazid and rifampicin.

DATA SOURCES

The current medical literature, including primary and secondary references. References were identified using electronic retrieval systems such as Medline and the Iowa Drug Information Systems as well as published abstracts from scientific meetings and discussions with experts in the field.

STUDY SELECTION

While data relating to the primary research questions were targeted, some background information was included so as to contextualise the study within the global and the South African context. Data supporting and disputing the specific study objectives are presented.

DATA SYNTHESIS

There is a global increase in the incidence of TB despite the availability of a sound scientific strategy for its control. The WHO has declared the TB crisis in South Africa to be the worst in the world. The human immunodeficiency virus (HIV) epidemic is a major contributor to this "global emergency". An increase in drug-resistant TB has also been noted globally although the exact extent of the problem in South Africa is not known.
Malabsorption of the anti-TB drugs in HIV+ and acquired immunodeficiency syndrome (AIDS) patients has been noted and has sound mechanistic explanations. However some studies have failed to confirm early case reports of malabsorption. If proved correct this has serious public health implications particularly in areas with a high prevalence of HIV infection. Apart from treatment failure in the individual patient, absorption of a single drug in a TB drug regimen equates to monotherapy and has been associated with the acquisition of drug resistance.
The available drugs against TB are being depleted as a result of drug resistance and there appears to be very few, if any, promising agents on the horizon. The importance of studying available agents to better understand their use is therefore emphasised.

Drug Resistant Tuberculosis – An Epidemiological, Clinical and Microbiological Perspective

INTRODUCTION

The chemotherapy of TB uses a combination of anti-TB drugs which has activity against one or more of several identified bacterial sub-populations depending on metabolic activity and environmental physicochemical properties. Mutations to drug resistance among the anti-TB drugs occurs at a low but constant rate. Thus the combination chemotherapy of 3-4 drugs assumes a low probability of simultaneous resistance to all drugs used in the regimen *(Davidson, 1987)*. As a result of this sound scientific strategy, the incidence of TB decreased and was thought to be nearing elimination in many developing countries *(Daniel, 1991)*.

However, recent years have seen a marked increase in the incidence of TB in general and MDR-TB in particular. This prompted the World Health Organisation (WHO) to make an unprecedented declaration of TB being a global emergency. The International Union Against Tuberculosis and Lung Disease (IUATLD) and the WHO estimate that approximately 1.7 billion persons, or one-third of the world's population is infected with Mycobacterium tuberculosis. They estimated the number of active TB carriers globally to be 20 million with more than 3 million deaths annually *(WHO, 1994)*.

In 1996 the WHO and the South African Department of Health conducted a combined review of the TB Control Programme in South Africa. They concluded that South Africa's high TB case rates, the emergence of MDR-TB and the growing HIV epidemic combine to make the country's TB crisis the most serious in the world *(WHO, 1996)*.

Why is this curable disease resurfacing as a public health nightmare?

This chapter reviews the history, epidemiology, clinical and microbiological manifestations of TB and of drug-resistant TB. A brief account of the pharmacotherapy of TB is also presented together with pharmacokinetic and pharmacodynamic considerations with particular emphasis on INH and RFA.

HISTORY

TB appears to have afflicted mankind from ancient times. The skeletal remains of a Neolithic man found near Heidelberg in Germany (5 000 BC) and that of several Egyptian mummies (3 700 to 1 000 BC) suggests the presence of TB of the spine.

Early physicians like Aristotle and Hippocrates called TB a "phthisis", a Greek word meaning wasting away. This was later translated into English as "consumption". Sylvius first applied the term "tubercle" to the typical nodular lesions he found at post mortem examinations and from this arose the term tuberculosis *(Metcalf, 1991)*.

In 1882 Robert Koch isolated and cultured *Mycobacterium tuberculosis* from crushed tubercles thereby establishing the true aetiology and infectious nature of the disease.

Soon after Koch's discovery, Farlanina indicated that pneumothorax was the sole and non-elective treatment for TB *(Grassi and Peona, 1995)*. In addition, TB treatment from the mid 1800's to the early 1900's consisted of placing patients in sanatoria. The prescription was one of fresh air and rest.

The discovery of streptomycin in 1943 by *Waskman* marked what is commonly referred to as the era of TB chemotherapy. The introduction of INH in 1952 and RFA in 1966 were 2 further important milestones in the treatment of TB *(Grassi and Peona, 1995)*. However, since 1970, there have been no new classes of antimicrobial agents specifically included into the armamentarium against TB *(Cole and Telenti, 1995)*. More recently, the macrolides and the quinolones hold some promise of becoming useful additions to the regimen *(Mandell and Petri, 1996)*.

INFECTION AND DISEASE

TB is an infectious disease caused primarily by *Mycobacterium tuberculosis*. Other species implicated in causing infection in humans include *M bovis* and *M africanum* although infection with these species is rare. The disease most commonly affects the lungs although any body site can be involved *(Daniel, 1991)*.

TB is nearly always transmitted through infectious particles that are released into the air by a patient with active pulmonary disease. This occurs when the patient coughs, sneezes, speaks or sings. These droplets stay suspended in the air for several hours. If inhalation of these particles by another person occurs, then the tubercle bacillus enters the lymphatics and bloodstream and spreads throughout the body.

Within 2-6 weeks, most immunocompetent individuals develop cell-mediated immunity to the infection. Lymphocytes and macrophages infiltrate the lesions containing the bacillus, killing most of the bacilli and walling off the infection. At this point, the infected person is usually asymptomatic and may remain so for life. Within 2 to 10 weeks after infection, most infected individuals show a positive reaction to the purified-protein derivative (PPD) skin test, and may show a healed, calcified lesion on the chest radiograph – a condition known as latent infection *(Daniel, 1991)*.

Those infected have a 10% lifetime risk of developing active TB disease. The organism remains dormant in the remaining 90% of people who are not considered to be contagious *(Zeind et al. 1996)*.

Physical or emotional stress can destroy the balance between the immune system and the infection, leading to clinically active disease. Conditions associated with progression to clinical disease include previously untreated infection, intravenous drug abuse, diabetes mellitus, prolonged corticosteroid use, immunosuppressive therapy, various carcinomas, gastrectomy, cachexia and HIV infection *(Zeind et al. 1996)*.

The progressive loss of immune function as a result of HIV infection is a powerful risk factor for the activation of latent TB. In HIV+ patients the lifetime risk of developing active TB is increased to 8% per year. Recent molecular epidemiology reports suggest that in HIV+ and AIDS patients, TB occurs more often as recently acquired infection rather than as reactivation of a latent focus *(Zeind et al. 1996)*.

CLINICAL PRESENTATION

The vast majority of TB patients have pulmonary TB. In the USA, extra-pulmonary TB accounts for 15% of cases and symptoms depend on the site of involvement *(Zeind et al. 1996)*. In pulmonary TB, the typical generalized symptoms are weight loss, malaise, fever and night sweats. With progression of the disease, the patient may develop a persistent cough that often produces sputum.

However, these "classical" symptoms are frequently absent, and the onset is insidious, with the diagnosis only being considered when a chest radiograph is performed. The typical radiological findings include patchy or nodular infiltrates in the apical areas of the upper lobes or the superior segment of the lower lobes. As the infection progresses, cavitation is often seen. Patients often only present for medical attention when dramatic symptoms such as haemoptysis occur. At this point, patients typically have large cavitary lesions with high mycobacterial loads *(Daniel, 1991)*.

In the early stages of TB in patients with HIV infection, chest radiographs are indistinguishable from patients who are seronegative. However, as the level of immune compromise progresses, there is less necrosis and cavitation, the chest radiographs show predominantly diffuse or miliary infiltrates while the immunocompetent patients show focal infiltrates and/or cavitation *(Wilkinson and Moore, 1996b; Zeind et al. 1996)*.

MYCOBACTERIOLOGY

The tubercle bacillus is a slender, straight, or slightly curved aerobic bacillus, less than 0.5 μm in diameter and 1 to 4 μm in length. It differs from bacteria in 2 notable ways. First, the TB bacillus replicates at a much slower rate – generally every 24 hours instead of every 20-40 minutes. Second, because of its waxy outer layer, the bacillus does not stain well with Gram's stain. Instead Ziehl-Neelsen or fluorochrome stains must be used to detect the organism in biological specimens – usually sputum. It is this property that gives rise to the term acid-fast bacillus (AFB) when referring to mycobacteria *(Daniel, 1991)*.

EPIDEMIOLOGY OF TUBERCULOSIS

"The 2 essential factors for the rapid spread of TB are crowded living conditions favouring the spread of infection and a population with little native resistance" *(Des Prez and Heim, 1990)*

The early European colonialists and travellers are credited with introducing TB into South Africa. This occurred during the course of their journeys to the East via the Cape of Good Hope and during the early days of colonisation. In 1867, the English epidemiologist *William Budd* wrote in *The Lancet*:

"Everywhere along the African sea-board, where blacks have come into contact and intimate relations with the whites, phthisis causes a large mortality among them. In the interior, where intercourse with the whites has been limited to casual contact with a few great travellers or other adventurous visitors, there is reason to believe that phthisis does not exist."

Budd 1867 cited by (Metcalf, 1991)

During the early 1900s, the migrant labour system and the conditions in the gold and diamond mines of South Africa contributed to the increase in TB incidence in the Black population. Medical examinations were cursory and failed to identify those unfit for work on the mines. The mines were poorly ventilated and very humid. Mine workers worked for long hours in close proximity to each other. They were housed in overcrowded compounds without partitions and their diets were woefully inadequate. The practice of repatriating mine workers who developed TB played a key role in disseminating the disease to the rural areas *(Metcalf, 1991)*.

However, the exact size of the TB problem in this country has probably always been under-estimated. Notification systems in most countries are known to have the problem of under-reporting. In South Africa this was compounded by the fragmented health systems implemented by the apartheid policies of the previous regime.

The introduction of chemotherapy saw a global decrease in TB morbidity and mortality *(Daniel, 1991; Dooley et al. 1992)*. In the USA, in 1953, the first year of national reporting, there were 84 304 cases of TB. By 1985, only 22 201 cases were reported *(Zeind et al. 1996)*, the lowest number recorded over the previous 3 decades. However around 1989, just when projections for the total elimination of TB were being calculated, a global increase in TB incidence was noted. In the USA, in 1993 there were 26 287 cases i.e. a 14% increase over the 1985 figures *(Zeind et al. 1996)*.

A similar pattern to the global epidemiological picture of a decline and a subsequent increase in TB incidence has also been seen in South Africa *(Department of Health, 1992)*.

This reversal in the downward trend and its underlying causes has been referred to as the "U-shaped curve of concern" *(Reichman, 1991)*. The HIV epidemic is probably the heaviest contributor to the rise in TB cases worldwide.

The overall incidence of TB has been estimated to be around 311 per 100 000 in South Africa *(WHO, 1996)*. However, the true incidence of TB for 1996 was 362 per 100 000 *(Department of Health 1998)*. There is an uneven distribution of the case loads across the 9 provinces of the country. The highest rates are reported in the Western Cape (737 per 100 000) and the lowest rates in the Northern Province (44 per 100 000). KwaZulu-Natal ranks fifth with an estimated annual incidence of 120 per 100 000. The racial distribution shows that the highest risk is among the so-called "Coloured" people of the Western Cape. The Black population has a high and stable prevalence while the Indian and White populations have low and falling rates *(WHO, 1996)*.

The HIV epidemic in South Africa is escalating at an alarming rate. Over a period of 7 years the prevalence of HIV infection rose more than 14-fold from 0,76 % in 1990 to 14.07 % in 1996 *(Abdool Karim et al. 1997)*. This can be expected to increase the TB case notifications dramatically.

EPIDEMIOLOGY OF DRUG-RESISTANT TUBERCULOSIS

In 1994 *Neville et al* warned of the "third epidemic" that threatens to add to the already dismal combination of TB and HIV i.e. MDR-TB. This concern was in response to numerous reports of MDR-TB occurring in the USA.

Frieden et al (1993) reported the emergence of drug-resistant TB in New York City. These authors examined drug resistance in 466 isolates (90%) available from 518 patients with positive cultures for *M. tuberculosis* during April 1991. Overall, 33% had isolates that were resistant to at least one drug. Of these, 26% of isolates were resistant to INH, 22% to RFA, and 19% had MDR-TB as defined in this study.

The Centers for Disease Control and Prevention (CDC), USA suggested a number of factors that could have contributed to the outbreaks of MDR-TB in hospitals and correctional facilities from 1990 through 1992 *(Anonymous1991; Doole et al. 1992)*. There was a high mortality among these patients, ranging from 43% to 89%. In addition, the median interval from diagnosis to death was very short, only 4 to 16 weeks. The factors identified bear general applicability outside the USA. They include patient non-adherence with therapy, sub-optimal treatment regimens, overall poor response to therapy in MDR-TB, prolonged infectiousness, severe immunosuppression, inadequate infection control measures, and susceptible contacts in close proximity to increased numbers of TB patients *(Anonymous1991; Doole et al. 1992)*.

The first three of these factors were emphasised by *Goble et al (1993)* who retrospectively reviewed treatment of 171 patients with MDR-TB . This primarily immunocompetent group had the disease for a median of six years and received a median of six anti-TB drugs. All patients were noted to have an isolate that was resistant to a median of six drugs. Despite individually tailored regimens, an overall response rate of only 56% was seen. RFA resistance was associated with suboptimal or irregular dosing and/or the administration of RFA as the single effective agent.

Edlin et al (1992) evaluated an outbreak of MDR-TB among hospitalised patients with AIDS in New York, and emphasised the last three factors i.e. severe immunosuppression, inadequate infection control measures, and susceptible contacts in close proximity to increased numbers of TB patients. The authors compared exposure among 18 AIDS patients who had developed MDR-TB with that among 30 controls who had contracted a susceptible strain of TB. The patients with MDR-TB were more likely to have been hospitalised in the same wards and in rooms near patients with infectious drug-resistant TB. In addition, of 16 patient's rooms that were tested with airflow studies, only one had the recommended negative pressure ventilation required for efficient infection control in this setting *(Edlin et al. 1992)*.

There is a paucity of good quality prevalence data on resistance to the anti-TB drugs in South Africa. This is unfortunate, as surveillance of drug resistance is vitally important for purposes of TB programme evaluation. An increase in drug resistance is generally indicative of poor programme management – irrespective of whether the resistance noted is initial drug resistance or acquired drug resistance.

The South African Medical Research Council (MRC) has been monitoring drug resistance trends as part of their National TB Research Programme. In a study of

newly admitted black TB patients in South African hospitals, *Weyer and Kleeberg (1992)* from the MRC demonstrated a marked decrease in primary and acquired drug resistance over the period 1965 to 1988. The drugs tested were INH, streptomycin, RFA, ethionamide and ethambutol. The authors noted that the prevalence of MDR-TB was below 2% in 1988.

At a sentinel surveillance site at Hlabisa Health Ward, in rural KwaZulu-Natal, using a careful study design to eliminate bias, only 1 case (0.3%) of MDR-TB in 335 consecutive incident cases of pulmonary TB was noted during 1994 *(Wilkinson et al. 1996c)*. The results from this site are probably reflective of its good programme management that includes directly observed therapy (DOT) *(Wilkinson et al. 1996a)*.

A report by *Weyer et al (1995)* noted that MDR-TB was between 1.1 and 4% (primary and acquired drug resistance respectively) in the Western Cape during 1994. This is the province with the highest prevalence of TB in South Africa.

MECHANISMS FOR DRUG-RESISTANT TB

Drug resistance in *Mycobacterium tuberculosis* occurs by random, one step spontaneous mutations at the gene loci of the chromosomes, at a low but predictable frequency. Clinically, drug-resistant TB arises slowly, usually as a result of selection pressure exerted by inadequate therapy. Fortunately resistance to one drug is not associated or linked with another unrelated drug. However, cross-resistance among members of a similar class, such as rifamycins and fluoroquinolones does occur. The mutations may affect one or more genes responsible for drug action. This may manifest as increased synthesis of a target enzyme for drug action thus rendering the drug less effective to inhibit growth of the microorganism. Alternately, they may alter a primary drug target or the drug's transport system into the microorganism *(Rastogi and David, 1993)*.

The probability of drug-resistant mutants is depicted by the formula $P = 1 - (1-r)^n$, where P is the probability of drug-resistant cases, r is the probability of drug-resistant mutants, and n is the number of bacilli in the lesion. The value of r for RFA is 10^{-8}, that for INH, streptomycin, ethambutol, kanamycin and para-aminosalicylic acid is of the order of 10^{-6}, and that for ethionamide, capreomycin, cycloserine and thiacetazone is 10^{-3}. When 2 drugs are used in combination, the value of r becomes the product of the individual r's e.g. the probability of initial combined resistance to INH and RFA is 10^{-8} x $10^{-6} = 10^{-14}$. When 3 drugs are used as the combination regimen, the value of r becomes very low indeed (10^{-18} to 10^{-20}) - emphasising the potential role of combination chemotherapy in reducing the probability of drug resistance in TB chemotherapy.
The role of the number of bacilli (n) in the lesion also influences the probability of drug-resistance as shown in the formula. Cavitary lesions usually contain from 10^8 to 10^9 organisms. This is thus the potential location at which maximal drug resistance can occur.

Drug resistance is categorised into initial, primary or acquired resistance *(Kochi et al. 1993)*.

Initial resistance is defined as the presence of drug resistance to 1 or more drugs in a new patient with TB presenting for treatment. This category includes patients with primary resistance, as well as those with undisclosed acquired resistance, who either cannot recall or who conceal prior therapy.

Primary resistance is defined as resistance to anti-TB drugs in a patient who has never received chemotherapy. It can be caused by infection with drug-resistant organisms from another patient with acquired drug-resistance, or because of infection with naturally resistant wild strains. Due to the difficulty in differentiating between primary resistance from undisclosed acquired resistance, the term initial resistance is preferred.

Acquired resistance is defined as resistance to anti-TB drugs that arises as a result of poor adherence to the recommended regimen or poor prescribing.

FACTORS FAVOURING THE EMERGENCE OF DRUG-RESISTANT TB

Poor patient adherence is widely reported to be a major cause of drug-resistant TB. Other causes include inadequate therapy, in particular monotherapy, addition of a single drug to a failing regimen, an insufficient number of active drugs in the regimen, erratic drug taking, sub-optimal drug dosages and poor absorption *(Frieden et al. 1993; Iseman, 1993; Yew and Chau, 1995).*

In developing countries, socio-economic factors such as poor nutrition, poverty, unemployment, overcrowding, illiteracy, social stigmatization and minimal access to medical care are all contributory factors to failure of effective treatment *(Sumartojo, 1993).* These will predispose to drug resistance.

Further problems peculiar to many developing countries are issues related to drug acquisition, distribution and supply. Concerns have also been expressed about the production and marketing of sub-standard formulations with poor bioavailability of the component ingredients *(Gangadharam, 1993; Yew and Chau, 1995).*

Epidemiological studies have identified several factors that favour the emergence of drug resistance in general. They include a history of previous anti-TB drug therapy, patient non-adherence with therapy, sub-optimal treatment regimens, overall poor response to therapy in MDR-TB, prolonged infectiousness, severe immunosuppression, inadequate infection control measures, and susceptible contacts in close proximity to increased numbers of TB patients *(Iseman, 1993; Davidson, 1987; Yew and Chau, 1995).*

The concurrent increase in both HIV infection and drug-resistant TB and the occurrence of MDR-TB in patients with HIV infection in the USA suggests that HIV may favour the emergence of drug resistance. There are several reasons for the association apart from HIV infection *per se.* Many of the outbreaks occurred in settings where HIV+ patients received care. It is now well described that HIV+ patients who are exposed to TB (drug-sensitive or drug-resistant) are much more likely to develop active TB rapidly compared to those who are HIV- *(Daley et al. 1992; Zeind*

et al. 1996). The HIV+ patients are likely to have higher mycobacterial loads and thus have a higher probability of harbouring more drug-resistant mutants.

Pharmacotherapy of Tuberculosis with particular emphasis on the Pharmacology and Pharmacokinetics of INH and RFA

RATIONALE FOR THE PHARMACOTHERAPY OF TUBERCULOSIS

In considering the susceptibility of *M tuberculosis* to antimicrobial agents and the development of rational drug therapy, it is important to appreciate the physicochemical properties of the tissues in which the bacilli reside and their metabolic activity. These are embodied in Mitchison's separate populations hypothesis which proposes the following theoretical model for TB infection *(Mitchison, 1992).*

M tuberculosis grows very rapidly and the bacilli load is high in the cavitary lesions, where growth conditions are favourable because of high oxygen content and a neutral pH. This subpopulation is particularly vulnerable to INH and to a lesser degree to RFA, streptomycin and ethambutol.

A second slow-growing subpopulation exists in an acidic environment located mainly intracellularly. Pyrazinamide is particularly active in an acidic medium (pH 5.3 to 5.5) and thus is very effective in killing this subpopulation. The other drugs in the regimen INH, RFA and ethambutol are less active against these organisms. The extended action of pyrazinamide is explained by theorising that the acidic environment favourable to its action also exists extracellularly where there is an inflammatory response.

The third subpopulation is located mainly in caseous material where the pH is neutral but the oxygenation is poor, and these organisms grow very slowly with occasional spurts of active growth. They are killed most efficiently by RFA due to the rapidity with which its bactericial action commences.

The fourth subpopulation is completely dormant and anti-TB drugs have no activity against this subpopulation.

On the basis of animal experiments and clinical trials, the anti-TB drugs may be classified into 3 categories *(Mitchison, 1985):*

Drugs with Resistance Prevention Activity

These agents, when combined with others, can prevent the emergence of resistant mutants to the companion drug. In this category, INH and RFA are the most effective, followed closely by ethambutol and streptomycin. Pyrazinamide and thiacetazone are less effective in preventing the emergence of resistance.

Drugs with Early Bactericidal Activity

These agents induce a rapid decrease in the number of living bacilli in the sputum at the beginning of treatment. They rapidly reduce the bacillary load in the patient and quickly convert the sputum cultures to negative, thus reducing the risk of transmission. INH is the most effective drug in this category, followed by ethambutol and RFA.

Drugs with Sterilising Activity

These drugs have the ability to kill all the tubercle bacilli in the lesions of experimental TB in animals and probably also in human disease. They reduce the relapse rate to a minimum within a short period. RFA and pyrazinamide have the greatest sterilising activity; INH is weaker; streptomycin, ethambutol, and thiacetazone have little activity.

Thus the best anti-TB drug regimen is one that combines drugs that are effective against all the identified subpopulations and have properties of early bactericidal activity, prevention of resistance and sterilising acitvity.

The crucial role played by INH and RFA in anti-TB drug regimens is clearly evident. Most short course chemotherapy regimens included these 2 drugs with streptomycin or ethambutol and pyrazinamide included at least for the first 2 months *(Davidson and Le, 1992)*. More recently, due to its side effects and the inconvenience of parenteral therapy, streptomycin has been largely removed from the regimen. Further, it has been recommended that ethambutol be used only in areas with a high prevalence of INH resistance.

PHARMACOLOGY AND PHARMACOKINETICS OF RIFAMPICIN

Rifampicin (rifampin) is a brick red crystalline powder that is a synthetic derivative of a natural antibiotic rifamycin B produced by *Streptomyces mediterranei*. Its activity against mycobacteria is characterised by a high sterilising activity and an ability to eliminate semi-dormant or persisting organisms *(Reynolds, 1993)*. When included in multi-drug combination regimens, RFA has the ability to prevent the emergence of resistance to its companion drugs *(Mitchison, 1985)*.

Microbial Spectrum of Activity

RFA owes its antimicrobial action to interference with the synthesis of nucleic acids by inhibiting DNA-dependent RNA polymerase. Its selective toxicity is due to its ability to inhibit the enzyme at relatively low concentrations in relation to the concentration required to inhibit mammalian RNA synthesis *(Mandell and Petri, 1996)*.

RFA inhibits the growth of a wide range of gram positive bacteria especially *Staphylococci* but is less active against gram negative organisms. The sensitive gram negative organisms include *Neisseria meningitidis* and *N gonorrhoea* and *Legionella spp*. It also has activity against *Chlamydia trachomatis* and some anaerobic bacteria *(Reynolds, 1993)*. At high concentrations (500 to 1000 X that against bacteria), RFA has activity against viruses such as herpes, adenovirus and pox virus *(Mandell and Petri, 1996)*. Although it has no activity against fungi, it does enhance the action of amphotericin. In addition to activity against *Mycobacterium tuberculosis* and *M leprae*,

RFA also has activity against atypical *mycobacteria* such as *M kansasii, M scrofulaceum* and *M intracellulare.* The MIC for susceptibile mycobacteria ranges from 0.1 to 2 µg/ml while that for other organisms (*Chlamydia* and *Staphylococci*) is lower at 0.01 to 0.02 µg/ml *(Dollery, 1991b; Reynolds, 1993).*

Resistance to RFA occurs rapidly if the drug is used alone due to the occurrence of naturally occurring resistant mutants which is of the order of 1 in 10^7 to 10^8. Thus in TB and leprosy regimens, RFA is always used in combination with other drugs to delay or prevent the emergence of drug resistance *(Reynolds, 1993).*

Pharmacokinetics

Table 2.1 is a summary of the pharmacokinetic parameters for RFA reported in the literature. The data presented is illustrative rather than exhaustive e.g. data from the excellent review by *Kenny and Strates (1981)* is not included in the table. This was because the authors of that review recalculated many of the parameters. This could have introduced a source of error as the present review also involved calculation of CL using data from the references cited.

Absorption
RFA is well absorbed from the GIT with peak plasma concentrations of 7 to 9 µg/ml being achieved 1 to 4 hours after a dose of 600 mg. There is however, considerable interindividual variation in absorption characteristics *(Reynolds, 1993; Kenny and Strates, 1981; Gelman and Rumack, 1998).*

Food may delay the rate of RFA absorption. Although some have suggested that the extent of absorption may also be affected, all researchers have not consistently observed this *(Kenny and Strates, 1981).* Gastric pH is also of importance and acidification of the gastric juice increases solubility and hence absorption and serum concentrations while alkalinity has the opposite effect *(Kenny and Strates, 1981).*

The bioavailability of RFA has been reported to be 90 to 95% *(Gelman and Rumack, 1998).* The serum concentrations of RFA are influenced not only by GIT absorption but also by the rate of biliary and renal excretion *(Kenny and Strates, 1981).* There is no appreciable first pass effect following a first dose but this might occur after repeated doses as a consequence of hepatic enzyme induction.

Distribution
RFA is highly lipid soluble and at physiological pH, only about 25% of the drug is ionised. It thus undergoes rapid tissue distribution into most organs, tissues, bone and body fluids including exudates into tuberculous lung cavities. High concentrations appear in the lachrymal glands and tears where a reddish colour is often noted. Cerebrospinal fluid concentrations are approximately 10 to 20% of serum concentrations but may be increased when the meninges are inflamed *(Kenny and Strates, 1981; Gelman and Rumack, 1998).* There is evidence to suggest that RFA may cross the placenta. Foetal levels approximately 33% that of the mother have been noted *(Kenny and Strates, 1981)* and RFA may also appear in the breast milk. Plasma protein binding has been estimated at 60-80% with approximately 30 to 41% of that being bound to albumin *(Kenny and Strates, 1981).* The volume of distribution is approximately 0.9 L/kg *(Gelman and Rumack, 1998)* to 1 L/kg *(Dollery, 1991b).*

Table 2.1 – Pharmacokinetic parameters for rifampicin reported in the literature

Description	n	Dose/Study Day	tmax (hours)	Cmax (μg/ml)	V (L/kg)	AUC μg.hr/ml	CL* (L/hr)	t½ (hours)	Reference
Crossover bioavailability study in healthy volunteers. Free versus fixed dose triple drug combination formulation. 1 week washout period. Other drugs studied were INH and PZA. Study was conducted on Day 1 of drug administration i.e. pre-enzyme auto-induction.	10	600 mg	2.1 - 2.3	10.6 - 11.6		72.6 - 76.7	7.8 – 8.3	1.7 – 2.3	(Acocella et al. 1988a)
Review of the literature		600 mg Day 1 Day 2 Day 6 Day 14	2 – 4	7 - 10	1	 131.4 84.6 100.5 87.7	 4.6 7.1 6.0 6.8	1 - 6 3.4 2.9 2.5 2.1	(Dollery, 1991b)
Review of the literature		600 mg Day 1 Post induction	2-4	7-9			.	 2 – 5 2 – 3	(Reynolds, 1993)
Review of the literature		600 mg Day 1 Post induction	1 - 4	8 - 9	0.9			 1.5 - 5 2 - 3	(Gelman and Rumack, 1998)
Prospective study in pulmonary TB patients (mean mass 58; range 45 - 87kg). Bioavailability of a fixed dose triple drug combination formulation determined periodically over a 60 day period. Other drugs studied were INH and PZA.	13	11.5 mg/kg Day 1 Day 15 Day 30 Day 60	 2.6 2.8 2.6 2.2	 9.9 10.2 8.6 9.5		 71.0 49.7 44.6 47.9	 9.4 13.4 15.0 13.9	 3.6 1.4 1.9 2.0	(Acocella et al. 1988b)

Table 2.1 – Pharmacokinetic parameters for rifampicin reported in the literature (*continued*)

Description	n	Dose/Study Day	tmax (hours)	Cmax (μg/ml)	V (L/kg)	AUC μg.hr/ml	CL* (L/hr)	t½ (hours)	Reference
Prospective study in pulmonary TB patients. 43 - 60kg. Crossover bioavailability study of free and fixed dose triple drug combination formulation. 2 study centres one using daily treatment and 1 using 3 x a week (intermittent) treatment. Other drugs studied were INH and pyrazinamide. Treated for ≥14 days prior to the study.	8 8	600mg daily 600mg 3xweekly	≤ 4	± 8		45.5 - 51.4 56.7 – 68.6	11.7 – 13.2 8.8 – 10.6	± 3	*(Ellard et al. 1986)*
Healthy volunteers (mean mass 73 kg; range 62 – 100 kg). Subgroups of subjects received RFA alone, and in free and fixed form with INH, PZA. Latter 2 preparations given in crossover design with a 1 week washout period. Various groups analysed separately. Other drugs studied were INH, pyrazinamide and streptomycin. RFA auto-induction not mentioned.	12	8.8 mg/kg	1.7 - 3.2	6.3 - 11.8		36 - 70.2	9.2 – 17.8	2.5 - 4.7	*(Acocella et al. 1985)*

*Calculated from data in the cited literature using CL = Dose/AUC

Metabolism

RFA is metabolised in the liver to desacetylrifampicin, a more polar compound that is more amenable to biliary secretion. The other important metabolite is 3-formyl rifampicin, which is formed by hydrolysis and is excreted in the urine. There are suggestions that the excretory capacity of the liver may be subject to saturation since increasing the dose above 450 mg does not increase the bile concentrations of the drug *(Kenny and Strates, 1981; Dollery, 1991b)*.

After RFA and its main metabolite desacetylrifampicin are excreted in the bile, RFA is reabsorbed into the blood while the metabolite is not i.e. RFA undergoes enterohepatic recycling *(Kenny and Strates, 1981; Reynolds, 1993)*. The quantity of RFA secreted into the bile is significant with 44-54, 60 and 84 mg being obtained 12-16 hours after oral administration of 150, 300 and 600 mg of RFA respectively *(Kenny and Strates, 1981)*.

After first administration of an oral dose, serum concentrations are similar to that after IV administration suggesting little first-pass metabolism. However, repeated administration induces the enzymes of the endoplasmic reticulum with resultant increase in the metabolism of both RFA (auto-induction) as well as other drugs undergoing hepatic biotransformation. In published reports of RFA auto-induction, this effect manifested as a decrease in serum concentrations, AUC and half-life with repeated administration *(Kenny and Strates, 1981)*.

Self-induction of RFA metabolism during daily and intermittent chemotherapy was studied by monitoring the changes in the serum half-life of the drug over a 4-week period in patients with pulmonary TB. RFA 450 mg was administered to 8 patients who received treatment daily, 7 on thrice weekly and 7 others on twice-weekly treatment. Serum half-life was computed from concentrations of the drug determined at 3, 4.5 and 6 hours after drug administration, on admission and at 1, 2 and 4 weeks after start of treatment. In the daily series, the mean serum half-life decreased from 4.9 hours on admission to 3.6 hours at 1 week and treatment beyond this had no further effect. In the thrice-weekly series, maximal induction was observed at the 2nd week, the mean values on admission and at 2 weeks being 5.8 and 3.7 hours, respectively. In the twice-weekly series, maximal induction was observed only at the 4th week, the mean values on admission and at 4 weeks being 4.9 and 3.7 hours, respectively *(Immanuel et al. 1989)*. The results of other studies examining RFA auto-induction are shown in Table 2.1.

The increased clearance of RFA noted upon repeated administration returns to the un-induced values as early 1 week after termination of drug therapy *(Kenny and Strates 1981; Zeind et al 1996)*

Excretion

The principal excretion pathway for RFA is via the bile where the drug undergoes enterohepatic recycling until eventual excretion into the faeces. The kidney is a secondary excretion pathway and the urine appears orange to brick red in colour in the presence of RFA. This provides a useful indicator of patient adherence to therapy. When the biliary excretion pathway is saturated (e.g. with doses in excess of 450mg), the urinary concentrations may increase. However, while dose modification may be

necessary in patients with impaired hepatic function or impaired biliary excretion, it is not usually necessary in patients with impaired renal function *(Dollery, 1991b)*.

Clinical Uses

RFA has good activity against a wide range of pathogens, and has demonstrated success in infections due to these organisms. However, in order to preserve a valuable drug for mycobacterial infections, and delay the emergence of drug resistance, RFA is largely reserved for use in a few specific indications viz.

1. *Mycobacterium tuberculosis* infections at all sites.
2. Prophylaxis against meningococcal meningitis.
3. Opportunistic mycobacterial infections.
4. Leprosy
5. Prophylaxis in tuberculin positive children.

Use in TB

RFA forms part of the standard short course chemotherapy for the treatment of drug-sensitive TB. It is used in a daily dose of 450mg (patients < 50kg) or 600mg (patients ≥ 50kg) for the first 2 months (intensive phase) in combination with INH, pyrazinamide and ethambutol followed by a further 4 months (continuation phase) in combination with INH. In the continuation phase, some countries use a regimen that involves larger doses of RFA administered on an intermittent schedule as opposed to daily administration. In children, a dose of 10 mg/kg is administered *(Dollery, 1991b)*.

Precautions and Contra-indications

RFA should be used with caution in liver function impairment. While some authorities contraindicate its use in patients with jaundice, others advise cautious use with monitoring of liver function *(Dollery, 1991b)*.

RFA affects haem and bilirubin metabolism in man. Serum unconjugated bilirubin concentration may increase together with other liver enzymes such as a transient moderate elevation in alkaline phosphatase during the first 24 hours of treatment. Unless they continue to rise thereafter or there are other signs of liver function impairment, they are not an indication to discontinue treatment *(Mandell and Petri, 1996)*.

Thrombocytopaenia, purpura, haemolytic anaemia and renal failure are indications for withdrawal of treatment.

RFA may colour the faeces, saliva, sputum, sweat, tears, urine and other body fluids orange red. This coloration of body fluids may cause alarm if unexpected. Contact lens users may note permanent discoloration of their soft contact lenses *(Mandell and Petri, 1996)*.

Adverse Reactions

RFA is generally well tolerated. Side-effects are seen more often during intermittent therapy or when restarting interrupted therapy. They are usually due to sensitisation or enzyme inducer effects and consequent drug interactions.

A cutaneous syndrome which presents 2-3 hours after daily treatment manifests as facial flushing, itching, rash or rarely eye irritation. A "flu-like syndrome" with fever, chills, headache and malaise is well described after intermittent RFA treatment. Hypersensitivity and sensitisation as well as a shock-like syndrome, and acute renal failure have been described.

More serious side-effects necessitating drug withdrawal include effects on the blood such as thrombocytopaenia and purpura and are seen especially with intermittent regimens. There may also be leucopaenia and haemolytic anaemia.
Hepatitis occurs rarely but may be aggravated by concomitant drugs or the presence of pre-exisiting liver disorders. An increased risk of venous thrombosis has been reported.

Less severe but of irritation to the patient are the GIT adverse reactions which include anorexia, nausea, abdominal discomfort, diarrhoea and vomiting *(Dollery, 1991b; Mandell and Petri, 1996).*

Drug Interactions

RFA is well known for its ability to cause induction of the cytochrome P450 mixed function oxidase system – more specifically the cytochrome P450IIIA system *(Dollery, 1991b).* Consequently a wide range of drugs have been reported to interact with RFA. Table 2.2 is a selected summary of these interactions. When RFA treatment is removed, the enzyme induction effect slowly returns to normal after 1 to 2 weeks *(Zeind et al. 1996)* or up to 30 days *(Kenny and Strates, 1981).*

Table 2.2 – Summary of some selected clinically important drug interactions involving rifampicin and suggestions for their therapeutic management

Agent	Mechanism/Effect	Management
Antacids	Increased pH reduces dissolution of RFA and hence absorption. Also adsorption effect due to chelation with aluminium and magnesium ions.	Administer at least 2 hours apart.
Anticoagulants e.g. warfarin	Enzyme induction. Decreased hypoprothrombinaemic effect.	Increase anticoagulant dose based on monitoring of prothrombin time.
Beta-Blockers e.g. propranolol and metoprolol	Enzyme induction. AUC of metoprolol increased; CL of propranolol increased.	Monitor therapeutic response and increase dose if necessary.
Contraceptives, oral	Enzyme induction. Increased metabolism of both the oestrogenic and the progesteronogenic components. Breakthrough bleeding and pregancies have been reported.	Use an alternate non hormonal form of contraception.
Cyclosporine	Enzyme induction. Low concentrations of cyclosporin with rejection of allografts reported.	Monitor serum cyclosporine concentrations. Increased dose or frequency of dosing. Consider use of an alternative to RFA.
Digoxin	More likely to be significant in patients with decreased renal function since digoxin is primarily eliminated by the kidneys.	Monitor serum digoxin concentrations. Monitor for arrhythmia control and signs and symptoms of heart failure.
Glucocorticoids e.g. prednisolone	Enzyme induction. Acute adrenal crisis and adrenal insufficiency has been reported in patients with Addison's disease given both drugs.	Increase glucocorticoid dosage twofold to threefold.
Antifungals e.g. ketoconazole and fluconazole	Enzyme induction and/or decreased ketoconazole absorption. Reduction in RFA concentrations has also been reported. Some suggest that there may be less of an effect on fluconazole.	Avoid this combination if possible. Some suggest that RFA and ketoconazole doses be spaced 12 hours apart. Monitor clinical response to ketoconazole, and increase dose if necessary
Phenytoin	Enzyme induction.	Monitor phenytoin serum levels upon starting and stopping RFA treatment. Adjust dose if necessary.
Hypoglycaemic agents e.g. tolbutamide and other sulphonylureas.	Enzyme induction.	Monitor blood glucose control on starting and stopping RFA . Adjust dose of hypoglycaemic agent while monitoring blood glucose concentrations.
Theophylline	Enzyme induction. Decreased efficacy on starting RFA and toxic reactions on stopping RFA are possible.	Monitor serum theophylline levels on starting or stopping therapy.
Verapamil	Enzyme induction.	Use of an alternative agent is recommended. If utilized, monitor patient for clinical response to verapamil.
Diltiazem	Enzyme induction.	Alternative agent recommended because even a very large increase in oral diltiazem dose may not be sufficient (similar interaction with verapamil).
Diazepam	300% increase in diazepam oral clearance has been reported	Monitor clinical response. Increase diazepam dose if necessary.

Compiled from various reviews including (*Venkatesan, 1992; Zeind et al. 1996; Borcherding et al. 1992; Stockley, 1996; Gelman and Rumack, 1998; Mandell and Petri, 1996*).

PHARMACOLOGY AND PHARMACOKINETICS OF ISONIAZID

INH is a white, odourless powder that is prepared by chemical synthesis. It is rapidly bactericidal to actively dividing *Mycobacterium tuberculosis* but only bacteriostatic to semi-dormant organisms *(Reynolds, 1993)*. The drug has high activity in preventing the emergence of resistance against companion drugs in a regimen, as well as having good early bactericidal activity. It is however less active than RFA and pyrazinamide as a sterilising agent *(Mitchison, 1985)*.

Microbial Spectrum of Activity

Although the exact mechanism of action is unknown, INH appears to inhibit the biosynthesis of mycolic acids, which are important and unique constituents of the mycobacterial cell wall. INH may also have effects on nucleic acid biosynthesis and glycolysis. INH has specific activity against mycobacteria with minimal effects on other bacteria or any pharmacological effects in man. It may have some activity against other mycobacteria e.g. *M kansasii*. Its greatest bactericidal effect is on actively dividing bacilli, the effect on semi-dormant organisms being only bacteriostatic *(Mandell and Petri, 1996)*.

The MIC for *M tuberculosis* is 0.02 to 0.2 μg/ml *(Reynolds, 1993)*.
Resistance to INH develops rapidly if used alone in active clinical disease. This does not appear to be a problem when used in prophylaxis where the organism load is low. In any bacterial population *in vivo*, naturally occurring resistant mutants exist with a probability of 1 in 10^6 organisms *(Dollery, 1991b; Mandell and Petri, 1996)*.

Pharmacokinetics

Table 2.3 records a summary of the pharmacokinetic parameters for INH reported in the literature. The list is illustrative rather than exhaustive – the intention is to provide an idea of the range in values reported. Several studies were excluded as the assay methodology used could have resulted in drug breakdown prior to assay. This is illustrated by the results of the study by *Ellard et al 1986* (Table 2.3) in which relatively low AUC values are reported. These authors stored their sample at –20°C for an undisclosed period of time prior to analysis. Our own experience (unpublished) and that of several other authors *(Hutchings et al. 1983; Weber et al. 1983)* is that storage at this temperature results in significant and rapid drug breakdown.

Table 2.3 – Pharmacokinetic parameters of isoniazid reported in the literature.

Description	n	Dose/Study Day	tmax (hours)	Cmax (μg/ml)	V (L/kg)	AUC μg.hr/ml	CL (L/hr)	t½ (hours)	Acetylator Status	Reference
Crossover bioavailability study in healthy volunteers. Free versus fixed dose triple drug combination formulation. 1 week washout period. Other drugs studied were RFA and pyrazinamide. Study was conducted on Day 1 of drug administration.	10	250 mg	1.1 – 1.6	6.6 – 6.8		30.8 – 33.6	7.4 – 8.2	7.2 – 8.4	Not determined	(Acocella et al. 1988a)
Review of the literature		300 mg	1 – 2	3 – 7	0.6 – 0.8			0.5 – 2 2 – 6.5	Rapid Slow	(Dollery, 1991a)
Review of the literature		300 mg	1 – 2	3 – 8				1 - 4		(Reynolds, 1993)
Review of the literature		5 mg/kg	1 – 2	1 – 5	0.6 – 0.75			0.75 – 1.8 2.3 – 3.5	Rapid Slow	(Gelman and Rumack, 1998)
Prospective study in pulmonary TB patients (mean mass 58; range 45 - 87 kg). Bioavailability of a combination triple drug fixed dose formulation determined periodically over a 60 day period. Other drugs studied were RFA and pyrazinamide.	13	4.8 mg/kg Day 1 Day 15 Day 30 Day 60	 1.5 2.1 1.9 1.5	 8.0 7.6 7.1 7.6		 34.7 33.5 30.6 34.2	 8.0 8.3 9.1 8.1	 2.5 2.5 2.3 2.5	Not determined	(Acocella et al. 1988b)

Table 2.3 – Pharmacokinetic parameters of isoniazid reported in the literature *(continued)*.

Description	n	Dose/Study Day	tmax (hours)	Cmax (μg/ml)	V (L/kg)	AUC μg.hr/ml	CL (L/hr)	t½ (hours)	Acetylator Status	Reference
Prospective study in pulmonary TB patients (mean mass 58 kg; range 43 - 60 kg). Crossover bioavailability study of free and fixed dose triple drug combination formulation. 2 study centres one using daily treatment and 1 using 3 x a week (intermittent) treatment. Other drugs studies were RFA and pyrazinamide.	6 2 7 1	250 mg daily 250 mg daily 750 mg 3 x weekly 750 mg 3 x weekly	< 2 <2	2.5 4.5 10		9.1 – 9.2 16.1 – 28.2 33.8 – 34.8 89.4 – 91.4	27.2 – 27.5 8.9 – 15.5 7.2 – 7.4 2.7 – 2.8	2 3 2	Fast Slow Fast Slow	*(Ellard et al. 1986)*
Healthy volunteers (mean mass 73 kg; range 62 – 100 kg). Subgroups of subjects received INH alone, and in free and fixed form with RFA and pyrazinamide. Latter 2 preparations given in crossover design with a 1 week washout period. Various groups analysed separately. Other drugs studied were RFA, pyrazinamide and streptomycin.	12	5.3 mg/kg	1.5 – 2.2	6.4 – 9.2		34.2 – 49.7	7.8 – 11.3	3.4 – 4.2	2 Fast acetylators but combined in the analysis	*Acocella 1985*

Absorption

After oral administration, INH is rapidly and completely absorbed from the gastrointestinal tract. Peak plasma levels of 3 - 8 μg/ml occurs within 1-2 hours after oral administration of normal therapeutic doses to adults (Table 2.3). Food interferes with the rate of oral absorption *(Dollery, 1991a; Zent and Smith, 1995)*. High carbohydate meals and various antacids reduce bioavailability. There is evidence of appreciable presystemic metabolism i.e. gut wall and liver (first-pass) metabolism. This may result in plasma concentrations in fast acetylators that are half those of slow acetylators after normal (300mg) doses *(Dollery, 1991a)*.

Distribution

INH is distributed in total body water with an apparent volume of distribution of 61% ± 11% of body weight. The volume of distribution is unrelated to acetylator status. INH has been detected in CSF, pleural effusions, faeces, saliva, placenta, breast milk, peripheral nerves and red blood cells in humans. High concentrations of the drug have been found in lung and skin which suggests that these organs may serve as storage sites. Binding of INH to plasma proteins is between 4 to 30% *(Gelman and Rumack, 1998)*.

Metabolism

INH is extensively metabolised in the gut wall and the liver to metabolites devoid of anti-TB activity. Metabolism via the hepatic cytochrome P450 mixed function oxidase system accounts for 70 to 90% of the elimination of INH. Several metabolic pathways are involved. N-acetylation to acetyl isoniazid is the most important and the main determinant of the individual genetic differences in drug disposition. After ingestion, the urine contains INH, pyruvic acid hydrazone, α-ketoglutaric acid hydrazone, acetylisoniazid, isonicotinic acid, isonicotinyl glycine, monoacetylhydrazine and diacetylhydrazine *(Dollery, 1991a)*.

Individuals are categorized as either fast or slow acetylators, depending on the rate of acetylation of INH in the liver. Approximately 50 to 60% of the South Indian, European or American negro populations are classified as slow acetylators. Inuit (Eskimos) and Orientals (Chinese and Japanese) are primarily fast acetylators *(Ellard, 1984)*. In South Africa, *Bach et al (1976)* found that 59% of Black South African patients were fast acetylators while *Buchanan et al (1976)* noted that 73% were fast acetylators. More recently, *Parkin et al (1997)* studied the phenotype and genotype of INH acetylation and noted that a larger proportion of South African patients were fast acetylators.

The elimination of INH depends on acetylator phenotype with half-lives of approximately 0.5 to 2 hours being observed in the fast acetylators and 2 to 6.5 hours in slow acetylators (Table 2.3). Patients who are slow acetylators may be more susceptible to the side effects related to higher concentrations, such as peripheral neuropathy. On the other hand it has been postulated that hepatoxicity may be more common in fast acetylators, owing to the production of larger amounts of the metabolite acetylhydrazine which is thought to be involved in the development of this side-effect. However *Ellard (1984)* evaluated the literature and concluded that clinically important hepatic toxicity is unrelated to acetylator status.
The use of INH in daily and 2-3 times weekly regimens in fast acetylators are not associated with any negative effect on clinical efficacy *(Reynolds, 1993)*. However,

once weekly regimens in fast acetylators have an unacceptable failure rate *(Dollery, 1991a)*. Thus it appears as though the rate of acetylation is of limited clinical significance with the current mode of use of INH.

While most authorities report INH metabolism as being either fast or slow, evidence from genotyping studies now indicate that there may be a trimodal distribution in INH metabolism *(Parkin et al. 1997)*.

Excretion
Only a small amount of INH is eliminated unchanged by the kidneys. Therefore, dosage adjustments in patients with renal dysfunction are necessary only for individuals who are slow acetylators with a creatinine clearance less than 10 ml/min *(Zeind et al. 1996)*. Small quantities are also found in the faeces. The major portion of the drug appears in the urine as metabolites. INH is removed by dialysis *(Reynolds, 1993)*.

Use in TB
The almost sole indication for INH is in the prophylaxis and treatment of all forms of TB. As a prophylactic agent, doses of 4 – 8 mg/kg for 6 – 12 months have been used in high risk populations *(Reynolds, 1993)*.

In the treatment of TB, the vital role of INH in combination with RFA has been highlighted above. In the initial 2 month intensive phase of treatment, a dose of 5 mg/kg is recommended *(Reynolds, 1993)*, although in practice a standard dose of 300 mg is used for patients less than 50 kg and 400 mg for those greater than 50 kg. In the continuation phase, the same dose is administered 2 – 3 times a week.

Drug Interactions
Several drug interactions have been reported with the use of INH in combination with other agents. INH is an inhibitor of cytochrome P-450-dependent microsomal pathways and may thus interact with other drugs that use this same metabolic pathway. These drug interactions usually result in increased concentrations of the drug whose metabolism is inhibited. The clinical effect of the interactions may be related to acetylator phenotype. A summary of some selected drug interactions is presented in Table 2.4.

Table 2.4 – Summary* of Some Selected Clinically Important Drug Interactions Involving Isoniazid

Drug	Mechanism/Effect	Management
Phenytoin	Enzyme inhibition. Phenyytoin concentrations ↑. Occurs mainly in slow acetylators of INH. If RFA is given concurrently, phenytoin levels ↓ i.e. induction effect outweighs INH's inhibitory effect.	Monitor serum phenytoin levels. May need to decrease phenytoin dose.
Carbamazepine	Enzyme inhibition. Carbamazepine concentrations ↑. There may also be an ↑ in metabolism of INH to a - hepatotoxic metabolite.	Monitor serum carbamazepine levels. May need to decrease dose
Ketoconazole	Effect noted when INH was given with RFA. Reduced concentrations of ketoconazole noted but INH concentrations may also be reduced.	Monitor clinical response to the ketoconazole.
Antacids (aluminium hydroxide)	Adsorption effect. May delay and decrease absorption of INH.	Administer at least 2 hours apart. Monitor INH.
Anticoagulants	Reported only at a dose of 9 mg/kg/day.	May need to decrease anticoagulant dose.
Vitamin D	Enzyme inhibition	Monitor vitamin D levels as well as calcium phosphate levels in selected patients
Benzodiazepines	RFA's effect if given concomitantly should also be considered.	May need to decrease dose of selected benzodiazepines.
Ethionamide	INH concentrations temporarily raised. Mechanism unknown.	Monitor patients for signs of INH toxicity e.g. peripheral neuritis.
Prednisolone	INH metabolism is increased. INH concentrations may be decreased by 25% in slow acetylators and 40% in fast acetylators.	Monitor patient for efficacy of INH and adjust INH dose as required.
Theophylline	Potential decrease in theophylline clearance appears to occur at INH doses greater than 300mg daily. If RFA is given concurrently, theophylline serum concentrations decrease (ie. induction effect outweighs INH's inhibitory effect)	Monitor serum theophylline concentrations and adjust dose as required.

*Compiled from various reviews including *(Stockley, 1996; Zeind et al. 1996; Gelman and Rumack, 1998; Reynolds, 1993)*

Adverse Reactions

INH is generally well tolerated at the recommended doses.

Peripheral neuropathy appears to be a dose-dependent adverse effect of INH that is reported by patients. It is uncommon at a dose of 5 mg/kg, occurring in 2% of patients receiving INH. At higher dosages, peripheral neuropathy may develop in 10 to 20% of the patients. INH-induced depletion of pyridoxine is the most likely cause of peripheral neuritis. Pyridoxine (15 to 50 mg/day) should be given with INH to people who have conditions in which neuropathy is common (diabetes, uraemia, alcoholism and malnutrition). For pregnant women and people who have a seizure disorder, it is also recommended that pyridoxine be given with INH. As indicated above, patients who

are slow acetylators may be more susceptible to the development of peripheral neuropathy.

Hepatotoxicity associated with the use of INH occurs in approximately 1 to 2% of patients. Transient elevations in liver enzyme are often noted during INH treatment. However, in the majority of cases, these return to pretreatment values despite continuation of INH. In rare cases however, progressive liver dysfunction and severe and often fatal hepatitis have occurred. Although the mechanism of hepatitis is unknown, it is probably associated with hepatic metabolites. Other confounding factors such as age and alcohol use may also be implicated.

It is recommended that baseline measurement of liver enzymes should be performed in patients receiving INH and should be followed up periodically. Patients should be questioned monthly for signs and symptoms of liver disease. They should be instructed to report to their physician any of the prodromal symptoms of hepatitis (e.g. malaise, fatigue, weakness, anorexia, or nausea). Should these symptoms appear or if the signs that are suggestive of hepatic damage occur (e.g. liver enlargement with tenderness, jaundice, or dark urine), prompt discontinuation of INH is warranted. Some clinicians recommend discontinuation of INH if transaminase values exceed five times normal when treating TB and three times normal when INH is used as a prophylactic agent *(Zeind et al. 1996)*.

Other rare adverse effects that are reported with INH administration include various central nervous system (CNS) toxicities (e.g. hallucinations, convulsions), dermatologic (e.g. acne, allergic rashes), haematologic (various anaemias including aplastic anemia, agranulocytosis, thrombocytopaenia and eosinophilia), and gastrointestinal effects.

Pharmacokinetic and Pharmacodynamic Considerations in the Management of Tuberculosis

The intrinsic *in vitro* susceptibility of a microorganism to an anti-microbial drug has been quantified using various methods that include MIC testing, minimum bactericidal concentrations and examinations of the kinetics of killing (time-kill curves). The MIC has however been the most popular and widely used criterion since experience has shown a good correlation between susceptibility testing and clinical efficacy.

In a clinical isolate, the true MIC can fluctuate due to a wide variety of methodological and other variables. These variables include changes in media, incubation time, temperature or method employed. These need to be standardised during MIC determination and considered when interpreting MIC values so as to reduce some of this variability. Further, the slow growth of mycobacteria in culture of 4-6 weeks using conventional solid media is a very real impediment to the effective use of MIC determinations in TB chemotherapy. However, the BACTEC^R radiometric system may be useful in providing results more rapidly – within 3 weeks *(Yew and Chau, 1995)*.

Pharmacodynamics attempts to relate the drug concentrations achieved in the host to the concentrations required to inhibit or kill the microorganism. The most popular pharmacodynamic parameters include the Cmax:MIC ratio, the time above the MIC

and the AUC above the MIC. However historically, other parameters have been considered. Although theoretically feasible, most of these have the disadvantage of lack of prospective evaluation and correlation with clinical evidence of efficacy.

The *inhibitory quotient (Ellner and Neu, 1981)* relates the achievable antimicrobial concentration at the infection site to the MIC of the infecting organism. This laboratory index assumes that a standard dose has been administered to a standard patient and makes no provision for altered pharmacokinetics. It derives all its antibiotic concentration data from the literature. The inhibitory quotient does not make provision for dosage calculations for a patient, since there is no dosage reference point. Further disadavantages include the absence of considerations relating to the height of the Cmax over the MIC or the duration that *in vivo* concentrations exceed the MIC.

The *intensity index* is the ratio of the average steady-state concentration of the antimicrobial agent to the MIC, multiplied by the duration that concentrations exceed the MIC in a 72-hour period *(Schumacher, 1982)*. Thus this parameter incorporates both the average concentrations above the MIC and the time that concentrations are maintained above the MIC. The data for this parameter are obtained from population studies describing the median MIC for bacterial species (MIC_{90} values) and average steady state drug concentrations in volunteers. While this parameter seems to be logical, some unusual predictions were obtained during its application *(Schentag et al. 1986)*. Based on intensity index data alone, it was concluded that ampicillin was more useful than dicloxacillin for *Staphylococcus* and gentamicin was more useful than tobramycin for general gram negative bacteria including *Pseudomonas*. Across class comparisons yielded even stranger results e.g. oral first-generation cephalosporins with long half-lives had greater intensity index values than the aminoglycosides while tetracycline was equal to the aminoglycosides and inferior to ampicillin. Thus this parameter should be limited to comparisons of organisms with known susceptibility exposed to reasonable concentrations of drug. Like most of the other pharmacodynamic parameters, the absence of clinical correlation is a serious disadvantage.

The ratio of the area under the serum concentration versus time curve (AUC) to the MIC has been applied to the evaluation of the third generation cephalosporins *(Schentag et al. 1986)*. Population values for bacterial MIC were used and serum concentrations from volunteer studies. The advantage of this pharmacodynamic parameter is that no peak concentration is required and the time above the MIC is included in the drug exposure parameter of AUC. The disadvantage is that the data are of clinical use only if the patient in question has an organism with similar susceptibility and has a pharmacokinetic profile identical to that of the volunteers used in the pharmacokinetic analysis. Some have modified the AUC:MIC parameter by considering the concentrations of free drug only rather than both free and bound drug. This has implications for drugs that are highly protein bound. Although this parameter shows clinical promise, it has also not been tested prospectively.

The *serum bactericidal activity (Wolfson and Swartz, 1985)* is the only pharmacodynamic parameter that has been clinically tested. This method integrates serum concentrations in the actual patient with the bacterial susceptibility of that patient's pathogen. It involves serum dilutions of the patient's serum against the bacterial pathogen. A serum bactericidal activity of 1:8 has been correlated with

efficacy in patients with neutropaenia given multiple antibiotic regimens and in those with endocarditis.

The serum bactericidal activity obviates the need to measure the patient's antibiotic serum concentration or the bacterial MIC. Both factors are incorporated in the test method, even though neither is known exactly. Thus, bacteria of unusual susceptibility and patients with unusual pharmacokinetics can be evaluated by this technique. The technique can also evaluate the simultaneous effects of several concurrent antibiotics on one bacterium.

A major limitation however, is that while the bactericidal activity identifies a patient who has been incorrectly treated, it provides no guidance on how to treat correctly. Secondly, the timing of bactericidal activity measurements is a matter of controversy. Some determine the bactericidal activity at peak, midpoint, trough, or even randomly. This problem is aggravated when several antibiotics whose pharmacokinetics may not be matched, are used concurrently, as it is never clear when to determine bactericidal activity or how to adjust doses. There are methodological problems with serum dilutions and the diluent to use. Some who have criticised the method as unreliable have recently introduced the serum bactericidal rate *(Drake et al. 1983)*. This is a time-related subculturing method performed on the bactericidal activity dilution of 1:2. It appears to be a useful parameter of bactericidal activity, but no clinical trials have appeared to support its use as yet. Finally, with either method, it is always difficult to predict which bacterial culture a clinician will request bactericidal activity measurements – especially since such requests are likely to be made several weeks into treatment. Thus, the state of the art often remains ahead of the logistics of application with this technique. In spite of the problems, bactericidal activity methods are currently enjoying a renewed interest in both animal and patient studies.

It is significant that none of these methods have been applied to the clinical evaluation of anti-TB drug therapy. One of the few reports that describe the pharmacodynamic parameters of the anti-TB drugs was that by *Peloquin and Berning (1994)*. These authors calculated the pharmacodynamic parameters of Cmax:MIC, time above MIC and AUC>MIC using population data and literature values of MIC (Table 2.5). The pharmacokinetic parameters were taken as the midpoint of the values reported in the literature. In this way the authors demonstrated the importance of INH and RFA when their pharmacodynamic parameters were compared to other anti-TB drugs.

Studies conducted with bactericidal drugs against aerobic bacteria suggest that for the cell wall active drugs (e.g. beta-lactam antimicrobials), maintaining the serum concentration above the MIC for the entire dosing interval (t>MIC) is the most important parameter for eradicating the organism *(Peloquin, 1996)*. Among the anti-TB drugs, INH, cycloserine, ethambutol, ethionamide and thiacetazone act primarily against the cell wall and thus t>MIC would be an important parameter to optimise when dosing with this drug.

On the other hand, in the case of drugs that exert their effect on intra-cellular targets (e.g. aminoglycosides) the Cmax:MIC ratio is considered important as this ensures adequate penetration into the site of action. RFA, fluoroquinolones, capreomycin, pyrazinamide and clofazimine would fall into this category as they act on RNA polymerase within the cell *(Peloquin, 1996)*.

Table 2.5 – Pharmacodynamic parameters for the anti-tuberculosis drugs (Peloquin and Berning, 1994)

DRUG	Cmax:MIC	t>MIC	AUC>MIC
Drugs acting primarily against the cell wall			
Cycloserine	3.8	22.5	195.5
Ethambutol 25 mg/kg	10	13.0	23.4
Ethionamide	1.6	1.5	1.0
Isoniazid			
Fast acetylator	40	9.0	11.6
Slow Acetylator	40	18.0	19.2
Thiacetazone	1.3	5.5	1.2
Drugs acting primarily against intracellular targets			
Rifampicin	24.0	9.0	39.9
Streptomycin			
22-25 mg/kg 3 times a week	18.8	11.0	274.6
12-15 mg/kg 5 times a week	10.0	8.0	124.5
Ciprofloxacin	5.0	10.5	16.9
Ofloxacin	5.0	15.5	47.4

AUC = area under the serum concentration versus time curve, Cmax = maximum serum concentration, MIC = minimum inhibitory concentration, t>MIC = time serum concentrations remain above the minimum inhibitory concentration.

These authors suggest that research into establishing "normal" ranges for these pharmacodynamic parameters may facilitate the construction of novel dosing regimens that may take advantage of each drug's strengths. They allude to the possibility that these parameters may guide dosage in the treatment of MDR-TB. A disdavantage to the methodology used in their paper however is the use of literature values for the pharmacokinetic parameters and mean values for the MIC from possibly unrelated sources.

A critical analysis of these attempts at pharmacokinetic-pharmacodynamic integration in the design of antimicrobial regimens reveals several promising approaches, but with the exception of serum bactericidal activity, none of the indices of susceptibility have yet been clinically evaluated. Rather, they have been used to compare the potential efficacy of different antibiotics of the same class or to suggest dosing guidelines. Most methods rely on population data alone, or attempt to integrate pharmacokinetic data from one study with pharmacodynamic data from an unrelated study. This provides minimal guidance on dosage adjustments for the individual patient. Clearly the ideal would be to derive the parameters using both pharmacokinetic and pharmacodynamic information from the same study.

Altered Pharmacokinetics of the anti-TB drugs in the context of HIV infection and the acquisition of drug resistance

Researchers from the National Jewish Center for Immunology and Respiratory Medicine were the first to observe possible malabsorption of the anti-TB drugs and to

suggest its link with HIV infection and AIDS *(Berning et al. 1992)*. The physiological or pathological basis for alterations in pharmacokinetics as a consequence of HIV infection is presented hereunder followed by a review of the studies that examined the link between HIV and malabsorption.

REASONS FOR ALTERED PHARMACOKINETICS IN HIV INFECTION

In general, conditions likely to influence the pharmacokinetics of drugs are those which affect normal functioning of the gastrointestinal tract (GIT), hepatic and renal systems. Patients with HIV infection and/or AIDS have been reported to have altered functioning of all 3 of these systems *(Unadkat and Agosti, 1990)*. The severity of the alterations varies widely across individuals and often depends on the severity and stage of immune compromise.

Gastrointestinal System

Diarrhoea from any cause will result in a reduction in the extent of drug absorption. Diarrhoea is one of the most common GIT symptom reported in AIDS occurring in 50 to 90% of individuals. It ranges in severity with fluid loss up to 17L per day reported. In addition to the more common diarrhoeal pathogens found in immunocompetent hosts, there is a wide variety of organisms implicated as aetiological agents in the HIV+ patient. These include *Mycobacterium avium intracellulare*, the protozoa *Cryptosporidium* and *Isospora*, cytomegalovirus and *Salmonella*. The human immunodeficiency virus itself has been found to infect the GIT mucosa and is associated with enteropathy *(Unadkat and Agosti, 1990)*.

Opportunistic infections that affect immunocompromised hosts such as oropharyngeal candidiasis, hairy leukoplakia, aphthous stomatitis, necrotising gingivitis, Karposi's sarcoma and herpes simplex infection may also predispose to malabsorption *(Unadkat and Agosti, 1990)*. This may be due to coating or destruction of the intestinal walls by these organisms *(Peloquin, 1993)*.

The pH of the stomach of patients with AIDS may be higher than that of normal patients *(Unadkat and Agosti, 1990)* and achlorhydria has also been reported. This has implications for the absorption of drugs that are weak acids or weak bases as pH affects the degree of ionisation according to the Henderson-Hasselbalch pH-partition hypothesis *(Mandell and Petri, 1996)*. Weak acids will be ionised at alkaline pH and this will tend to retard absorption. In contrast drugs that are weak bases will be more unionised and their absorption will be promoted.

In a review by *Kenny and Strates (1981)* an increased pH after administration of sodium bicarbonate was noted to reduce the extent of RFA absorption. In the same study, a decreased pH was associated with better drug absorption. The authors attributed this to a better solubility of RFA in acid pH.

Hepatic System

Some of the opportunistic infections in HIV+ patients may result in aberrations in liver function. This may alter the pharmacokinetics of the anti-TB drugs. These include the frequent occurrence of hepatitis due to hepatitis B, non-A, non-B hepatitis,

Mycobacterium avium intracellulare, cytomegalovirus, Epstein-Barr virus, *Pneumocystis carinii* pneumonia, fungi, lymphoma, Karposi's sarcoma *(Unadkat and Agosti, 1990)*. Drugs used to treat these infections may also affect liver function and hence drug metabolism e.g. sulphonamides, ketoconazole, amphotericin and zidovudine *(Mandell and Petri, 1996)*. Biliary diseases seen in HIV infection include cholangitis secondary to cytomegalovirus or cryptosporidium, and obstruction secondary to lymphoma or Karposi's sarcoma *(Unadkat and Agosti, 1990)*.

The cytokines, interferon-α and tumour necrosis factor have been implicated in the inhibition of the metabolism of drugs via the cytochrome P450 oxidative pathways. Since patients with AIDS have been reported to have higher circulating concentrations of these 2 agents, it is reasonable to speculate that the oxidative metabolism of drugs in such patients is likely to be impaired. This has been suggested as the mechanism for the increased metabolism of antipyrine after zidovudine treatment. These authors suggest that circulating levels of interferon-α and tumour necrosis factor may decrease after zidovudine treatment resulting in an increase in the clearance of antipyrine *(Unadkat and Agosti, 1990)*. Since the major metabolic pathway for INH is acetylation and for RFA is desacetylation, this is unlikely to be of clinical significance with these drugs.

Patients with AIDS often suffer from a wasting syndrome that may result in hypoalbuminaemia. This latter effect may have implications for protein binding of drugs and hence affect the interpretation of the volume of distribution and clearance of drugs.

Renal Function

Renal diseases occur in 10 to 30% of AIDS patients. A distinct form of HIV-associated renal disease with a broad spectrum of severity and sometimes associated with proteinuria has been reported *(Unadkat and Agosti, 1990)*. However, with the exception of severe renal disease, this is unlikely to cause alterations in the pharmacokinetics of INH and RFA, as the kidneys constitute a minor route of drug elimination for these drugs.

REVIEW OF THE INVESTIGATIONS INTO THE PHARMACOKINETICS OF THE ANTI-TB DRUGS IN HIV INFECTION

Berning et al (1992) were the authors of the first case report of malabsorption of the antimycobacterial drugs in an AIDS patient . A further review of serum concentrations of samples submitted to their laboratory at the National Jewish Centre for Immunology and Respiratory Medicine, Denver, USA for anti-TB drug assay, revealed an additional 32 instances of drug malabsorption. Rifampicin showed the worst absorption with 19 of 20 RFA measurements recorded as abnormal *(Peloquin et al. 1993)*.

Following on these reports, *Patel et al 1995* reported on 2 patients with HIV infection being treated for TB who relapsed with drug-resistant isolates. In both patients, significantly low serum concentrations of anti-TB drugs were confirmed at the laboratories of the Denver group. In demonstrating that malabsorption was associated

with the acquisition of drug resistance, these authors endorse the warning by *Peloquin et al (1993)*, that *"... patients being treated for multidrug-resistant tuberculosis may be at particular risk for poor therapeutic responses if drug malabsorption occurs."* They emphasise that the absorption of only one drug in a multi-drug regimen may select for further drug resistance *(Patel et al. 1995)*.

Peloquin et al (1996) subsequently conducted a pilot study in which they compared the 2-hour post-dose concentration of anti-TB drug with the expected normal ranges that they propose. They noted that 21 of 26 patients (81%) had levels less than 90% of the predicted low end of the therapeutic range and 14 patients (54%) had levels less than 30% of the predicted minimum (Peloquin et al. 1996). They concluded that frequent and significant malabsorption occurred in HIV+, TB patients with or without AIDS.

These studies have limitations in that they report on retrospectively collected data or because they consisted largely of case reports. The 2-hours post-dose data point is at a highly variable part of the pharmacokinetic profile for drugs that display rapid absorption. It is subject to wide fluctuations and is difficult to interpret. This difficulty was acknowledged by *Patel et al (1995)* when they noted a peak RFA concentration at 4 hours rather than 2 hours in one of the 2 patients in their report.

In an attempt to resolve these difficulties, *Sahai et al (1997)* studied the malabsorption problem using a full prospective pharmacokinetic trial consisting of 13 serum concentration points per patient. These authors examined the bioavailability of INH, RFA and pyrazinamide in 4 groups of 12 volunteers each. Group I consisted of healthy volunteers, while Groups II, III and IV were HIV+ patients without TB. The patients in these latter 3 groups had CD4+ counts of $> 300/mm^3$ (Group II - asymptomatic HIV+), $< 200/mm^3$ (Group III – symptomatic HIV+) and $< 200/mm^3$ with ≥ 3 loose stools per day (Group IV – symptomatic HIV+ with diarrhoea).

In the case of RFA, the study demonstrated that in all HIV+ patients, the AUC was 68.4% lower (CI – 47.5 to 98.4%) and the Cmax was 58.8% lower (CI - 42.3 to 82%) than in healthy volunteers. A similar effect on AUC could not be demonstrated for INH although the HIV+ patients with diarrhoea (Group IV) displayed a lower Cmax for INH compared to the patients with symptomatic HIV but no diarrhoea (Group III).

The authors report a significant linear trend for decreasing Cmax values (μg/ml) from Group I to Group IV for both RFA (9.29, 5.48, 5.27, 5.24 – p=0.006) and INH (5.97, 5.12, 4.73, 3.69 – p=0.046) that apparently mirrors the stage of HIV disease. However, the results of this trend analysis are probably clinically irrelevant since all patients had levels above the MIC for sensitive organisms. Further, the large difference between Cmax for volunteers and HIV+ patients for RFA and that due to diarrhoea for patients on INH was responsible for the strong statistical significance (low p value) of the trend analysis.

These reports of reduced absorption in HIV+ patients were not confirmed by 2 recent pharmacokinetic studies in TB patients conducted in Africa (Choudhri et al. 1997; Taylor and Smith, 1998).

The study by *Choudhri et al (1997)* conducted in Nairobi, Kenya found that neither HIV infection nor diarrhoea accounted for the interpatient variability in the AUC, Cmax or the terminal half-life (t½) of INH or RFA. They studied the steady-state pharmacokinetics of INH, RFA and pyrazinamide in 29 adults (14 HIV+ and 15 HIV-) with TB. It was noted further that neither the AUC nor the t½ of any of these drugs reflected interpatient differences in CD4 lymphocyte counts i.e. the severity of immune compromise did not influence the pharmacokinetics of the drugs.

The study by Taylor and Smith (1998) was conducted in 13 HIV+ patients (with AIDS) and 14 HIV- patients hospitalised for TB in Cape Town, South Africa. These authors did not find the differences in AUC and Cmax that Sahai (1996) reported for RFA or any of the malabsorption problems previously reported. On the contrary, these authors noted better absorption of RFA in HIV+ patients as reflected in higher AUC values. They were unable to explain this discrepancy but speculated that it may have been due to altered serum protein binding or an underlying hepatic dysfunction. The study participants had received their medication under strict supervision for 3-5 days prior to the study. In addition they had received (unsupervised) daily doses of their anti-TB drugs for over 4 months prior to enrolment into the study. Although this information suggests that RFA enzyme auto-induction would have been maximal at the time of the study, the authors do not discuss or exclude this possible explanation for the higher AUCs in the AIDS group in their manuscript. It has been reported that RFA serum pharmacokinetic values return to the un-induced state within 30 days of termination of drug therapy (Kenny and Strates, 1981). Thus non-compliance and consequent absence of maximal enzyme auto-induction prior to recruitment into the study may offer a possible explanation for the higher AUCs of the patients in the AIDS group.

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Chapter 3

TEMPORAL TRENDS IN *MYCOBACTERIUM TUBERCULOSIS* DRUG RESISTANCE IN KWAZULU-NATAL, SOUTH AFRICA : 1983 to 1995

Summary

OBJECTIVE

To determine temporal trends in *Mycobacterium tuberculosis* drug resistance in KwaZulu-Natal, South Africa between 1983 and 1995.

DESIGN

Routine drug susceptibility data from the central provincial mycobacteriology laboratory in KwaZulu-Natal, South Africa for the 13 year period from 1983 to 1995 were analysed. The laboratory used the 1% proportional method in Lowenstein-Jensen medium for susceptibility testing throughout the study period.

RESULTS

A total of 21 704 sputum samples from which M. tuberculosis was cultured, were subjected to susceptibility testing. Multi-drug resistance (MDR-TB), defined as combined resistance to isoniazid (INH) and rifampicin (RFA) was 2.2% in 1983 and 3.7% in 1995 (p = 0.01). Examination of the temporal trends in MDR-TB revealed a downward trend from 2.6% in 1984 to 0.9% in 1987 (chi square for linear trend 9.34 ; p = 0.002). The prevalence did not change much between 1987 and 1990 followed by an upward trend from 1.1% in 1990 to 3.7% in 1995 (chi square for linear trend 30.56; p < 0.001). Resistance to INH alone was 6.7% in 1983 and 7.0% in 1995 (p = not significant) and for RFA alone 8.1% and 7.2% (p = not significant), respectively. All other drugs tested showed a statistically significant decrease in drug resistance between 1983 and 1995 viz. streptomycin (13.7% and 7.7%; p < 0.001), ethionamide (9.3% and 4.5%; p < 0.001), thiacetazone (7.8% and 3.2%; p < 0.001) and ethambutol (3.4% and 2.1%; p = 0.01).

CONCLUSIONS

Following an initial downward trend, a significant increase in the prevalence of MDR-TB was noted. It is particularly disconcerting that there was a reversal in a previous downward trend in MDR-TB. While these data cannot simply be extrapolated to all patients with TB due to the selective basis on which susceptibility tests were requested, they nevertheless provide valid temporal trends.

Introduction

The incidence of tuberculosis (TB) has been increasing globally in recent years, partially due to the human immunodeficiency virus (HIV) epidemic. More ominous, however, have been the reports of the rising prevalence of strains of *Mycobacterium tuberculosis* that are resistant to currently used chemotherapy *(Edlin et al. 1992; Iseman, 1993; Bloch et al. 1994; Neville et al. 1994).*

In a study of newly admitted Black TB patients in South African hospitals, *Weyer and Kleeberg (1992)* demonstrated a marked decrease in primary and acquired drug resistance over the period 1965 to 1988. There has been no investigation into the trends in drug resistance thereafter. In 1996 the WHO and the South African Department of Health conducted a combined review of the TB Control Programme in South Africa. They concluded that South Africa's high TB case rates, the emergence of MDR-TB and the growing HIV epidemic combine to make the country's TB crisis the most serious in the world *(WHO, 1996).*

KwaZulu-Natal, one of South Africa's 9 provinces, is reported as having the highest prevalence rate of HIV infection (27 %) compared to the other provinces which have prevalence rates that range from 6 % in the Western Cape to 23 % in Mpumalanga province *(Department of Health, 1998).* The province ranks fifth in terms of the estimated incidence of TB *(WHO, 1996).* There is currently no published data on the prevalence of MDR-TB in the province.

The purpose of this study was to determine the temporal trends in *M. tuberculosis* drug resistance using routine drug susceptibility data from the Regional Mycobacteriology Laboratory in KwaZulu-Natal, Durban, South Africa.

Methods

The Mycobacteriology laboratory at King George V Hospital (KGV) in Durban is the central referral laboratory at which susceptibility testing for the anti-TB drugs is performed for hospitals and clinics in KwaZulu-Natal. Susceptibility tests are routinely requested by clinicians when patients with TB do not respond to standard TB treatment regimens, require re-treatment or when the clinician suspects drug-resistant TB.

Tuberculosis drug susceptibility reports from KGV Mycobacteriology Laboratory that had been recorded in the laboratory register were computerised and analysed. Repeat or duplicate specimens with identical drug susceptibility patterns, presenting within a 3-month period of each other were identified using the first 4 characters of the patient's names and eliminated using a computer algorithm.

During the period under study, drug susceptibility testing was conducted using the method advised by the Tuberculosis Research Institute of the Medical Research Council of South Africa *(Nel et al. 1980).* This is based on the 1% proportional method using Lowenstein-Jensen medium. A standardized inoculum was sub-cultured

onto Lowenstein-Jensen medium containing the following concentrations (µg/ml) of drug: -

> Isoniazid (INH) - 0.1 and 5.0
> Streptomycin - 5
> Ethionamide - 20
> Ethambutol - 2.8
> Rifampicin (RFA) – 28
> Thiacetazone - 1

The laboratory reported results as resistant, sensitive or partially resistant based on growth of the organism in the drug-containing culture medium relative to growth on a drug-free control. In all analyses for this study, partial resistance was re-coded as sensitive for purposes of clinical interpretation.

The statistical analyses were conducted using Epi-Info Version 6.04b (Centers for Disease Control and Prevention (CDC), USA and WHO, Geneva). Comparisons and linear trends were evaluated using the extended Mantel Haenszel Chi-square test with the $p<0.05$ level regarded as statistically significant. This test reflects the departure of a linear trend from the horizontal i.e. from no trend *(Armitage 1955)*.

Results

From 1983 to 1995, a total of 26 441 *M. tuberculosis* strains were isolated from sputum specimens and were subjected to susceptibility testing. There were 4737 repeat specimens with identical drug susceptibility patterns presenting within a 3 month period of each other that were identified and eliminated from further analyses. The study sample thus consisted of 21 704 isolates. Table 3.1 records the resistance rates to the 6 drugs tested and the rates for combined resistance to INH and RFA (MDR-TB) over the 13 year period.

Table 3.1 – Number (%) of resistant Mycobacterium tuberculosis isolates and notified cases of Tuberculosis during the period 1983 to 1995 in KwaZulu-Natal, South Africa

	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995[1]
Isoniazid (INH)													
Number (%)	77 (6.7)	91 (8.1)	70 (7.3)	54 (5.9)	62 (6.0)	82 (6.8)	66 (5.5)	66 (5.4)	118 (7.8)	110 (8.0)	202 (8.4)	265 (7.9)	256 (7.0)
Number of isolates tested	1150	1125	959	912	1026	1214	1192	1214	1510	1373	2392	3363	3653
Rifampicin (RFA)													
Number (%)	93 (8.1)	98 (8.8)	84 (8.8)	54 (6.0)	26 (2.5)	51 (4.2)	40 (3.4)	44 (3.7)	104 (6.9)	104 (7.6)	171 (7.1)	220 (6.5)	264 (7.2)
Number of isolates tested	1149	1116	956	906	1026	1213	1186	1204	1504	1372	2396	3376	3666
Streptomycin													
Number (%)	158 (13.7)	150 (13.4)	110 (11.5)	48 (5.3)	57 (5.6)	50 (4.2)	52 (4.4)	41 (3.4)	101 (6.7)	123 (9.0)	168 (7.1)	219 (6.5)	279 (7.7)
Number of isolates tested	1152	1124	957	906	1026	1202	1188	1208	1503	1372	2379	3348	3644
Ethionamide													
Number (%)	74 (9.3)	58 (5.2)	34 (3.6)	20 (2.2)	10 (1.0)	18 (1.5)	38 (3.2)	40 (3.3)	45 (3.2)	62 (4.5)	105 (4.4)	144 (4.3)	162 (4.5)
Number of isolates tested	794	1119	956	906	1023	1204	1187	1202	1406	1368	2394	3352	3642
Thiacetazone													
Number (%)	90 (7.8)	46 (4.1)	12 (1.3)	11 (1.2)	2 (0.2)	1 (0.1)	4 (0.3)	19 (1.6)	19 (1.3)	36 (2.7)	61 (2.6)	86 (2.7)	110 (3.2)
Number of isolates tested	1148	1116	959	902	1024	1208	1186	1211	1492	. 1361	2374	3173	3448
Ethambutol													
Number (%)	39 (3.4)	34 (3.0)	17 (1.8)	4 (0.4)	1 (0.1)	5 (0.4)	6 (0.5)	4 (0.3)	10 (0.7)	30 (2.2)	25 (1.1)	55 (1.6)	77 (2.1)
Number of isolates tested	1147	1123	956	909	1025	1211	1189	1210	1504	1361	2374	3357	3647)
MDR-TB (INH + RFA)													
Number (%)	25 (2.2)	29 (2.6)	22 (2.3)	15 (1.7)	9 (0.9)	13 (1.1)	9 (0.8)	13 (1.1)	31 (2.1)	37 (2.7)	63 (2.6)	131 (3.9)	134 (3.7)
Number of isolates tested	1135	1114	953	900	1011	1204	1180	1199	1497	1364	2382	3354	3648
TB Case Rates at Hlabisa Health Ward[2]							301	336	312	624	703	827	839
TB Notifications in KwaZulu-Natal[3]	9706	8815	8795	5573	8588	10889	9255	11003	11202	11563	9704	10354	10226

Nearly all isolates were tested against the 6 drugs - minor differences in number of isolates tested per year were due to lack of growth of the organism in the control tube or to contaminated specimens.
[1]Drug resistance data for 1995 consists of only 10 months of data
[2](Wilkinson and De Cock, 1996a)
[3]Source – Epidemiological Comments – Department of Health

Multi-drug resistance, defined as combined resistance to *at least* INH and RFA increased from 2.2% in 1983 to 3.7% in 1995 (p = 0.01). Examination of the temporal trends in MDR-TB (Figure 3.1) revealed a downward trend from 2.6% in 1984 to 0.9% in 1987 (chi square for linear trend 9.34; p = 0.002). MDR-TB was stable between 1987 and 1990 and then an upward trend was noted from 1.1% in 1990 to 3.7% in 1995 (chi square for linear trend 30.56; p < 0.001).
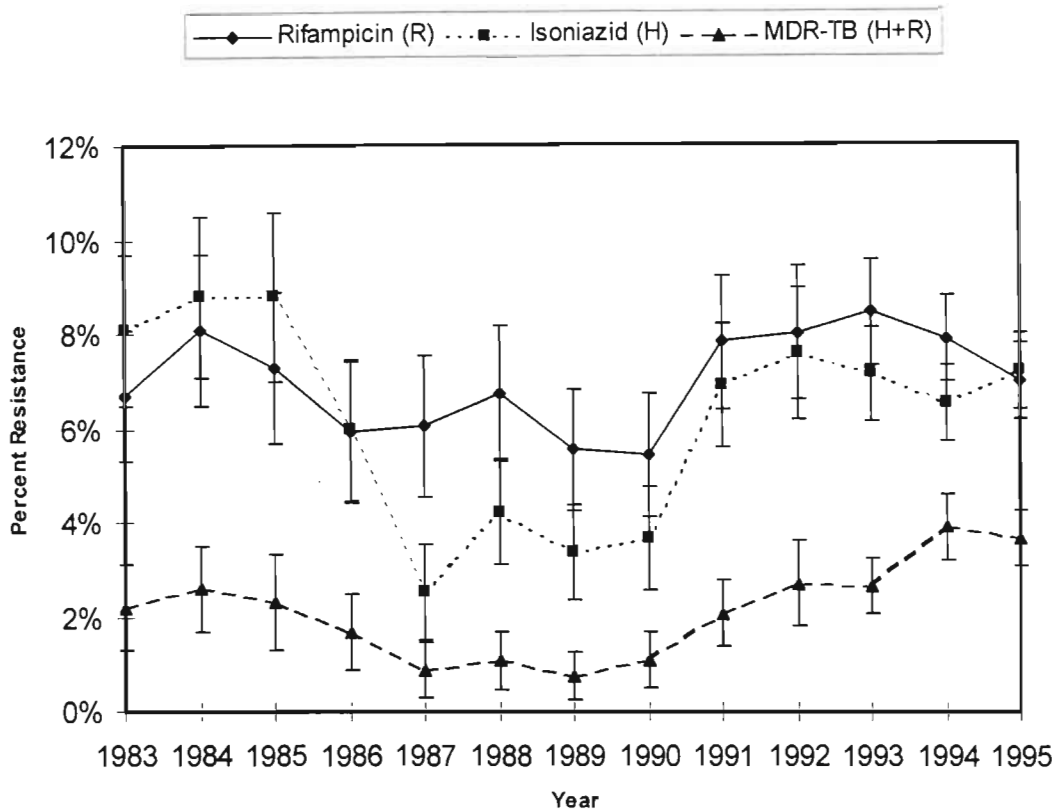
Figure 3.1 - Trends in Mycobacterium tuberculosis drug resistance to isoniazid (INH) and rifampicin (RFA) alone and in combination (MDR-TB) in KwaZulu-Natal, South Africa 1983-1995

Pulmonary TB notifications for whole of KwaZulu-Natal *(Epidemiological Comments 1983 to 1996)* and for the sentinel surveillance site at Hlabisa Health Ward, KwaZulu-Natal *(Wilkinson and De Cock, 1996a)*, are recorded in Table 3.1 and plotted with the number of requests for drug susceptibility received by the laboratory for the period under study (Figure 3.2).

47
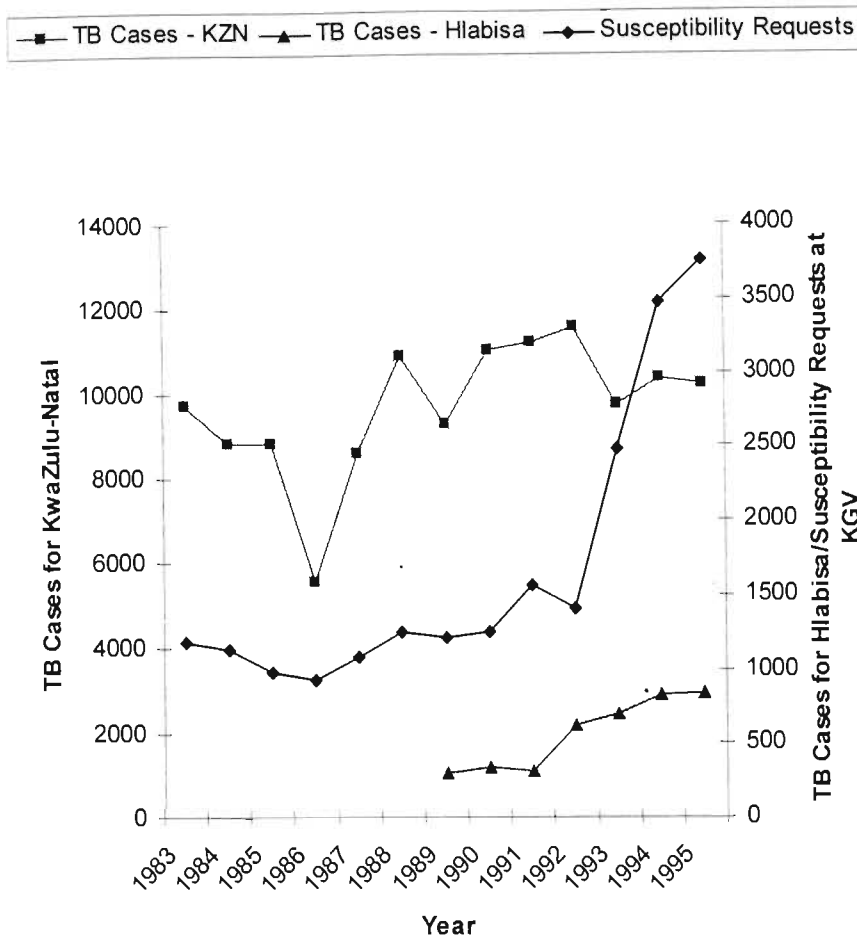
Figure 3.2 - Comparison of TB Case Loads for Hlabisa Health Ward and KwaZulu-Natal in relation to the number of requests for drug susceptibility testing received by King George V Mycobacteriology Laboratory 1983 - 1995

From 1983 to 1991, the number of requests for drug susceptibility runs approximately parallel with the number of cases of TB reported to the Department of Health. Since 1991 however, the number of requests for drug susceptibility have continued to increase while there has been an apparent decline in TB notifications in the province. The data from Hlabisa Health Ward, however, shows a continued increase in TB case loads over this period.

Discussion

A significant increase in MDR-TB was noted over the 13 year period: 1983 – 1995. It is particularly disconcerting that the increase followed an initial downward trend that was reversed in 1989. This increase in MDR-TB occurred against a backdrop of a general decline in resistance to individual anti-TB drugs.

While the proportion of MDR-TB is still relatively low, the absolute number of MDR-TB patients has been increasing. Thus while the percentage MDR-TB has increased from 2.2% in 1983 to 3.7% in 1995, the actual number of MDR-TB cases being cared for by

the KwaZulu-Natal Health authorities has increased more than 5 times from 25 in 1983 to 134 in 1995. This increased burden on State treatment facilities occurs in the context of a health care system that is stretched thin as it undergoes radical reform in post-apartheid South Africa.

Approximately 18% of the specimens submitted for susceptibility testing were identified as being repeat requests and were counted once only for the purposes of this study. In most cases these were isolates from patients with MDR-TB who tended to visit several different clinics serviced by the laboratory before finally being admitted for in-patient management at KGV.

Since the virulence of MDR-TB is similar to that of drug-sensitive TB *(Iseman, 1993)*, this means that each case is a potential source of spread of infection unless adequate steps are taken to control the disease. When compared to drug-sensitive patients, these patients are likely to remain infectious for longer periods of time after commencement of drug therapy and thus would require longer periods of treatment that may include hospitalization. The slow growth of *M tuberculosis* in cultures and consequent delays before microbiological confirmation of drug resistance further compromises timeous institution of appropriate chemotherapy. Indeed some TB researchers *(Veen, 1995)* have speculated on whether current regimens for drug-resistant TB have any impact at all on patient outcome when compared to the pre-chemotherapy era. However if sputum positive MDR-TB patients are allowed to remain in the community, or if patients are allowed back into the community before completion of a full course of treatment, a very real danger exists of creating clusters of MDR-TB patients in communities burdened by years of socio-economic impoverishment.

It is interesting to note that there has been a reversal in the downward trend in MDR-TB during the latter 5 years of the study (Figure 3.1). It is tempting to speculate that the HIV epidemic may be a contributory factor. Several researchers in the United States have observed an association between drug-resistant TB and HIV infection *(Anonymous 1991; Edlin et al. 1992; Doole et al. 1992)*. However, our own experience has not mirrored this. We examined the hospital records of 295 patients treated for tuberculosis at KGV between 1991 and 1994 and found no association between HIV status and drug resistance *(Anastasis et al. 1997)*. Whether the reversal in MDR-TB drug resistance trends is an usual cyclical biological event or the "third epidemic" *(Neville et al. 1994)* will be determined by ongoing surveillance of these trends.

This study has reported on temporal trends in drug-resistant TB in the most populous province of South Africa, but several limitations should be considered. Almost all of these are associated with the nature of retrospective analyses of routine laboratory data. The absence of demographic data or a differentiation between primary and secondary (acquired) infection in the laboratory records would have enabled the identification of potentially high-risk groups as has been recommended *(Chaulet et al. 1995)*. The proportions presented represents a mixture of primary and (mostly) acquired drug resistance. Due to the selective basis on which susceptibility testing was requested, the proportions calculated are not the true prevalence of drug-

resistant TB. However, comparisons for trend purposes are valid since the indications for drug susceptibility testing changed little over the past few years.

An added advantage is that all susceptibility testing was conducted at the same laboratory in which the senior laboratory staff and microbiological methods remained constant during the study period. The laboratory at KGV serves a large catchment area of over a hundred clinics. It is unlikely that the policy for drug susceptibility testing changed during the first 8 years of the study as the number of requests for drug susceptibility ran approximately parallel to the number of TB notifications for the province (Figure 3.2). After 1991, however, susceptibility requests continued to increase despite an apparent decrease in TB notifications. This corresponds to the time during which there was an increased level of awareness of the rising problem of MDR-TB and an increase in the number of patients co-infected with HIV and TB *(Wilkinson and Moore, 1996b)*. Thus clinicians may have been requesting drug susceptibility tests in an attempt to exclude MDR-TB in HIV+ patients who presented with poor clinical response to anti-TB treatment. It is reported that patients co-infected with HIV and TB have a similar response to treatment as patients without HIV infection, but have higher relapse rates *(Narain et al. 1992)*. They are likely to be generally less well clinically because of other associated opportunistic infections. While this may explain the increase in the number of tests requested, the dramatic fall in the number of TB notifications after 1991 warrants careful assessment of the accuracy of this data. Experience from the Hlabisa Health Ward, a typical but well monitored rural community of approximately 180 000 inhabitants within KwaZulu-Natal *(Wilkinson and Moore, 1996b)* showed a contradictory increase in TB notifications for this same period. Perhaps the decline in the province's TB notifications reflects the logistical and administrative difficulties experienced during the amalgamation of the racially segregated health ministries at the time of South Africa's emerging democracy.

Changes to National TB Control Programmes are often reflected epidemiologically on trends in drug resistance. In South Africa, the National Health Ministry determines TB treatment policies and there are usually only minor local differences in the adherence to these policies. There were no major changes in TB treatment policy such as the introduction of new drugs to the anti-TB drug regimen or the widespread implementation of directly observed therapy (DOT) throughout the province during the study period.

Surveillance on trends in drug resistance impact on TB control programs and at a regional level will also inform the policies for local choices of initial therapy *(Iseman, 1993; Bloch et al. 1994)*. While most agree that non-adherence (compliance) with TB treatment regimens is a major risk factor for drug resistance, studies should include a search for the specific risk factors that are likely to cause a differential increase in MDR-TB as opposed to TB in general.

Conclusions

This survey of trends in drug resistance at a regional referral Mycobacteriology Laboratory over a 13 year period (1983 – 1995) has revealed a significant increase in MDR-TB in KwaZulu-Natal, South Africa. It is particularly disconcerting that the increase in MDR-TB followed an initial downward trend that was reversed in 1989.

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SUMMARY of CHAPTERS 4 – 7
Pharmacokinetic and Pharmacodynamic Characteristics of Isoniazid and Rifampicin in Patients with Multi-drug-Resistant Tuberculosis

OBJECTIVE

To investigate if there is an association between the pharmacokinetic parameters of isoniazid (INH) and rifampicin (RFA) and multi-drug resistant tuberculosis (MDR-TB) in HIV positive (HIV+) and HIV negative (HIV-) patients.
To describe the pharmacokinetic and pharmacodynamic characteristics of INH and RFA in MDR-TB and drug-sensitive TB patients stratified according to HIV status.

DESIGN

Prospective case-control pharmacokinetic study.

SETTING

King George V Hospital, a large specialist referral TB in-patient treatment facility in Durban, KwaZulu-Natal, South Africa

PATIENTS

A total of 138 adult pulmonary tuberculosis patients: 62 MDR-TB (21 HIV+ and 41 HIV-) and 74 drug-sensitive TB (37 HIV+ and 36 HIV-; 1 not classified). A further 2 patients (1 HIV+ and 1 HIV-) could not be classified according to drug susceptibility status.

METHODS

Single daily doses of INH (300 or 400 mg) and RFA (450 or 600 mg) were administered under supervision for 2-5 days prior to the study. Any other drug treatment prescribed for TB or concomitant complaints was noted but not discontinued. Thereafter 6 blood samples were drawn over 2 dosing intervals at 0, 1, 2, 4, 8 and 12 hours after dose administration. Clinical, socio-demographic, radiological (extent and severity of lung involvement), clinical chemistry (liver function tests) and microbiological (drug susceptibility and minimum inhibitory concentration (MIC)) data were collected. Serum drug concentrations were determined using a validated high performance liquid chromatography (HPLC) assay.
Pharmacokinetic data analysis was conducted according to the population approach using the NONMEM program as well as with non-compartmental methods.

RESULTS

Results from the non-compartmental analysis were similar to those obtained from the population approach. Upon initiation of treatment, the average 54-kg patient had a CL/F for RFA of 7.7 L/hr. After continuous daily treatment, maximal enzyme auto-induction was reached at approximately 10 days at which time the CL/F was 15.6 L/hr.

The mean population V/F for RFA was 26.5 L at initiation of treatment and 42.1 L after 10 days of therapy. The inter-individual variability (% coefficient of variation [CV] for RFA was 39% for CL/F and 26% for V/F. Residual variability was described with a proportional component of 39% and an additive component of 0.05 μg/ml.

The proportion of INH fast acetylators in the population was found to be in the majority (85%). The mean population CL/F was 13.0 L/hr for fast acetylators and 4.7 L/hr for slow acetylators. The V/F for INH was 50.0 L. The inter-individual variability in INH CL/F was 32% for slow acetylators and 41% for fast acetylators. There was also a 41 % variability in V/F. Residual variability was described with a proportional component of 28% and an additive component of 0.02 μg/ml.

The pharmacokinetic parameters for both INH and RFA obtained in this study compare well with that reported in the literature.

Population pharmacodynamic parameters (maximum serum concentration [Cmax]:MIC ratios, time above the MIC and area under the curve [AUC] above the MIC) for INH and RFA were described and represent potential benchmarks for future prospective clinical evaluation.

CONCLUSIONS

There was no association between the pharmacokinetic parameters of INH and RFA and MDR-TB. Neither was there any association between HIV status or degree of immune compromise as determined using HIV viral loads and the pharmacokinetic or pharmacodynamic parameters of INH and RFA.

Chapter 4

PATIENTS AND METHODS

Ethical Considerations

The study protocol was approved by the University of Durban-Westville Ethics Committee (Approval Number 93018B).

In accordance with WHO guidelines, patients were provided with pre- and on going post-test counselling regarding the test for antibodies to HIV, by trained counsellors from KGV.

All information obtained during the study was treated as confidential. Patients were identified by code number only - the key to which was known only to the principal investigator.

Study Centre

The study was conducted at King George V (KGV) Hospital, a 1311-bed state hospital with a psychiatric and TB unit. The TB section of the hospital is the larger, comprising 901 beds. KGV is a referral hospital for patients primarily from the province of KwaZulu-Natal. Patients are referred from state or private hospitals, general practitioners and primary health care clinics.

Patient/Subject Selection

Using available microbiology reports in the patients' case notes, culture positive MDR-TB and drug sensitive adult TB patients (18 – 65 years) were identified at the TB wards of KGV hospital over a 5 month period. These patients were counselled in their home language either individually or in groups and their informed consent to participate in the study was obtained (Appendix A). Patients were excluded if there was a history of intolerance to INH or RFA, or if there were any contra-indications to multiple blood sampling such as mental confusion or poor venous access.

During patient recruitment, attempts were made to ensure balanced selection into the 4 study groups as shown in Figure 4.1. Once at least 25 patients had been recruited into a particular group, recruitment into that particular group stopped until 25 patients had been recruited into each of the other groups. Thereafter, the rest of the patients were recruited in a similar manner.

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                          ┌─────────────────────────────────────┐
                          │  Adult Pulmonary Tuberculosis Patients │
                          └─────────────────────────────────────┘
              ┌───────────────────────────────┴───────────────────────────────┐
    ┌───────────────────────────┐                         ┌───────────────────────────┐
    │   Multi-drug Resistant    │                         │     Drug-Sensitive        │
    │ (resistant to at least    │                         │ (not resistant to INH     │
    │     INH and RFA)          │                         │      or RFA)              │
    └───────────────────────────┘                         └───────────────────────────┘
      ┌──────────────┴──────────────┐            ┌──────────────┴──────────────┐
┌────────────────┐ ┌────────────────┐  ┌────────────────┐ ┌────────────────┐
│ HIV sero-positive│ │ HIV sero-negative│  │ HIV sero-positive│ │ HIV sero-negative│
└────────────────┘ └────────────────┘  └────────────────┘ └────────────────┘
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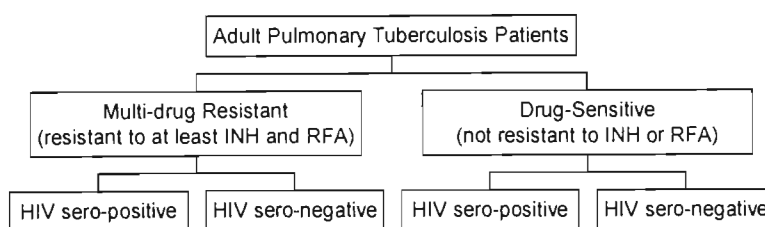
Figure 4.1 – Description of the 4 study groups of adult pulmonary tuberculosis patients recruited into the study to determine the pharmacokinetic and pharmacodynamic characteristics of isoniazid and rifampicin.

Sample Size

A target sample size of 20 per group was estimated to be required to detect a 50% difference between groups with 80% power and a 5% chance of incorrectly rejecting the null hypothesis (t-test). These calculations were based on a reported mean AUC ± standard deviation of 6.3 ± 3.5 µg.hr/ml for INH in HIV- patients (*Choudhri et al 1997*) .

The work of *Ette and Sun 1995* on sample sizes for population pharmacokinetic studies provided reassurance that this sample size would result in accurate and precise estimation of population pharmacokinetic parameters as well as the inter and intraindividual variability.

Study Procedure

After patient recruitment into the study based on the inclusion/exclusion criteria, their medical, family, social and drug history was recorded from their case records and from interviews with the patient.

The once a day dosing regimen of INH and RFA was recommenced in those patients in whom these drugs had been discontinued i.e. primarily the MDR-TB patients. This treatment was continued for a period of between 2-5 days prior to the pharmacokinetic study day. No other changes to the dosing regimens or normal ward routine were instituted. The dates and approximate times of administration of all medications were recorded during the 2-5 day period.

Samples were taken over two consecutive dosing intervals to obtain samples 8 and 12 hours after the dose on study day 1 and 24 hours (pre-dose), 1, 2 and 4 hours after the dose on study day 2 as follows: -

At 05h00 on the morning of study day 1, the nursing staff recorded the exact time of administration of INH and RFA. Later in the day, at approximately 13h00, the patients were weighed, an in-dwelling venous cannula (Vasofix[R]) was inserted into a forearm vein and the 8-hour blood sample (8ml) was drawn into an additive-free blood collection

tube. A further blood sample (12-hour sample) was drawn at 17h00. The in-dwelling venous cannula remained *in-situ* overnight.

At 05h00 on the morning of study day 2, a blood sample was drawn prior to the administration of any medication. The INH and RFA dose were then administered under direct supervision and the exact time recorded. Thereafter, further blood samples (8ml each) were withdrawn at 1, 2 and 4 hours after administration of medication. The exact times of administration of all medications and meals were recorded by nursing staff for the duration of blood collection. A standardised hospital breakfast was served approximately 3 hours after drug administration.

The motivation for drawing samples over 2 days was to minimise disruption of the ward at 05h00, the usual time of drug administration, in order to insert in-dwelling venous cannulas. This strategy was also advantageous with respect to obtaining more information on intra-individual variability.

After collection, samples were allowed to clot on crushed ice and then centrifuged for 15 minutes at 3000 r.p.m. The serum was separated and aliquots transferred into three appropriately labelled polypropylene tubes. All tubes were stored on solid CO_2 (dry ice) or in a -85°C freezer. The maximum time that samples remained on crushed ice was 5 hours. Frozen serum was stored at -85 °C for no longer than 30 days prior to drug concentration determination.

An early morning sputum sample was collected before drug administration on either the first or the second study day and submitted for drug susceptibility confirmation and determination of the minimum inhibitory concentrations (MIC) to both INH and RFA.

Radiology

All patient's "on admission" radiographs (single postero-anterior) were examined by a specialist radiologist and graded according to the extent of disease and the presence and size of any cavities *(Simon, 1966)*.

In brief, the method involved dividing the radiograph of each lung field into 6 segments. The extent of lung involvement was then graded on a 6 point scale. The lowest score of 1 was awarded to radiographs with less than 4 cm^2 of lung involvement and the highest score of 6 to radiographs involving greater than 1 lung field.

The lung cavitation was also graded according to a 6 point scale. A score of 0 was recorded if there were no cavities. Single cavities received scores of 1 to 3 while multiple cavities received scores of 4 to 6 depending on the diameter of the largest cavity.

History of Tuberculosis Therapy

All patients were questioned about previous anti-tuberculosis treatment and classified as either a new case or a re-treatment case. This classification was based on definitions outlined in the *Standard Diagnostic and Treatment Protocol*, Tuberculosis

Control Program, Department of Health, September 1996 *(Department of Health, 1996)*.

1. **New Case**: a patient who had **never received treatment** for tuberculosis before - excluding chemoprophylaxis.

2. **Re-treatment Case**: A patient who was **previously treated** for tuberculosis and who presented with **active tuberculosis again**. These cases were further divided as follows:

- **Re-treatment after previous cure or treatment completion**: A patient who was previously treated for tuberculosis and declared cured as demonstrated by negative bacteriology at six months or one who completed a course of treatment, but for whom no bacteriology results were available to demonstrate cure.

- **Re-treatment after previous treatment interruption or failure:** A patient who was previously treated for tuberculosis and who interrupted treatment for a cumulative period of two months or longer over the total six month treatment period or one whose sputum was still bacteriologically positive at six months. .

Methodology for Investigations

MYCOBACTERIOLOGICAL METHODS

All mycobacteriology procedures were conducted at the Department of Medical Microbiology, University of Natal.

Sputum samples were decontaminated using a solution containing n-acetyl-l-cysteine, sodium hydroxide and sodium citrate according to established standard operating laboratory procedures. Auromine or Ziehl-Neelsen stained slides of the decontaminated sample were then examined microscopically. If mycobacteria were observed, then a direct susceptibility test was set up – otherwise the sample was inoculated onto Lowenstein Jensen agar, Middlebrook 7H11 agar or Middlebrook 7H12 broth and incubated for approximately 6 weeks. Species identification was established using the niacin and nitrate methods *(Nel et al. 1980)*.

Prior to susceptibility testing or minimum inhibitory concentration (MIC) determinations, the cultures were allowed to grow in Dubos broth for 3-5 days at 37 °C and the turbidity of the resulting broth culture was adjusted to a No. 1 McFarland standard.

Susceptibility testing

A standardized inoculum from Dubos broth was introduced onto Middlebrook 7H11 agar plates containing the following concentrations of drugs: -

isoniazid 0.2 and 1.0µg/ml; rifampicin 1.0µg/ml; streptomycin 2.0 and 10.0 µg/ml; ethambutol 7.5 µg/ml; capreomycin 10.0 µg/ml; ethionamide 5.0 µg/ml; cycloserine

30.0 μg/ml; kanamycin 5.0 μg/ml; ofloxacin 10.0 μg/ml; ciprofloxacin 10.0 μg/ml and thiacetazone 1.0 μg/ml.

A drug-free control plate was also prepared. The agar plates were incubated at 37 °C in a CO_2 incubator for 3 weeks at which time the mycobacterial growth on the drug containing plates were compared to those on drug-free control plates. Resistance was reported if there was ≥ 1% of growth on the drug-containing plate relative to the drug-free control plate.

Minimum inhibitory concentrations (MIC)

MIC's for INH and RFA were determined radiometrically using the BACTEC[R] 320 TB (Bactlab Systems) instrument. This system uses Middlebrook 7H12B liquid medium that contains ^{14}C labelled palmitic acid as the carbon source. Metabolically active mycobacteria release $^{14}CO_2$ into the gaseous layer at the top of the test vial, and the instrument quantitates a growth index on a scale of 0-999 units.

Concentrated solutions of INH and RFA (Sigma Chemicals) were prepared and stored frozen at –70 °C until required for addition into the BACTEC[R] liquid media. A standardized inoculum of mycobacteria from Dubos broth was introduced into media containing the following drug concentrations (μg/ml): -

INH - 0.1; 1.0; 4.0; 8.0 and 16.0

RFA - 0.5; 2.0; 4.0; 8.0 and 12.0

The growth index in the drug-containing vials was compared to the growth index in a drug-free control vial. The instrument was operated according to the manufacturer's instructions and as per the standard operating procedures of the laboratory.

Microbiological Definitions

A **multi-drug resistant patient** was defined as a patient with resistance to at least both INH and RFA confirmed with drug susceptibility tests.
A **drug-sensitive patient** was defined as a patient who was sensitive to both INH and RFA regardless of the presence of resistance to the other drugs tested. Where this could not be confirmed using susceptibility tests, the classification was based on the presence of at least 3 consecutive monthly sputum smears that were negative for acid fast bacilli. This latter clinical definition was also applied if resistance to any drug other than INH and RFA was noted.
Patients who were resistant to either INH or RFA (but not both drugs concurrently) were not classified.

HIV SEROLOGY AND VIRAL LOAD

Patients' HIV status was determined using the AxSym[R] HIV-1/HIV-2 microparticle enzyme immunoassay (MEIA) System (Abbott Diagnostics). All positive results were checked using the Vironostika[R] HIV Uni-Form II plus O System (Organon Teknika), an enzyme linked immunosorbent assay (ELISA) for the determination of antibodies to HIV-1 and HIV-2.

The viral load of patients who were found to be HIV+ was determined using the Amplicor HIV-1 Monitor [R] Test (Version 1.5). This is a Polymerase Chain Reaction (PCR) nucleic acid amplification test for the quantitation of HIV-1 RNA in clinical specimens.
These tests were performed according to the manufacturer's instructions at the Department of Virology, University of Natal.

LIVER FUNCTION TESTS

Liver function tests (LFT) were conducted as part of the routine care of the patient and the results were obtained from the patients' case records. These tests were conducted at KGV laboratory on a Beckman CX5CE Autoanalyser (Beckman Instruments, United States).

TUBERCULOSIS DRUG ASSAYS

The assay of INH and RFA in patient sera was conducted using a validated high performance liquid chromatographic (HPLC) assay technique at the Department of Pharmacology, University of Cape Town. The assay procedure was developed in-house *(Zent and Smith, 1995)* and is described briefly below.

Both drugs were removed from serum by solid phase extraction on Bond Elut[R] C18 columns (Analytichem International). Unbound material was removed from the column with 2x2ml aliquots of methanol, followed by 2x2ml aliquots of water and finally 1ml of 0.05M sodium phosphate buffer pH4.5 (washing buffer). Thereafter, 0.5ml of patient serum was applied to the column, washed with 1ml of washing buffer and eluted using 0.5ml acetonitrile and 0.5ml methanol to extract the INH and RFA respectively.

RFA was assayed at room temperature by injection of the eluent onto a Spherisorb[R] S5 C8 (15 cm x 0.46 cm) column fitted with a Pelliguard[R] LC 8 (2.5 cm x 0.46 cm) guard column. The mobile phase comprised 80% acetonitrile and 20 % of 0.1% trifluoroacetic acid (TFA) in water. Ultraviolet detection occurred at a wavelength of 270 nm. A maximum of 10 samples was extracted per batch of samples to ensure stability of the RFA during the assay. Under the chromatographic operating conditions, the retention time for RFA was approximately 4 minutes.

For the assay of INH, 400 μl of eluent was first evaporated to dryness on a centrifugal vacuum concentrator and then reconstituted with 0.5 ml of mobile phase. The resulting solution was injected at room temperature onto a Spherisorb[R] S5 C8 reversed phase column (25 cm x 0.46 cm) fitted with a Pelliguard[R] LC 8 (2.5 cm x 0.46 cm) guard column. The INH was eluted isocratically using 4% acetonitrile and 96% of 0.6% TFA in water and subjected to ultraviolet detection at a wavelength of 270nm. The retention time for INH was approximately 3 minutes.

The regression curves for both drugs were linear over the concentration range 0.02 - 20 μg/ml. Quantitation of the chromatograms was by the peak area method. The limit of quantitation (LQ) was 0.05 μg/ml for both assays. The inter-day and intra-day

coefficient of variation for INH at 0.05 µg/ml was 4.3 % and for RFA at 0.05 µg/ml was 5.8 µg/ml.

Pharmacokinetic and Statistical Analysis

Data collected in the course of this study was organised and coded onto data collection forms before being captured into computer files. Computer management of the data utilised Microsoft Excel for Windows 95[R] Version 7. All data entries were cross-checked for accuracy of data capture.

Demographic, clinical and radiological characteristics of the groups were compared by using the chi-square test for categorical variables. Analysis of variance (ANOVA), the t-test or the Kruskal-Wallis test was used for continuous variables. Odds ratios were calculated as measures of association and 95% confidence intervals were used to indicate the precision of the point estimates.

The software programme, Epi-Info Version 6.04b (Centers for Disease Control and Prevention (CDC), USA and WHO, Geneva), was used for statistical analysis.

Unless otherwise indicated as more stringent, statistical significance was assumed at the $p < 0.05$ level.

NON COMPARTMENTAL PHARMACOKINETIC DATA ANALYSIS

Data

This analysis included only those patients in whom sufficient samples had been drawn to characterise the full concentration vs time profile (i.e. ≥ 5 drug concentration data points). In addition, any patient who had received additional unscheduled extra doses of INH or RFA were excluded from this data-set. Patients who had received INAT[R] (containing INH in combination with thiacetazone) on a three times-a-day schedule were also excluded as blood samples were not drawn at "descriptive" times in these patients.

Calculation of Parameters

Pharmacokinetic parameters i.e. area under the serum concentration-time curve (AUC), the highest observed serum concentration (Cmax) and the time to the Cmax (tmax) were obtained using noncompartmental analysis (NCA) methods (WinNonlin Professional Edition Ver 1.5, Scientific Consulting Inc.).

A 24-hour AUC was calculated as follows: steady state was assumed, the sample times for the 2 dosing intervals were combined and the concentration at the end of the dosing interval was assumed to be equal to that measured prior to drug administration. All serum drug concentrations reported as below the limits of quantitation (LQ) of the assay were recorded as 0.5xLQ i.e. 0.025µg/ml. The terminal elimination half-life (t½) was determined by curve stripping of the terminal elimination portion of the serum concentration versus time curve. Any concentration recorded as below LQ was excluded from the calculation of t½. Clearance (CL/F) and apparent steady state volume of distribution (Vss/F) were calculated using non-compartmental methods and were

scaled to bioavailability (F) since there was no data from an intravenous reference product.

The pharmacokinetic parameters were compared using ANOVA with Group status (Figure 4.1) as the treatment effect. If significant differences were found then pairwise comparisons were made using Duncan's multiple range test.

POPULATION PHARMACOKINETIC ANALYSIS

Data
All patients receiving the drugs of interest were included in the NONMEM dataset.

Covariates
In addition to the primary research variables of drug susceptibility and HIV status, there were several other covariates that were tested for an influence on the pharmacokinetics of INH and RFA. The demographic characteristics investigated were age, weight and sex. The effect of severity of TB on the pharmacokinetics was evaluated using the radiographic severity score, associated disease states and the number of drugs to which an MDR-TB patient was resistant. All liver function results were also tested. Any concomitant medications administered within 48 hours of the pharmacokinetic study day were grouped together according to pharmacological class. These included enzyme inducers, enzyme inhibitors, antacids, drugs affecting gastric motility, non-steroidal anti-inflammatory drugs, iron preparations and antihistamines. The influence of other anti-TB drugs was tested individually. In the case of RFA, the number of days since starting treatment was used to determine the time of maximal enzyme auto-induction. Miscellaneous covariates tested for an influence on the pharmacokinetics of INH and RFA included the history of previous TB treatment.

Missing Data
During the NONMEM analysis, missing continuous data were re-coded to the median value. Those with missing indicator variables ('yes/no' data) were regarded as being negative for the covariate of interest. While this procedure is unlikely to influence the estimation of any parameter estimate, it may inflate the inter-individual variability. This should not have had any significant effect on the final results due to the low prevalence of missing data in this study.

Description of Population Pharmacokinetic Models

Compartment Model
One- and two-compartment linear models were tested in order to determine the basic structural pharmacokinetic model for the INH and RFA population pharmacokinetic analysis.

This was communicated to NONMEM by the choice of the appropriate ADVAN subroutines from the PREDPP library of subroutines. For the one-compartment model, ADVAN 1 or 2 was selected for the zero order or first order absorption models, respectively. In the case of the two-compartment model, ADVAN 3 or 4 was chosen for the zero order or first order absorption, respectively.

In the case of the one-compartment model, the parameters of CL and V were obtained by choosing the TRANS 2 program, which re-parameterises the elimination rate constant (KE) in terms of CL and V. The two compartment model was re-parameterized as CL, central compartment volume (V2), inter-compartmental clearance (Q) and peripheral volume (V3) by means of the TRANS 4 subroutine.

The first order absorption models parameterized the absorption process in KA while the zero order absorption models estimated the duration of the absorption process in the parameter D1.

Assuming that the one- and two-compartment models are stated in more generic terms, viz. as y_{ij} which is the jth observation (i.e. a drug concentration at a specified time) from the ith individual in a population of individuals (i =1,2,...N) then the structural pharmacokinetics are described in a non-specific way by *Equation 1*.

$$y_{ij} = f(x_{ij}, \phi_i)$$

Equation 1

The symbol f represents the structure of the compartment model, which is a function of the known quantities, x (i.e. dose and time), and the parameters, ϕ (e.g. KA, V and CL). The quantities in x are measurable in the experiment and are therefore called fixed effects, in contrast to effects that are not known and are called random effects (see below). The parameters in the parameter vector ϕ are called fixed effect parameters because they quantify the influence of the fixed effects on the dependent variable.

Parameter model

A general model for the fixed effects parameters ϕ_i may be written as:

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

Equation 2

Here, g is a structural type model that is a function of the fixed effects z_i (e.g. continuous variables such as weight or categorical variables such as presence or absence of MDR-TB) and the fixed effects parameters θ.

Pharmacostatistical Model

The deviations of the parameters ϕ_i for a particular individual from the true (but unknown) population mean values (inter-individual variability) were modelled using the exponential model.

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

Equation 3

Here η_i are random variables which are assumed to be multivariate normally distributed, with zero means and whose squares represent the respective variances of

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the parameter. These estimates were reported by NONMEM in its OMEGA matrix.

The deviation of the observed concentration from that predicted by the model (intra-individual or residual variability) was estimated using a combined additive and constant co-efficient of variation model.

$$y_{ij} = f(x_{ij}, \phi_i) * \varepsilon(1)_{ij} + \varepsilon(2)_{ij}$$

Equation 4.

Here $\varepsilon(1)_{ij}$ represents the proportional component of the deviation while $\varepsilon(2)_{ij}$ represents the additive part of the error. These error terms are also assumed to be random variables which are assumed to be multivariate normally distributed, with zero means and whose squares represent the respective variances of the concentration. These estimates were reported by NONMEM in its SIGMA matrix.

Mixture Model

The proportion of slow and fast acetylators of INH and the typical values of their pharmacokinetic parameters were determined using a mixture model (Appendix L) in the NONMEM program.

A mixture model assumes that the population consists of 2 or more sub-populations, each approximating a normal distribution and each sub-population having its own model. It is not known *a priori* into which sub-population each individual in the population belongs, although it is assumed that 1 of the models describes the observations for that particular individual. NONMEM computes mixing probabilities corresponding to the sizes of the sub-populations.

In this case of slow and fast acetylators, it was assumed that some fraction p of the population has one set of typical values for the pharmacokinetic parameters and the remaining fraction 1-p has another set of typical values.

The parameter models were described as:

Subpopulation 1:

$$CL = \theta_1 exp\eta 1$$
$$V = \theta_3 exp\eta 2$$

Subpopulation 2:

$$CL = \theta_2\theta_1 exp\eta 1$$
$$V = \theta_4\theta_3 exp\eta 2$$

The parameters θ_2 and θ_4 are the fractional differences in the typical values between the 2 sub-populations.

With a particular set of values for the population pharmacokinetic parameters and their estimates of variability, NONMEM classifies each individual into 1 of the 2 sub-populations. For each possible model, the empirical Bayes posterior probability that the relevant model describes the observations is computed by the NONMEM likelihood function. The individual gets classified into the most probable of the 2 sub-populations.

Computer Software and Hardware

Population pharmacokinetic data analysis was conducted using Nonlinear Mixed Effects Modelling with the first-order method as implemented in the computer program, NONMEM[R] Version IV Level 2.1. The companion programs to NONMEM[R] were also used i.e. NMTRAN[R], the data pre-processor and PREDPP[R] Version III Level 1.0 with its library of population pharmacokinetic models *(Boeckmann et al. 1994)*. All NONMEM[R] analyses were performed in DOS[R] windows on a Pentium 200MMX IBM[R]-compatible personal computer operating under Windows 95[R]. All NONMEM[R] and related subroutines were compiled using the Lahey F77-EM32 Fortran 77 Language System (Lahey Computers Systems Inc 1992).

Exploratory data analysis, model building and graphical presentations utilised Xpose[R] Version 2.0 *(Jonsson and Karlsson, 1997)* within the S-Plus for Windows[R] Version 3.3 Release 1 environment (Mathsoft, Inc).

Model building was conducted using a combination of the procedure described by *Mandema et al (1992)* and the NONMEM[R] Users Guides *(Boeckmann et al. 1994)*. The criteria for assessment of the different models are described in the Model Diagnostic Tools.

Model Diagnostic Tools

During model building, the effect of a change to the model was assessed by several goodness of fit characteristics. The primary criterion was the minimum value of the objective function (OBF). In addition, the effect on unexplained variability in the model and on graphical plots was also considered.

Change in the Objective Function
This global measure of goodness of fit is based on the final parameter estimates and is equal to minus twice the log likelihood of the data. A difference in the minimum value of the objective function (DOBF) between 2 models is asymtotically chi square distributed with degrees of freedom equal to the number of parameters added to the model. A decrease in OBF of ≥ 3.84 is significant at $p \leq 0.05$ when a single parameter has been added into the model. This was used as the acceptance criterion in the univariate analysis (Step 3) and in the model build-up (Step 4). An increase in OBF of ≥ 11 is significant at $p \leq 0.001$ when a single parameter has been deleted from the model. This was used as the acceptance criterion during the model deletion stage (Step 5) as a "penalty" for the multiple comparisons.

Decrease in Unexplained Variability
This means that the addition of the parameter into the model "explains" some of the interindividual variability that was previously part of the random "noise". Sometimes

this could also have manifested as part of random intraindividual variability prior to elaboration of the model.

Graphical Plots
Model Prediction and Individual Predictions vs Dependent Variable
The data should be distributed as close as possible to the line of identity i.e. the line corresponding to perfect correlation between predicted and observed concentrations.

Model Predictions and Individual Predictions vs Independent Variable
These graphs should mimic the graph of observed concentration vs time plot.

Weighted Residuals vs Dependent and Independent Variable
These plots should show no pattern. The unexplained part of the data should manifest as featureless random noise. Outlying values should remain within 3 weighted residual units.

Stages in Population Pharmacokinetic Model Building

Step 1 – Data Checkout
A basic NONMEM run with no covariates was conducted to produce the tables necessary for data checkout in Xpose[R].

Step 2 – Structural Pharmacokinetic Model Development
The basic structural pharmacokinetic model was developed using the Model Diagnostic Tools described above. One and two compartment models were compared as well as various options to characterise the absorption profile i.e. first order, zero order or fixed absorption rate constant with estimated variability. If the estimate of any parameter (e.g. KA) required confirmation or needed to be fixed to a particular value (e.g. due to a absence of an adequate amount of data), then the sensitivity analysis procedure *(Wade et al. 1993)* was performed. In this procedure, the parameter was fixed to various values and the OBF and parameter estimates obtained were compared. This was with a view to determining a value of the parameter which resulted in stable parameter estimates and/or the lowest OBF.

The exponential error model was used for inter-individual variability while the combined additive and exponential error model was used for the intra-individual variability.

Step 3 – Exploratory Data Analysis and NONMEM Univariate Analysis
At the end of Step 2, collections of the individual Bayesian estimates for each parameter in the model with no added covariates were obtained using the 'POSTHOC' feature in NONMEM.
Each collection was treated as data and its distribution and relationship with covariates were investigated using graphical displays and generalized additive models (GAM) in Xpose[R]. The primary intention was to identify non-normal parameter distributions and to examine potential relationships between covariates and parameters.
Simultaneously, each covariate was added into the parameter model (KA, CL/F and V/F) one-at-a-time and its effect on the model was determined using the Model

Diagnostic Tools described above. The OBF value obtained from the final model in Step 2 was used as the reference in this univariate analysis procedure

Step 4 – Step-wise Build-up to Full Model
The covariates that caused a statistically significant improvement in the fit of the data in Step 3 were ranked according to the size of the DOBF. Covariates were then progressively added into the model starting with the covariate that caused the greatest DOBF and proceeding down the rank order established in the univariate analysis. The effect of each new addition was evaluated and it was retained only if the Model DiagnosticTools suggested an improvement in the fit of the data. Typically Step 4 results in a fairly large full model.

Step 5 – Step-wise Deletion to Final Model
The complete model from Step 4 was likely to contain redundant, confounded or imprecisely estimated terms. The stepwise deletion of covariates was done in 2 stages.
Covariates in the full model were set to their NULL values (i.e. that value which effectively removes the parameter from the model) one-at-a-time (with replacement of the previously removed covariate) and the effect on the model fit was evaluated. The covariates were ranked according to the size of the increase in DOBF caused by their removal from the model.
The covariates in the full model were subsequently removed in a step-wise manner according to the established rank order. At this stage of the model building, a covariate was only retained in the model if its removal caused an increase in DOBF of ≥ 11 as discussed above.
Finally, the covariance step was implemented on the final NONMEM model to establish parameter precision and correlations. The relative importance of the additive and constant coefficient of variation error estimates was evaluated with a view to determining whether a simpler error model would have been appropriate.

Step 6 – Confirmation of Final Model
The final NONMEM run was confirmed. The strategy employed was to delete those parameters with large relative standard errors (RSE) and whose confidence intervals included the NULL values. The validity of the deletion was confirmed if the magnitude of the standard errors and RSE's of the other parameters decreased.

Interpretation of the NONMEM output

The precision (RSE in percent) in estimating the parameters of the model was calculated by dividing the standard error of the estimate of each parameter by its value and expressing the result as a percentage *(Boeckmann et al. 1994)*.

The inter-individual variability in the primary pharmacokinetic parameters (KA, CL, V, TLAG) was calculated by taking the square root of the individual elements of the OMEGA matrix and expressing the result as a percentage of the population mean values.

The intra-individual variability was estimated by fixing the estimate of the SIGMA matrix to unity and redirecting the estimate into 2 θ parameters, 1 for the CCV error component and the other for the additive error. These were coded in such a way as to

provide the estimates of the variability directly (Appendix H and K) , i.e. without the need to obtain the square root of the parameter estimate. This code was also necessary in order to obtain some of the diagnostic plots in Xpose2[R] *(Jonsson and Karlsson, 1997).*

The 95% confidence intervals (CI) for the parameters were calculated by adding the parameter estimate to ± 1.96 X the standard error of the estimate.

CALCULATION OF THE DERIVED PHARMACODYNAMIC PARAMETERS

The relationship between the pharmacokinetic parameters and the minimum inhibitory concentration is illustrated in Figure 4.2.

Assumptions

For purposes of these calculations, if the MIC was greater than the highest concentration tested, then the MIC was assumed to be equal to the highest concentration tested. If the MIC was lower than the lowest concentration tested, then the MIC was assumed to be equal to this lowest concentration tested. In the case of INH, this was 16 and 0.1 µg/ml respectively. For RFA, the corresponding assumed MICs were 12 and 0.5 µg/ml respectively.
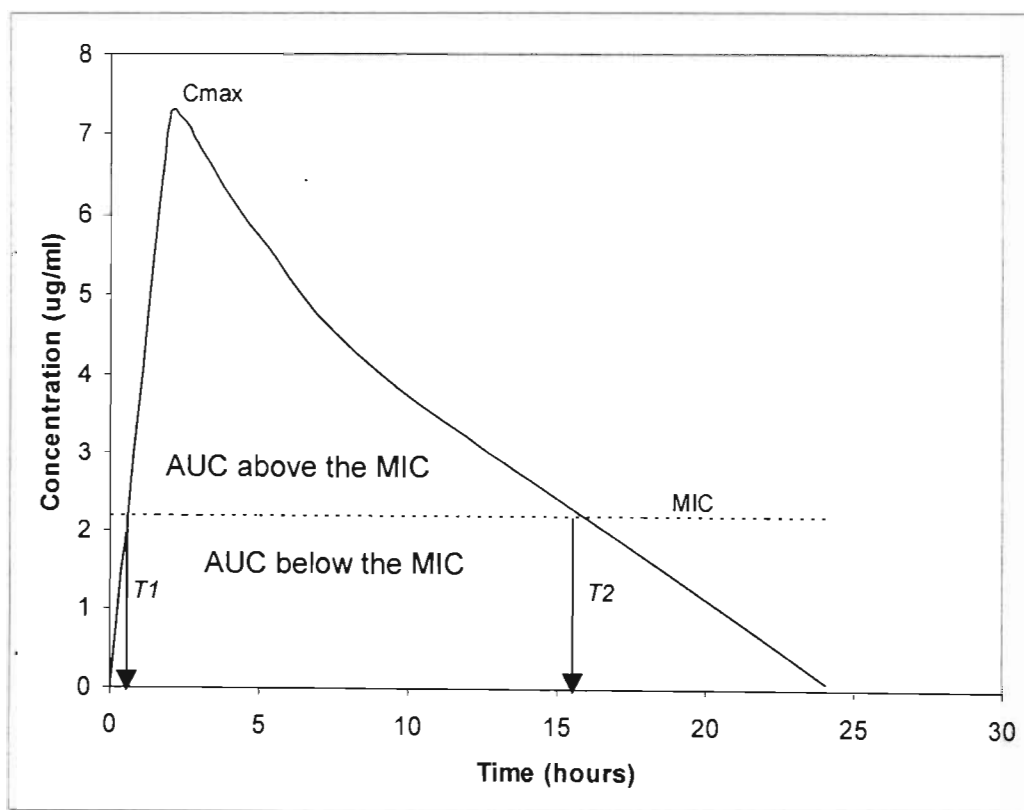
Figure 4.2 - A hypothetical serum concentration versus time curve, displaying the maximum serum concentration (Cmax), the area under the curve (AUC), the time above the MIC ($T2 - T1$), and the relationship between these parameters and the minimum inhibitory concentration (MIC). Solid line = drug concentration; Dashed line = MIC *(Peloquin, 1996)*

Cmax:MIC

The pharmacokinetic parameters used in these calculations were the Bayesian individual estimates obtained using the *post-hoc* feature within NONMEM. Cmax was calculated using *Equations 5* and *6 (Gibaldi and Perrier, 1982)*.

$$t_{max} = \frac{\ln \left(KA \ / \ KE \right)}{KA \ - \ KE} + TLAG$$

<div align="right">*Equation 5*</div>

Where t_{max} is the time at which Cmax is achieved, KA is the absorption rate constant and KE=CL/V.

$$C_t = \frac{Dose \ * \ KA}{V \ * \ (KA \ - \ KE)} * \left(\left(e^{- KE \ * t} \right) - \left(e^{- KA \ * t} \right)\right)$$

<div align="right">*Equation 6*</div>

Where $t = t_{max}$ and C_t = Cmax i.e. the concentration at t_{max}.

The Cmax was divided by the MIC to obtain the ratio of Cmax:MIC.

T>MIC

The time above the MIC (T>MIC) was determined using the Newton-Raphson iterative technique.
In brief, the value of C_t in *Equation 6* was replaced with the MIC value for the .
individual patient. Thereafter, the 2 time points (T1 and T2, before and after attainment of the Cmax respectively) at which the patient's serum concentrations were equal to the MIC, were computed.

AUC>MIC

The AUC > MIC was calculated using *Equation 7*. In this equation, the total area between T1 and T2 is computed by integration of *Equation 6*. Thereafter the area below the MIC, given by the rectangle formed by the MIC and T2-T1, is subtracted out.

$$AUC > MIC = \left(\left(\int_{T1}^{T2} \frac{Dose \ * \ KA}{V \ * \ (KA \ - \ KE)} * (e^{- KE \ * t} - e^{- KA \ * t}) dt \right) - \left(MIC \ * \ (T2 - T1) \right) \right)$$

<div align="right">*Equation 7*</div>

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Chapter 5

RESULTS

Demographics and Clinical Characteristics

One hundred and forty six patients were invited to participate in this study. Eight patients declined and thus 138 patients were recruited into the study. Their demographic and clinical characteristics are summarised in Table 5.1 and listed in detail in Appendix B.

The study population comprised of 135 African patients, the remaining 3 being 1 White patient and 2 so-called "Coloured" patients. There were 62 MDR-TB patients and 74 drug-sensitive TB patients. The drug susceptibility status of 2 patients (1 HIV+ and 1 HIV-) was not classified as they were found to be resistant to INH alone. Therefore the sample for the comparative study of MDR-TB and drug-sensitive TB consisted of 136 patients. The remaining 2 patients were, however, included in the data set for the population pharmacokinetic analysis where covariates other than MDR-TB were studied. In 10 of the patients, the organism failed to grow during drug susceptibility confirmation and their classification into the drug-sensitive TB group was based on the presence of at least 3 consecutive monthly sputum smears that were negative for acid fast bacilli.

The mean age of the HIV+ patients was lower than that of the HIV- patients (31.0 ± 8.5 versus 39.4 ± 12.1 years; p<0.0001). There was no significant difference in the mean weight of the patients in the different groups. Although the HIV+ patients had a lower mean weight than the HIV- patients, this was not statistically significant (53.74 ± 10.21 vs 56.45 ± 11.81 kg).

There were more females than males recruited into the study, a larger proportion of whom were HIV+ (53% of females versus 31% of males; p=0.01). There was no significant difference with regard to the sex distribution of subjects into the MDR-TB or drug-sensitive TB groups.

There were significantly more MDR patients who had a history of previous treatment for TB (79%) compared to new cases (21%) (odds ratio 7.85, CI 3.34 to 18.77; p<0.0001). Among the re-treatment cases that developed MDR-TB, there was a larger proportion of patients whose previous treatment had been interrupted or failed. However this was not statistically significant (odds ratio 2.34, CI 0.74 to 7.48, p= 0.08).

The clinical condition most frequently encountered in association with TB was anaemia (35%). A large number of the HIV+ patients presented with oral candidiasis (24%). Two patients had genital herpes (viral load unmeasurable in 1 and 2.7×10^6 copies per millilitre in the other) and 1 had Karposi's sarcoma (viral load 2.7×10^5 copies per millilitre).

Table 5.1 - Demographic and Clinical Characteristics of Multi-drug Resistant and Drug-Sensitive Pulmonary Tuberculosis Patients

	ALL PATIENTS (n = 138)[1]	MDR-TB HIV+ (n = 21)	MDR-TB HIV– (n = 41)	DRUG-SENSITIVE TB HIV+ (n = 37)	DRUG-SENSITIVE TB HIV– (n = 36)
Age (years) – mean ± sd	35.7 ± 11.5	33.2 ± 9.3	39.2 ± 10.5	29.2 ± 7.1	40.3 ± 13.5
Weight (kg) – mean ± sd	55.2 ± 11.2	53.2 ± 11.3	56.1 ± 11.4	54.3 ± 9.7	57.2 ± 12.4
Sex - number (%) of patients					
Males	61 (44)	8 (38)	24 (59)	10 (27)	18 (50)
Females	77 (56)	13 (62)	17 (41)	27 (73)	18 (50)
History of Prior TB - Number (%) of patients					
New Case	64 (46)	7 (33)	6 (15)	29 (78)	21 (58)
Re-treatment:-					
After previous cure or treatment completion	24 (17)	4 (19)	9 (22)	2 (5)	9 (25)
After previous treatment interruption or failure	50 (36)	10 (48)	26 (63)	6 (16)	6 (17)
HIV Viral Load - copies of RNA/ml					
Geometric mean	6.2×10^4	7.6×10^4	-	6.3×10^4	-
Median	6.9×10^4	9.3×10^4	-	7.2×10^4	-
Minimum	200	1.4×10^3	-	200	-
Maximum	2.7×10^6	2.4×10^6	-	2.7×10^6	-
Other Disease States - Number (%) of patients					
Anaemia	48 (35)	7 (33)	3 (7)	23 (62)	14 (39)
Oral Candidiasis	18 (13)	3 (14)	2 (5)	11 (30)	2 (6)
Diabetes mellitus	10 (7)	-	6 (15)	-	4 (11)
Hypertension	7 (5)	-	5 (12)	-	2 (6)
Epilepsy	6 (4)	-	-	5 (14)	1 (3)
Congestive Cardiac Failure	3 (2)	-	1 (2)	-	2 (6)
Genital Herpes	2 (1)	-	-	2 (5)	-
Karposi's Sarcoma	1	-	-	1 (3)	-

[1] The total number of MDR-TB and Drug-sensitive TB patients does not add up to total number of patients included in the study. This is because the drug susceptibility status of 2 patients (1 HIV+ and 1 HIV-) could not be classified as they were found to be resistant to INH alone. The HIV status of 1 drug-sensitive patient could not be determined as the patient withdrew consent for HIV testing after recruitment into the study.

HIV Status and Viral Loads

There were 59 HIV+ patients and 78 HIV- patients. The HIV status of 1 drug-sensitive TB patient could not be determined as the patient withdrew consent for HIV testing after recruitment into the study. A summary of the viral load data is presented in Table 5.1 and reported in detail in Appendix C.

A wide range in viral load was noted in the HIV+ patients. The majority of samples produced good viral RNA recovery. However, in samples from 2 patients, the assay repeatedly produced inadequate RNA internal controls while in a third patient, the system recorded a level of <200 copies of RNA per millilitre. One of these patients had concurrent genital herpes and severe oral candidiasis. In all of these 3 patients, their HIV status was confirmed using at least 2 immunoassay tests. These patients were retained as HIV+, a viral load of 0 copies per millilitre was recorded for the 2 patients with inadequate RNA controls while the patient with a level of <200 was recorded as having a viral load of 200 copies per millilitre.

There was no significant difference in the HIV viral load of the MDR-TB and the drug-sensitive TB patients in the HIV+ sub-groups.

Radiology

Table 5.2 describes the severity of pulmonary involvement with TB after examination of a single posteroanterior (PA) chest radiograph. The radiographs for 3 patients could not be located at the time of this assessment and were therefore excluded. The majority of the patients included in the study had extensive lung field involvement with multiple cavitary lesions. There was no statistically significant difference between the 4 groups or between MDR-TB and drug-sensitive TB patients with respect to the severity scores. However, the HIV+ patients (from both the MDR-TB and drug-sensitive TB groups combined) had lower radiological severity scores than the HIV- patients from these groups (extent 4.8 ± 1.1 vs 5.2 ± 1.0 p = 0.02; cavitation 3.8 ± 1.6 vs 4.3 ± 1.4 p = 0.06).

Table 5.2 - Radiological Description of Disease Severity in Multi-drug Resistant and Drug-Sensitive Pulmonary Tuberculosis Patients

SEVERITY SCORE	DESCRIPTION	ALL PATIENTS (n = 135)[1]	MDR-TB		DRUG-SENSITIVE TB	
			HIV+ (n = 20)	HIV– (n = 39)	HIV+ (n = 37)	HIV– (n = 36)
	Extent of Lung Involvement (Number of patients)					
1	$\leq 4cm^2$	2	-	-	2	-
2	$> 4cm^2$ to < 1/6 of 1 lung field	1	-	1	-	-
3	$\geq$ 1/6 to <1/3 of 1 lung field	9	2	2	1	4
4	$\geq$ 1/3 to < 2/3 of 1 lung field	23	4	4	11	4
5	$\geq$ 2/3 to the whole of 1 lung field	44	10	13	12	8
6	larger than 1 lung field	56	4	19	11	20
	Mean Severity Score ± sd for extent of lung involvement	5.0 ± 1.1	4.8 ± 0.9	5.2 ± 1.0	4.7 ± 1.2	5.2 ± 1.1
	Classification of Cavitation (Number of Patients)					
0	No cavities	7	1	-	5	1
	Single Cavity:					
1	$\leq$ 2cm in diameter	4	-	2	1	1
2	>2cm to < 4cm in diameter	6	1	2	-	3
3	$\geq$ 4cm in diameter	7	2	2	2	1
	Multiple Cavities:					
4	largest cavity $\leq$ 2cm in diameter	62	9	15	21	16
5	largest cavity > 2cm to < 4cm in diameter	22	2	10	5	5
6	largest cavity $\geq$ 4cm	27	5	8	3	9
	Mean Severity Score ± sd for lung cavitation	4.1 ± 1.5	4.2 ± 1.5	4.4 ± 1.3	3.6 ± 1.7	4.3 ± 1.5

[1] The radiographs for 3 patients could not be located at the time of this assessment. The total number of MDR-TB and Drug-sensitive TB patients do not add up to total number of patients included in the study. This is because the drug susceptibility status of 2 patients (1 HIV+ and 1 HIV-) could not be classified as they were found to be resistant to INH alone. The HIV status of 1 patient could not be determined as the patient withdrew consent for HIV testing after recruitment into the study.

Liver Function Tests

There were no significant differences among the 4 groups with regard to the serum liver enzyme measurements (Table 5.3). However, in the case of the gamma glutamyl transpeptidase (GGT), alanine transaminase (ALT) and the aspartate transaminase (AST) results, although the median values remained within the normal range, there were individual patients with results above the upper limit of the normal range.

In the case of the measurements concerned with protein metabolism, the HIV–, MDR-TB patients had significantly higher albumin levels than patients from the other groups (p < 0.001). This observation could not be extended into a statistically significant difference between either the MDR-TB and Drug-sensitive TB patients or the HIV+ and HIV- patients.
It was also noted that HIV- patients had lower globulin levels than the HIV+ patients (p < 0.001).

Table 5.3 – Liver Function Test Results (median and range) in Multi-drug Resistant and Drug-Sensitive Pulmonary Tuberculosis Patients

	NORMAL RANGE	ALL PATIENTS (n=123)[1]	MDR-TB		DRUG-SENSITIVE TB	
			HIV+ (n=19)	HIV– (n=36)	HIV+ (n=32)	HIV– (n=34)
Alkaline phosphatase (ALKP) u/l	80-306	83 (43-261)	79 (43-261)	79 (47-131)	89 (47-202)	81 (45-232)
Total bilirubin (BILI) µmol/l	3-17	10 (2-33)	9 (3-21)	10 (4-33)	11 (2-23)	11 (4-22)
Albumin (ALB) g/l	38-48	25 (14-88)	24 (14-36)	30 (19-88)	23 (15-36)	24 (16-46)
Globulin (GLOB) g/l	20-32	52 (24-107)	65 (48-82)	48 (24-87)	56 (37-107)	47.5 (24-80)
Gamma glutamyl transpeptidase (GGT) u/l	11-49	37 (12-233)	47 (21-150)	32.5 (18-156)	78.5 (12-134)	59 (19-233)
Alanine transaminase (ALT) u/l	0-41	16 (5-112)	17 (7-37)	16 (5-66)	16.5 (9-112)	16 (8-52)
Aspartate transaminase (AST) u/l	0-37	31 (15-104)	33 (16-66)	25 (15-94)	34 (17-87)	31 (15-104)

[1] Liver function test results were not available for 15 of the 138 patients in the study. The total number of MDR-TB and Drug-sensitive TB patients does not add up to the total number of patients included in the study. This is because the drug susceptibility status of 2 patients (1 HIV+ and 1 HIV-) could not be classified as they were found to be resistant to INH alone. The HIV status of 1 patient could not be determined as the patient withdrew consent for HIV testing after recruitment into the study.

Appendix C is a detailed listing of the liver function test results for the individual patients.

Mycobacteriology

A summary of the mycobacteriology results is presented in Table 5.4 while individual patient's susceptibility results are recorded in Appendix D.

In the MDR-TB patients, resistance to 2 drugs only, i.e. INH and RFA, was rare - the majority of patients, 52 of 62 (84%), patients displayed resistance to ≥ 5 drugs. There was no significant difference in the mean number of drugs to which isolates showed resistance in the re- treatment and drug-naive groups (6.7 ± 2.3 vs 6.6 ± 1.5; p = 0.73).

Table 5.4 - Summary of anti-TB drug susceptibility results in Multi-drug Resistant and Drug-Sensitive Pulmonary Tuberculosis Patients

	All cases (n=136)[1]	Re-Treatment Cases		Odds Ratio and 95% Confidence interval (CI)		p
		Yes (n=73)	No (n=63)	Point Estimate	CI	
	Number (%) of isolates					
DRUG-SENSITIVE TB	74 (54)	24 (33)	50 (79)			
MULTI-DRUG RESISTANT TB	62 (46)	49 (67)	13 (21)	7.85	3.34 - 18.77	<0.0001
2 drug resistant (i.e. INH & RFA only)	1 (0.7)	1 (1.4)	0 (0)			
3 drug resistant	6 (4.4)	6 (8.2)	0 (0)			
4 drug resistant	3 (2.2)	2 (2.7)	1 (1.6)			
5 drug resistant	5 (3.7)	4 (5.5)	1 (1.6)			
6 drug resistant	14 (10.3)	9 (12.3)	5 (7.9)			
> 6 drug resistant	33 (24.3)	27 (37)	6 (9.5)			
Mean ± SD number of drugs to which the MDR-TB isolates were resistant	6.8 ± 2.2	6.7 ± 2.3	6.6 ± 1.5			0.73 (ns)

[1] The drug susceptibility status of 2 patients was not classified as they were found to be resistant to INH alone.

By definition, patients with drug-sensitive TB were those who **did not** have combined resistance to INH and RFA but who may have had resistance to other anti-TB drugs. This group included 51 patients with isolates sensitive to ALL drugs tested and 23 patients with isolates resistant to 1 or more second-line anti-TB drugs other than INH and/or RFA. This latter subgroup consisted of 3 patients with resistance to streptomycin, 17 with resistance to ethionamide, 14 with resistance to cycloserine and 5 with resistance to thiacetazone. These 23 patients complied with the clinical definition for drug-sensitive TB i.e. at least 3 consecutive monthly sputum smears that were negative for acid fast bacilli.

The drug susceptibility profiles of the patients included in the study are recorded in Table 5.5.

Table 5.5 – Drug Susceptibility Profiles in Pulmonary Tuberculosis patients

Drug	Number of isolates tested	Number (%) Resistant
Isoniazid 0.2 ug/ml	115	55 (48)
Isoniazid 1.0 ug/ml	129	64 (50)
Rifampicin	129	61 (47)
Ethionamide	119	61 (51)
Streptomycin 2.0 ug/ml	123	47 (38)
Streptomycin 10.0 ug/ml	116	37 (32)
Ethambutol	129	45 (35)
Thiacetazone	118	38 (32)
Cycloserine	118	33 (28)
Kanamycin	119	8 (7)
Capreomycin	118	7 (6)
Ciprofloxacin	119	4 (3)
Ofloxacin	119	3 (2.5)

The drugs most frequently (greater than 33%) associated with combined resistance to INH and RFA were ethionamide, streptomycin, ethambutol, thiacetazone and cycloserine. A lower prevalence of resistance (less than 8%) to kanamycin, capreomycin and the quinolones (ciprofloxacin and ofloxacin) was noted.
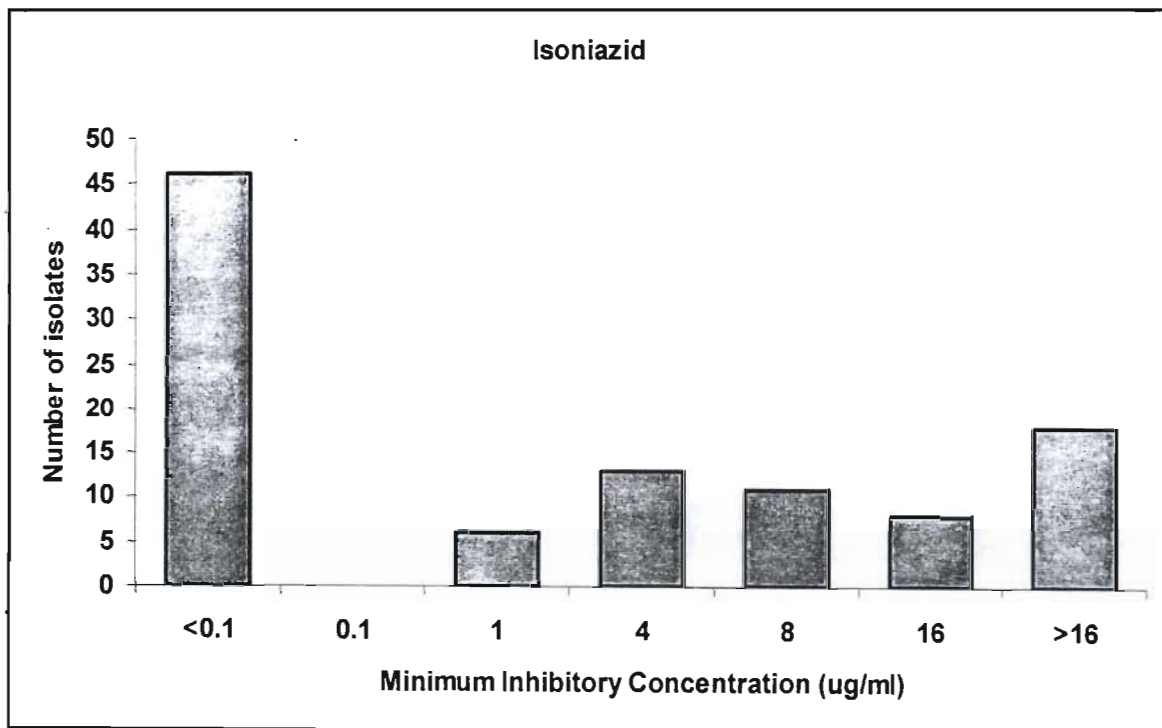
Figure 5.1 - Bar chart of isoniazid minimum inhibitory concentrations for isolates of *Mycobacterium tuberculosis* obtained from multi-drug resistant and drug-sensitive tuberculosis patients (n=102).
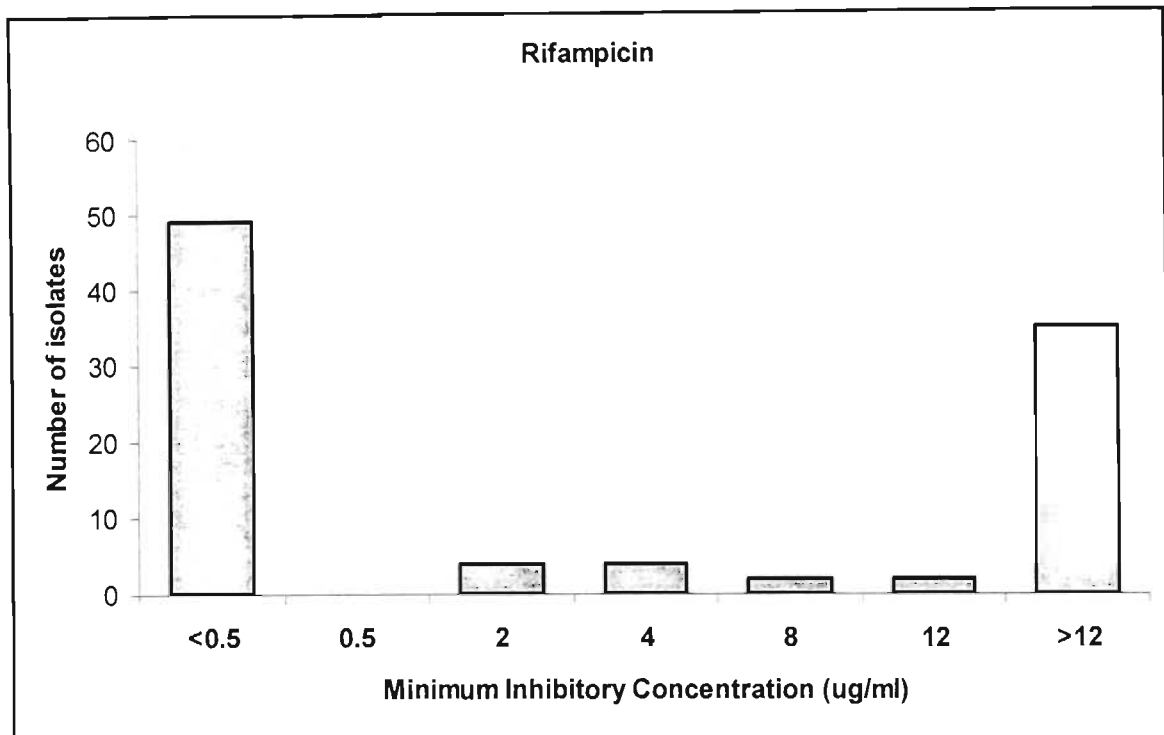
Rifampicin

Figure 5.2 - Bar chart of rifampicin minimum inhibitory concentrations for isolates of *Mycobacterium tuberculosis* obtained from multi-drug resistant and drug-sensitive tuberculosis patients (n=103).

The majority of drug-sensitive TB patients had MICs below the lowest drug concentration tested for both INH (0.1 μg/ml) and RFA (0.5 μg/ml) - Figure 5.1 and Figure 5.2. Similarly, the majority of MDR-TB patients had MICs above the highest concentration tested for both drugs (INH – 16 μg/ml and RFA 12 μg/ml). A mean MIC for INH of 7.2 ± 5.2 μg/ml was calculated among 39 isolates in which a MIC was recorded within the range of breakpoint concentrations tested. In the case of RFA, there were only 12 isolates with a MIC within the range of breakpoint concentrations tested. The mean MIC among these isolates was 5.3 ± 3.8 μg/ml.

Drug Treatment Regimens and Drug Interactions

The majority of the patients with drug-sensitive TB were treated with INH, RFA, pyrazinamide and ethambutol. The same regimen was administered to 12 of the MDR-TB patients who were awaiting drug susceptibility test results at the time of this study, and to the 2 patients whose drug susceptibility status was not classified. Another 2 patients received ethionamide instead of ethambutol.

The MDR-TB patients received a regimen that contained at least pyrazinamide, an aminoglycoside (either streptomycin or kanamycin) and a quinolone (either ciprofloxacin or ofloxacin). MDR-TB patients received between 3 to 6 anti-TB drugs (mean 4.2 ± 0.8) – the other drug(s) in the regimen being ethambutol, ethionamide or thiacetazone.

In all cases, INH and RFA were not part of the anti-TB drug regimen for these patients and were added on for purposes of the present study.

The majority of the anti-TB drugs were given as a single daily dose either before meals (INH and RFA) or after meals (pyrazinamide, ETH, EMB, ciprofloxacin and ofloxacin). The aminoglycosides were administered by intra-muscular injection at a dosing frequency of between 3 to 5 times per week. While most patients who had been prescribed the quinolones received single daily doses, there were some who were given twice daily doses.

Thiacetazone (60 mg per tablet) was administered with INH (133 mg per tablet) in the combination preparation INAT^R and given 3 times a day. The patients who received ethionamide were prescribed a dose of 250 mg 3 times a day.

In most cases, 2 dose levels of anti-TB drugs were prescribed - the patient's body mass of 50 kg dividing the low and the high dose level. Patients weighing less than 50 kg received 300 mg of INH, 450 mg of RFA, 1000 or 1500 mg of pyrazinamide and 500 mg of kanamycin or streptomycin. Those weighing above 50 kg received 400 mg of INH, 600 mg of RFA, 2000 mg of pyrazinamide and 750 or 1000 mg of the aminoglycoside respectively. The dose of ethambutol was between 800 and 1200 mg, ciprofloxacin between 750 and 1000 mg per day and ofloxacin 800 mg per day.

A wide variety of concurrent medications were administered to the subjects in the study as part of their routine care. Table 5.6 records the number of individuals receiving drugs that were considered to have the potential to interfere with the pharmacokinetics of INH and RFA.

Table 5.6 – Concurrent Drug Therapy with the Potential to Interfere with INH and RFA Pharmacokinetics Administered within 48 Hours of the Pharmacokinetic Study Day

Drug	Number (%) of Patients		Effect/Mechanism	Reference
	MDR-TB (n=62)	Drug-Sensitive TB (n=74)		
Enzyme inducers Rifampicin auto-induction *Before maximal RFA auto-induction (≤ 7 days treatment)*	41 (66)	6 (8)	Induction of the cytochrome P450IIIA enzyme system.	*(Kenny and Strates, 1981; Dollery, 1991)*
After maximal RFA auto-induction (> 7 days treatment)	21 (34)	68 (92)		
Carbamazepine	1 (2)	3 (4)	Main effect is to reduce carbamazepine concentrations but INH metabolism may also be increased.	*(Gelman and Rumack, 1998)*
Corticosteroid	1 (2)	1 (1)	Prednisolone may ↓ INH concentrations by 25% in slow acetylators and 40% in fast acetylators	*(Gelman and Rumack, 1998)*
Ketoconazole	0	1 (1)	Main effect is to ↓ ketoconazole concentrations but INH serum concentrations may be ↓ by 25 - 40% and RFA serum concentrations may be ↓ by 40 - 50%	*(Stockley, 1996; Gelman and Rumack, 1998)*
Enzyme inhibitors Ethionamide	28 (45)	2 (3)	INH serum concentrations temporarily increased – not necessarily an enzyme inhibitor effect	*(Gelman and Rumack, 1998)*
Quinolones (ciprofloxacin/ofloxacin)	40 (65)	0	Known inhibitor of microsomal enzymes but no reports of drug interactions with the anti-TB drugs.	
Drugs affecting gastro-intestinal function Antacids	5 (8)	3 (4)	Absorption of INH decreased especially by aluminium containing antacids	*(Stockley, 1996; Gelman and Rumack, 1998)*
Hyoscine butyl bromide	4 (5)	0	Decreased gastrointestinal motility but no reports of interactions with the anti-TB drugs	
Metoclopramide	2 (3)	4 (5)	Increased gastrointestinal motility but no reports of interactions with the anti-TB drugs	

Protocol Deviations

There was a low incidence of protocol deviations in this study. An extra dose of medication was administered to 9 patients on the morning of the second study day. In another patient, the RFA dose was inadvertently omitted.

Liver function tests and radiographs were not specifically performed for this study but formed part of the routine care of the patient. There were 15 patients with no liver function test results in their file and the radiographs of 3 patients were not available at the time of this study.

Non-Compartmental Pharmacokinetic Analysis

DATA

The patients participating in the non-compartmental analysis consisted of 106 patients on RFA and 92 patients on INH.

The excluded patients consisted of 22 patients who had insufficient data to characterise the full pharmacokinetic profile. Nine patients were excluded because they had received additional doses of drug. A further 15 patients who received INAT[R] were excluded from the INH analysis while 1 patient was excluded from the RFA analysis because his dose had been omitted.

A full listing of the dose and serum concentration data for RFA and INH is recorded in Appendix E and F respectively.

RIFAMPICIN

The pharmacokinetic parameters for RFA calculated using non-compartmental methods are recorded in Table 5.7.

MDR-TB patients on RFA had higher AUC and Cmax values and lower CL/F values than patients with drug-sensitive TB ($p < 0.01$) – Table 5.7. This was also noted in the weight and dose corrected AUC and Cmax values. There were no significant differences with the other pharmacokinetic parameters. The MDR-TB patients had recent commencement of RFA (and INH) therapy and were likely to be in a pre-enzyme induced state. After patients had been separated according to the likelihood of maximal RFA enzyme auto-induction, i.e. before and after 7 days of daily treatment, the differences in the parameters were no longer evident.

Table 5.7 – Pharmacokinetic Parameters (mean ± sd) for Rifampicin (before and after auto-induction) in Multi-drug Resistant and Drug-Sensitive Pulmonary Tuberculosis Patients calculated using Non-Compartmental Analysis Methods

| PARAMETER | ALL PATIENTS[1] | MDR-TB | | DRUG-SENSITIVE TB | | ANOVA |
		HIV+	HIV-	HIV+	HIV-	p
All Patients (n=106)						
	n=106	n=16	n=35	n=25	n=27	
Dose (mg/kg)	9.70 ± 1.30	9.52 ± 1.12	9.98 ± 1.24	9.57 ± 1.50	9.55 ± 1.36	0.5 (ns)
AUC (µg.hr/ml)	45.47 ± 24.92	50.37 ± 21.16	57.81 ± 32.27	33.81 ± 13.51	38.32 ± 15.99	<0.001
cAUC (kg.hr/L)	4.74 ± 2.65	5.29 ± 2.18	5.88 ± 3.46	3.56 ± 1.34	4.12 ± 2.02	0.001
Cmax (µg/ml)	8.32 ± 3.92	8.73 ± 2.73	10.20 ± 5.13	7.17 ± 2.41	7.10 ± 2.81	0.02
cCmax (kg/L)	0.87 ± 0.43	0.91 ± 0.26	1.05 ± 0.59	0.74 ± 0.23	0.76 ± 0.33	0.05
Tmax (hr)	2.32 ± 1.34	1.88 ± 0.34	2.42 ± 0.93	1.99 ± 0.78	2.46 ± 1.86	0.27 (ns)
$t_\frac{1}{2}$ (hr)	2.20 ± 0.56	2.53 ± 0.26	2.26 ± 0.56	2.02 ± 0.62	2.10 ± 0.68	0.03 (ns)
CL/F (L/hr)	14.24 ± 9.15	12.79 ± 9.58	11.42 ± 6.47	16.19 ± 7.35	17.00 ± 12.41	0.003
V/F (L)	43.81 ± 33.33	45.54 ± 30.91	37.13 ± 26.11	45.20 ± 21.25	50.97 ± 49.85	0.09 (ns)
Patients on rifampicin treatment for < 7 days (n=38)						
	n=38	n=9	n=25	n=1	n=2	
Dose (mg/kg)	9.79 ± 1.29	9.58 ± 1.24	9.95 ± 1.31	10.79	9.16; 7.30	0.27 (ns)
AUC (µg.hr/ml)	64.04 ± 28.85	60.22 ± 18.19	65.60 ± 33.72	77.80	42.06; 65.64	0.89 (ns)
cAUC (kg.hr/L)	6.63 ± 3.12	6.34 ± 1.90	6.69 ± 3.66	7.20	4.59; 9.00	0.99 (ns)
Cmax (µg/ml)	10.97 ± 4.57	9.86 ± 2.14	11.29 ± 5.45	13.30	12.47; 10.87	0.83 (ns)
cCmax (kg/L)	1.15 ± 0.54	1.03 ± 0.2	1.17 ± 0.64	1.20	1.36; 1.49	0.80 (ns)
Tmax (hr)	2.16 ± 0.70	1.96 ± 0.31	2.27 ± 0.81	2.20	2.12; 1.15	0.48 (ns)
$t_\frac{1}{2}$ (hr)	2.34 ± 0.43	2.57 ± 0.19	2.29 ± 0.47	2.50	1.33; 2.46	0.19 (ns)
CL/F (L/hr)	9.15 ± 4.07	8.76 ± 2.72	9.34 ± 4.64	5.80	13.49; 9.14	0.74 (ns)
V/F (L)	30.99 ± 16.58	32.57 ± 11.28	31.23 ± 19.32	20.70	25.80; 32.43	0.93 (ns)
Patients on rifampicin treatment for ≥ 7 days (n=68)						
	n=68	n=6	n=10	n=24	n=25	
Dose (mg/kg)	9.65 ± 1.31	9.45 ± 1.03	10.04 ± 1.13	9.52 ± 1.51	9.65 ± 1.33	0.75 (ns)
AUC (µg.hr/ml)	35.09 ± 14.45	41.89 ± 16.33	38.33 ± 17.71	31.97 ± 10.14	37.07 ± 15.60	0.4 (ns)
cAUC (kg.hr/l)	3.68 ± 1.58	3.93 ± 1.79	3.85 ± 1.80	3.40 ± 1.12	3.91 ± 1.84	0.69 (ns)
cCmax (kg/L)	0.71 ± 0.26	0.74 ± 0.25	0.75 ± 0.30	0.73 ± 0.21	0.71 ± 0.28	0.97 (ns)
Cmax (µg/ml)	6.84 ± 2.53	8.02 ± 2.35	7.44 ± 2.94	6.92 ± 2.09	6.73 ± 2.58	0.77 (ns)
Tmax (hr)	2.41 ± 1.58	1.77 ± 0.41	2.78 ± 1.16	1.98 ± 0.80	2.53 ± 1.92	0.38 (ns)
$t_\frac{1}{2}$ (hr)	2.12 ± 0.65	2.47 ± 0.34	2.2 ± 0.76	2.00 ± 0.62	2.12 ± 0.68	0.39 (ns)
CL/F (l/hr)	17.09 ± 9.95	13.54 ± 5.74	16.63 ± 7.63	16.63 ± 7.17	17.45 ± 12.78	0.70 (ns)
V/F (l)	50.98 ± 37.99	48.11 ± 17.36	51.86 ± 35.26	46.22 ± 21.07	52.71 ± 51.46	0.95 (ns)

ANOVA = analysis of variance; AUC = area under the serum concentration versus time curve; cAUC = weight and dose corrected AUC; Cmax = serum concentration at tmax; cCmax = weight and dose corrected Cmax; tmax = time at which maximum serum concentration is achieved; $t_\frac{1}{2}$ = serum half-life; CL/F = apparent clearance; V/F = apparent volume of distribution

[1]The total number of MDR-TB and Drug-sensitive TB patients does not add up to total number of patients included in the study. This is because the drug susceptibility status of 2 patients (1 HIV+ and 1 HIV-) could not be classified as they were found to be resistant to INH alone. The HIV status of 1 drug-sensitive patient could not be determined as the patient withdrew consent for HIV testing after recruitment into the study.

ISONIAZID

The results of the non-compartmental pharmacokinetic analysis for INH are recorded in Table 5.8. The HIV+, MDR-TB patients had higher AUC and Cmax values than the patients from the other 3 groups (p ≤ 0.03). These patients also received a higher mean dose but this was not significantly higher than that received by patients in the other 3 groups. Using an INH half-life of 2 hours as the boundary between the slow and fast acetylators *(Gelman and Rumack, 1998)*, there were 7 (8%) slow acetylators and 85 (92%) fast acetylators in the present study.

Table 5.8 – Pharmacokinetic Parameters (mean ± sd) for Isoniazid in Multi-drug Resistant and Drug-Sensitive Pulmonary Tuberculosis Patients calculated using Non-Compartmental Analysis Methods

| PARAMETER | ALL PATIENTS (n = 92)[1] | MDR-TB | | DRUG-SENSITIVE TB | | ANOVA p |
		HIV+ n=13	HIV- n=24	HIV+ n=25	HIV- n=27	
Dose (mg/kg)	6.53 ± 1.23	7.23 ± 2.09	6.61 ± 0.96	6.41 ± 1.15	6.24 ± 0.94	0.2 (ns)
AUC (µg.hr/ml)	34.45 ± 17.83	52.03 ± 29.91	32.40 ± 12.37	29.65 ± 11.16	33.47 ± 15.78	0.03
cAUC (kg.hr/L)	5.26 ± 2.20	7.02 ± 2.32	5.01 ± 2.00	4.67 ± 1.75	5.36 ± 2.37	0.03
Cmax (µg/ml)	6.74 ± 2.26	8.59 ± 2.42	6.75 ± 2.28	6.32 ± 1.50	6.53 ± 2.37	0.02
cCmax (kg/L)	1.05 ± 0.36	1.23 ± 0.31	1.06 ± 0.44	0.99 ± 0.23	1.05 ± 0.36	0.28 (ns)
Tmax (hr)	1.35 ± 0.55	1.26 ± 0.46	1.43 ± 0.48	1.33 ± .068	1.33 ± 0.48	0.81 (ns)
$t_{\frac{1}{2}}$ (hr)	3.17 ± 0.88	3.55 ± 1.07	3.15 ± 0.91	3.10 ± 0.66	3.11 ± 0.99	0.47 (ns)
CL/F (L/hr)	12.39 ± 5.28	8.56 ± 3.76	12.95 ± 4.33	13.23 ± 5.10	12.87 ± 6.16	0.05 (ns)
V/F (L)	55.28 ± 26.40	39.86 ± 13.69	57.38 ± 20.55	59.83 ± 27.83	56.22 ± 31.10	0.09 (ns)

ANOVA = analysis of variance; AUC = area under the serum concentration versus time curve; cAUC = weight and dose corrected AUC; Cmax = serum concentration at tmax; tmax = time at which maximum serum concentration is achieved; $t_{\frac{1}{2}}$ = serum half-life; CL/F = apparent clearance; V/F = apparent volume of distribution

[1]The total number of MDR-TB and Drug-sensitive TB patients does not add up to total number of patients included in the study. This is because the drug susceptibility status of 2 patients (1 HIV+ and 1 HIV-) could not be classified as they were found to be resistant to INH alone. The HIV status of 1 drug-sensitive patient could not be determined as the patient withdrew consent for HIV testing after recruitment into the study.

Population Pharmacokinetic Analysis

DATA

All 138 patients participated in the population pharmacokinetic analysis for INH while 1 patient was excluded from the RFA analysis because his dose had been omitted.

A full listing of the dose and serum concentration data for RFA and INH is recorded in Appendix E and F respectively.

Missing Data

The drug susceptibility status of 2 of the 138 patients could not be classified as they were found to be resistant to INH alone. Another patient's HIV status could not be determined as the patient withdrew her consent for HIV testing after recruitment into the study. The former 2 patients were regarded as drug sensitive and the latter patient as HIV- for purposes of the population pharmacokinetic analysis.

There were 15 patients with no liver function test results in their files. There was no record of any clinical features of liver function abnormality in these patients. The radiographs of 3 patients were not available at the time of the study and thus a radiographic severity score in these patients could not be obtained. In all these cases, the missing data was recorded as the group median value.

Separate NONMEM runs were conducted in which the 15 patients with missing liver function test results were either included (with the missing data recorded as the group median values) or excluded from the dataset. In these runs the influence on the decision to include or exclude the relevant covariate from the model was evaluated.

In the case of INH, there was no significant difference in the OBJ for these 2 alternate scenarios. For RFA, there were significant differences in OBJ when the missing liver function test results were recorded as the group median as opposed to excluding the relevant subjects. However, the decision to include or exclude the relevant covariate was not altered.

RIFAMPICIN

Basic Structural Pharmacokinetic Model

The models tested in the development of the basic structural pharmacokinetic model for RFA are shown in Table 5.9. The complete list of NONMEM runs conducted during the analysis is recorded in Appendix G. An example of a NONMEM control stream used during the RFA analysis is shown in Appendix H.

Table 5.9 - Development of the Basic Structural Pharmacokinetic Model for the Population Pharmacokinetic Analysis of Rifampicin in Pulmonary Tuberculosis Patients (n=137)

RUN	MODEL	INTER-INDIVIDUAL VARIABILITY				RESIDUAL VARIABILITY		OBF	COMMENTS
		η_{KA} %	$\eta_{CL/F}$ %	$\eta_{V/F}$ %	η_{TLAG} %	σ_{CCV} %	σ_{ADD} µg/ml		
3	One compartment model with first order absorption	60	51	<1	-	45.6	0.53	1474.664	Model was parameterised as KA, CL/F and V/F with exponential error terms on the inter-individual variabilty in the parameters. Residual variability was modelled with combined additive and constant coefficient of variation error terms. Model is under-predicting but appears concentrated at PRED=11. Outliers were identified.
9	One compartment model with first order absorption	54	53	19	-	49.2	0.05	863.349	Removed data points for patient 122 and 123 that was swapped during sample preparation. No difference with respect to concentration of points now at PRED=7.5
14	One compartment Model with zero order absorption	-	55	29	32	49	0.05	860.823	Implemented zero order bolus input and the duration parameter was estimated. Concentration of points at PRED=7.5 a little better.
15	One compartment model with first order absorption	57	51	24	59	41	0.05	804.652	Introduction of a lag time. Model predictions are much better. The OBF value from this run was used as the reference against which all subsequent runs were compared in the univariate covariate addition process. Concentration of points at PRED=7.5 gone.
27	Two compartment Model with first order absorption	51	53	18	-	50	0.05	858.735	Although the OBF is smaller than RUN 13, goodness of fit plots are not any better than with the one compartment model. The variability in parameter estimates are also not very much better.
42	One compartment model with first order absorption	47	42	<1	69	37	0.05	773.334	Estimated F1 – relative bioavailability for the 2 dose amounts i.e. 450mg and 600mg dose. Data can no longer estimate the variability in V/F once it is partitioned into F1.

η - inter-individual variability; KA – absorption rate constant, CL/F – apparent clearance, V/F – apparent volume of distribution, σ - intra-individual variability, CCV – constant coefficient of variation, ADD – additive, OBF – minimum value of the objective function

A one-compartment linear model parameterised in clearance (CL/F) and volume of distribution (V/F), was chosen to describe the pharmacokinetics of RFA in this population (Figure 5.3). The absorption profile was characterised by a first order process with an absorption lag time (TLAG). The sensitivity analysis procedure conducted on various fixed values of KA confirmed that the NONMEM estimate (0.7 hour^{-1}) was appropriate since the lowest value of OBF corresponded to the same fixed value of 0.7 hour^{-1}.

Initial indications were that there was a difference in the relative bioavailability (F) of the 2 different dose amounts (450mg and 600mg) of RFA. However, this model was not selected as the base model for examination of covariate effects in view of the inability to estimate any variability in V/F after inclusion of the F term.
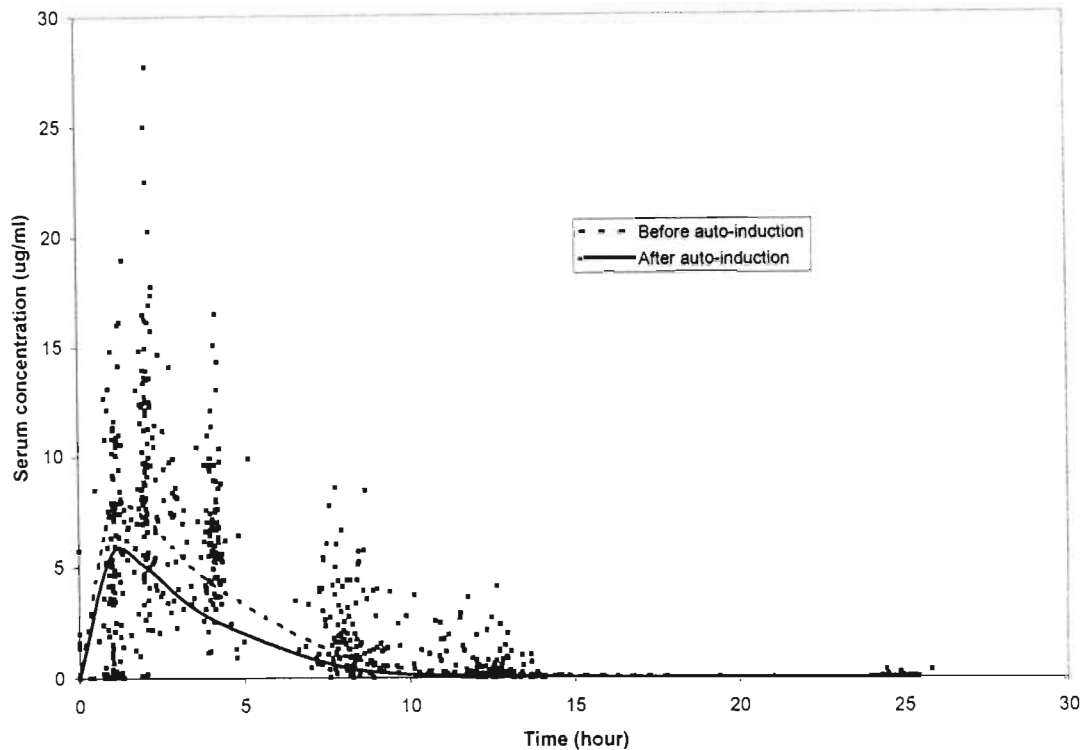
Figure 5.3 – Time course of rifampicin serum concentrations before (broken line) and after maximal enzyme auto-induction (solid line). The scatter of data points shows the rifampicin concentrations measured in 137 pulmonary tuberculosis patients. All serum concentration values have been normalised to a 600 mg dose.

Covariate Model Development

The univariate analysis of RFA identified 23 potentially important covariates with DOBFs of ≥ 3.84 (Table 5.10 and Appendix I). The effect of RFA auto-induction on CL/F caused the largest decrease in the OBF compared to the basic model. Several other covariates that also caused large DOBFs during the univariate analysis were found to be confounded e.g. patients on quinolones and ethionamide were also those who had MDR-TB. The MDR-TB patients were most likely to have received RFA for a period of time insufficient to allow for maximal RFA enzyme auto-induction. These covariates were excluded during the forward model building stage (Table 5.10), as they did not cause a significant DOBF of ≥ 3.84 when added into the model that already included the effect of RFA auto-induction.

Table 5.10 - Development of the Covariate Pharmacokinetic Model for the Population Pharmacokinetic Analysis of Rifampicin in Pulmonary Tuberculosis Patients (n=137) – Univariate Analyses and Step-wise Build-up to the Full Model

Significant Covariate Effects Identified During the Univariate Analysis in rank order of DOBF[1]	DOBF[1] during Step-wise Addition of covariates	Covariate Retained in Full Model
Rifampicin auto-induction on CL/F	74.5	Yes
Quinolones on CL/F	0.7	No
Enzyme inhibitor on CL/F	+0.3	No
MDR-TB on CL/F	3.2	No
Ethambutol on CL/F	0.2	No
Severity score for extent of lung cavitation on CL/F	2.1	No
Log HIV viral load on V/F	19.5	Yes
HIV on V/F	0.7	No
Weight on CL/F	58.1	Yes
Globulin on CL/F	4.4	Yes
Thiacetazone on CL/F	0.2	No
Globulin on V/F	2.0	No
Sex on V/F	4.4	Yes
Iron preparations/anaemia on CL/F	2.0	No
Drug resistance severity score on CL/F	2.4	No
Rifampicin auto-induction on V/F	9.3	Yes
Age on CL/F	2.3	No
Enzyme inhibitor on V/F	+0.4	No
Iron preparations/anaemia on V/F	1.2	No
HIV on CL/F	2.1	No
Aspartate transaminase (AST) on CL/F	0.5	No
Non-steroidal anti-inflammatory drug use on CL/F	1.3	No
Log HIV viral load on CL/F	1.1	No

[1]DOBF – difference in the minimum value of the objective function between 2 NONMEM runs. Chi square distributed – DOBF ≤ 3.84, p ≤ 0.05 df=1, CL/F – apparent clearance, V/F – apparent volume of distribution

The forward model building stage retained only 6 out of the 23 covariates for inclusion in the full model. These were the effect of RFA auto-induction on CL/F and on V/F, log HIV viral load on V/F, weight on CL/F, globulin on CL/F and sex on V/F. Several possible splits for the time to maximal RFA enzyme auto-induction were investigated. These were 7 and 14 days as suggested by the literature *(Kenny and Strates, 1981)*, 4.5 days as suggested by tree-based modelling, 10 days as suggested by the GAM analysis and an arbitrary value of 30 days. Tree based modelling and GAM analyses are options available within the Xpose[R] program that assist in selecting important covariates in a NONMEM model. The split of 10 days produced the best fit of the data to the model.

It was noted during the forward model building stage that several parameters had significant effects on both CL/F and V/F. The covariance between these 2 parameters

was therefore modelled by implementation of a BLOCK OMEGA rather than a DIAGONAL OMEGA before the backward deletion stage.

The 6 covariates included in the full model were deleted one-at-a-time and their relative importance was ranked according to the size of the DOBF caused by their deletion. Covariates were removed from the full model according to this rank order as recorded in Table 5.11. During the backward deletion of covariates, only 2 covariates caused an increase of ≥ 11 in DOBF thereby confirming their retention in the final model. These were the effect of RFA auto-induction on both CL/F and on V/F.

Table 5.11 - Development of the Covariate Pharmacokinetic Model for the Population Pharmacokinetic Analysis of Rifampicin in Pulmonary Tuberculosis Patients (n=137) – Step-wise Deletion to the Final Model

Model	DOBF[1]	Comments
CL/F = f(DAYS-10;WT;GLOB) V/F = f(DAYS-10;VIR;SEX)	-	Full Model
CL/F = f(DAYS-10;WT) V/F = f(DAYS-10;VIR;SEX)	6.9	Globulin does not significantly influence CL/F
CL/F = f(DAYS-10;WT) V/F = f(DAYS-10;VIR)	6.1	Sex does not significantly influence V/F
CL/F = f(DAYS-10;WT) V/F = f(DAYS-10)	6.8	Log HIV viral load does not significantly influence V/F
CL/F = f(DAYS-10;WT)	45.8	Rifampicin auto-induction has a significant influence on V/F
CL/F = f(DAYS-10) V/F = f(DAYS-10)	8.4	Weight does not significantly influence CL/F
V/F = f(DAYS-10)	76	Rifampicin auto-induction has a significant influence on CL/F

[1]DOBF – difference in the minimum value of the objective function between 2 NONMEM runs. Chi square distributed – DOBF ≥ 11, p ≤ 0.001 df=1, CL/F – apparent clearance, V/F – apparent volume of distribution, f – 'a function of ...', DAYS-10 – number of days since starting rifampicin therapy with a minimum of 0 days and a maximum of 10 days, WT – patient weight centred on the median of 54 kg, GLOB – serum globulin concentration centred on the median of 52 g/L, VIR – log HIV viral load, SEX – male or female sex

Figure 5.4 compares plots of weighted residuals for the basic model with that for the final optimal model. The fit for the final model is shown to be superior with no biased clustering of data points.

The final regression model for the typical values of CL/F and V/F for RFA was as follows:

CL/F = 15.60 + 0.79 x (DAYS - 10)
V/F = 42.10 + 1.57 x (DAYS - 10)

Where DAYS - 10 is the number of days since starting RFA therapy with a minimum of 1 day and a maximum of 10 days.

Rifampicin: Basic Model

Rifampicin: Final Model
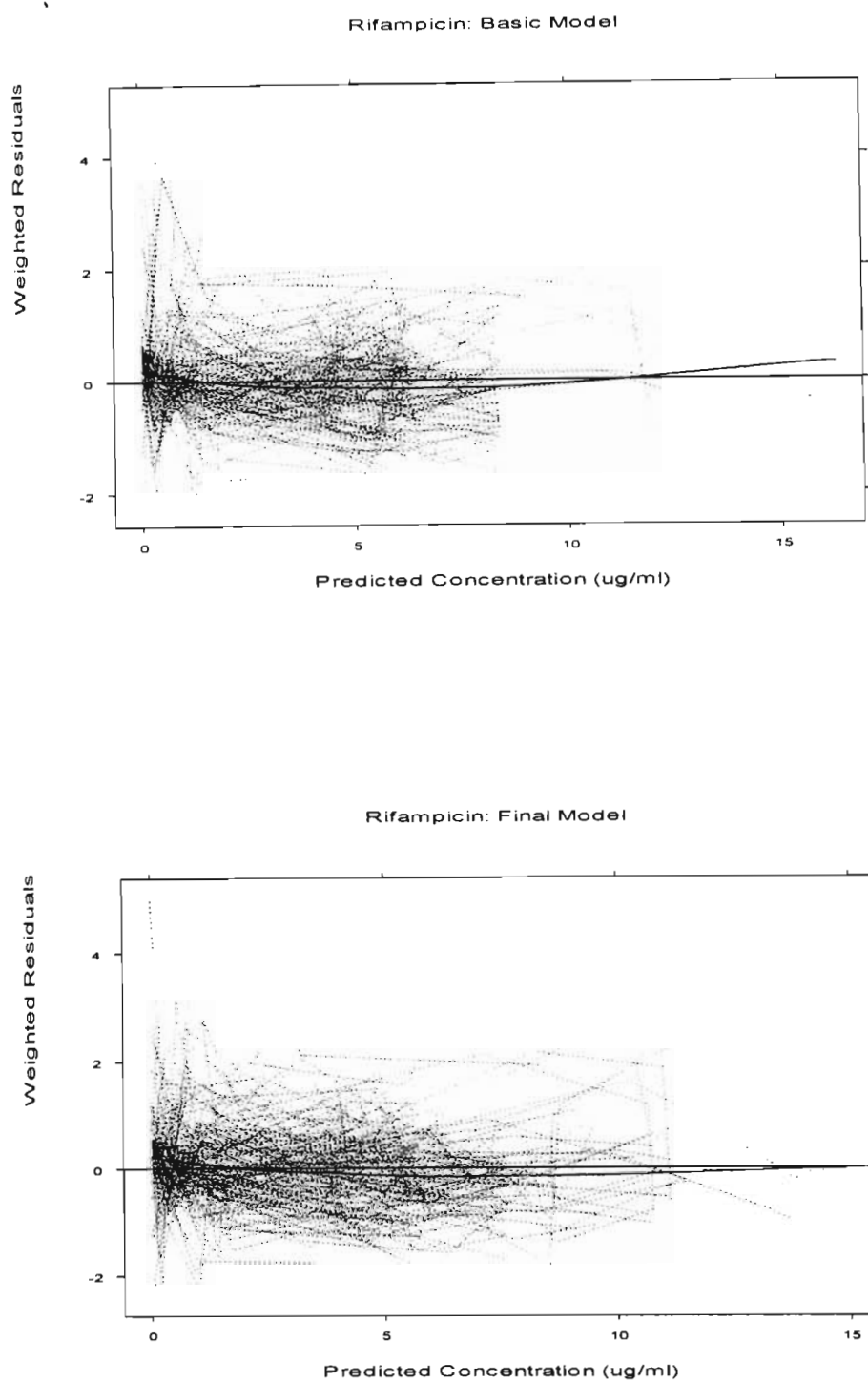
Figure 5.4 – Assessment of goodness of fit: Plots of weighted residuals versus predicted rifampicin concentrations from the basic (top) and the final (bottom) NONMEM models. The individual lines connect the concentration points measured in 137 patients with pulmonary tuberculosis.

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Table 5.12 – Population Pharmacokinetic Parameters for Rifampicin in Pulmonary Tuberculosis Patients (n=137) .

PARAMETER	COVARIATE INFLUENCE	MEAN POPULATION PARAMETER ESTIMATE (CI)	RELATIVE STANDARD ERROR %
CL/F (L/hr)	At commencement of rifampicin therapy	7.70 (6.20,9.20)	18
	After 10 days of daily rifampicin therapy	15.60 (14.10,17.10)	5
Inter-individual Variability in CL/F (%CV)		39 (29, 47)	23
V/F (L)	At commencement of rifampicin therapy	26.40 (20.64, 32.16)	30
	After 10 days of daily rifampicin therapy	42.10 (36.34, 47.86)	7
Inter-individual Variability in V/F (%CV)		26 (0, 43)	100
KA (hr^{-1})		0.89 (0.59,1.19)	17
Inter-individual Variability in KA (%CV)		52 (0, 73)	51
TLAG (hr)		0.50 (0.35, 0.65)	15
Inter-individual Variability in TLAG (%CV)		69 (0, 102)	60
Residual (intra-individual) Variability			
Proportional error (%CV)		39 (34, 45)	7
Additive (µg/ml)		0.05 (0.03, 0.07)	19

KA – absorption rate constant, CL/F – apparent clearance, V/F – apparent volume of distribution, TLAG – absorption lag time; CI – 95% confidence interval; %CV – percent coefficient of variation

ISONIAZID

Basic Structural Pharmacokinetic Model

Various models were tested in order to determine the basic structural pharmacokinetic model for the INH population pharmacokinetic analysis (Table 5.13). The complete list of NONMEM runs conducted during the analysis is recorded in Appendix J. An example of a NONMEM control stream used during the INH analysis is shown in Appendix K.

Table 5.13 – Summary of Models tested in the Development of the Basic Structural Model for the Population Pharmacokinetic Analysis of Isoniazid in Pulmonary Tuberculosis Patients (n=138).

RUN	MODEL	INTER-INDIVIDUAL VARIABILITY				RESIDUAL VARIABILITY		OBF	COMMENTS
		η_{KA} (%)	$\eta_{CL(1)/F}$ (%)	$\eta_{CL(2)/F}$ (%)	η_V (%)	σ_{CCV} (%)	σ_{ADD} (μg/ml)		
2	One compartment Model with first order absorption	2102	27	-	15	23	0.5	851.055	Model was parameterised as KA, CL/F and V/F with exponential error terms on the inter-individual variability in the parameters. Residual variability was modelled with combined additive and constant coefficient of variation error terms. KA estimate and variability are very large.
9	One compartment Model with first order absorption	118	45	-	36	18	0.5	882.867	Results of the sensitivity analysis procedure with KA fixed to 3.0. Variability on KA is more realistic than RUN 2.
17	One compartment Model with zero order absorption	-	45	-	34	23	0.53	901.258	Duration parameter for drug absorption is unrealistically small.
19	Two compartment Model with first order absorption	68	52	-	37	20	0.46	800.274	2 compartment model. Although the OBF is smaller, goodness of fit plots are not any better than with RUN 9.
23	One compartment Model with first order absorption	127	43	43	43	29	0.02	289.890	Implemented Mixture modelling on CL/F. Significant ↓ in OBF. Lag time on absorption.
32	One compartment Model with first order absorption	146	43	38	43	29	0.02	289.640	Results of the sensitivity analysis procedure with Ka fixed to 3.5 and no lag time. The OBF value from this run was used as the reference against which all subsequent runs were compared in the univariate covariate addition process.

η - inter-individual variability; KA – absorption rate constant, CL/F – apparent clearance; CL(1)/F– apparent clearance (fast), CL(2)/F – apparent clearance for slow acetylators, V/F – apparent volume of distribution, σ - intra-individual variability, CCV – constant coefficient of variation, ADD – additive, OBF – minimum value of the objective function

A one-compartment linear model parameterised via clearance (CL/F) and volume of distribution (V/F), was chosen to describe the pharmacokinetics of INH in this population. Implementation of the Mixture Modelling feature of NONMEM (Appendix L) resulted in a dramatic decrease in the OBF (-593). The distribution of individual

Bayesian *posthoc* CL/F values before and after implementation of mixture modelling is shown in Figure 5.5 and 5.6 respectively.

The non-compartmental analysis suggested that the absorption of INH (tmax=1.35 hour) was much more rapid than RFA (tmax=2.32 hour). Further, the model building process to determine the best pharmacokinetic structural model for INH was characterised by a very large inter-individual variability in KA and an occasional shifting of this variability into the inter-subject variability in V. These observations emphasized that there were insufficient samples taken during the absorption phase to accurately estimate the absorption characteristics of INH.

In view of this, an estimate of KA was determined and fixed using the sensitivity analysis procedure. In this procedure, KA was fixed to various values and the OBF's and parameter estimates obtained were compared. This was with a view to determining a value of KA that resulted in stable parameter estimates and/or the lowest OBF. In all subsequent runs the KA was constrained to a value of 3.5 hr^{-1}.

In 7 patients, the pre-dose INH serum concentration was found to be higher than the last concentration measured approximately 12 hours previously (mean 0.98 vs 0.025 µg/ml, respectively). There was no evidence to suggest that any doses had been administered during that interval. The aberrant higher concentrations were attributed to assay error and were deleted from the data file as their retention caused the Bayesian estimate for V/F to be very large ($\geq$ 120 L) in these patients.
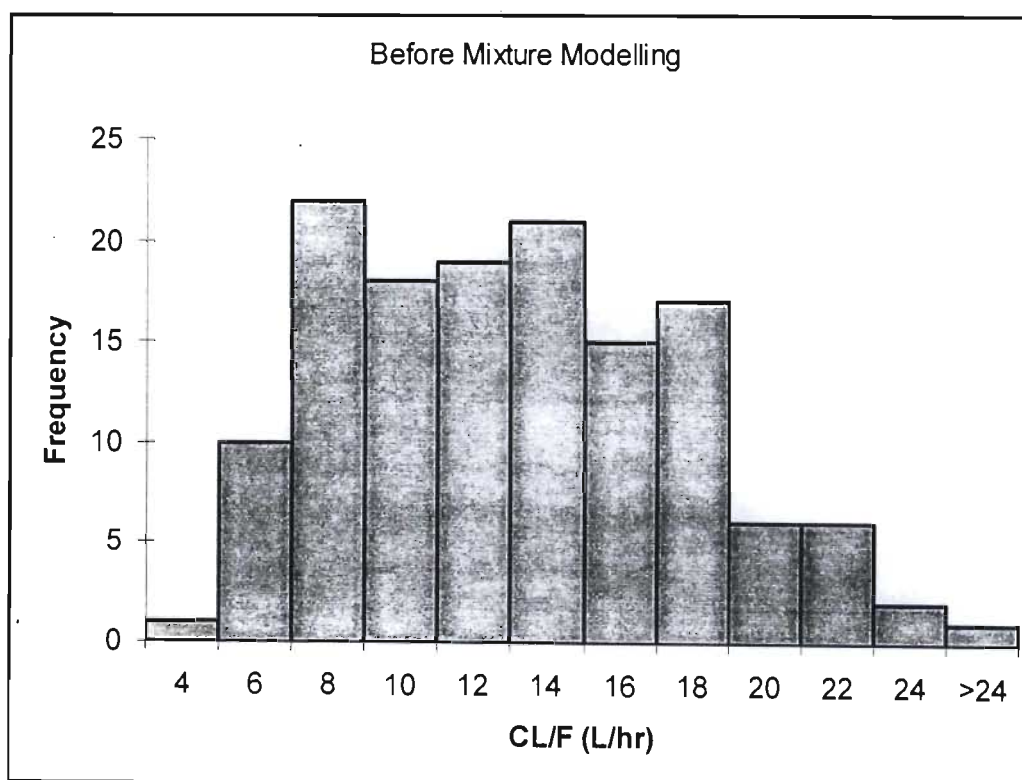
Figure 5.5 – Histogram of Bayesian posthoc individual clearance values (L/hr) for isoniazid in patients with pulmonary tuberculosis (n=138) before implementation of NONMEM's mixture modelling.
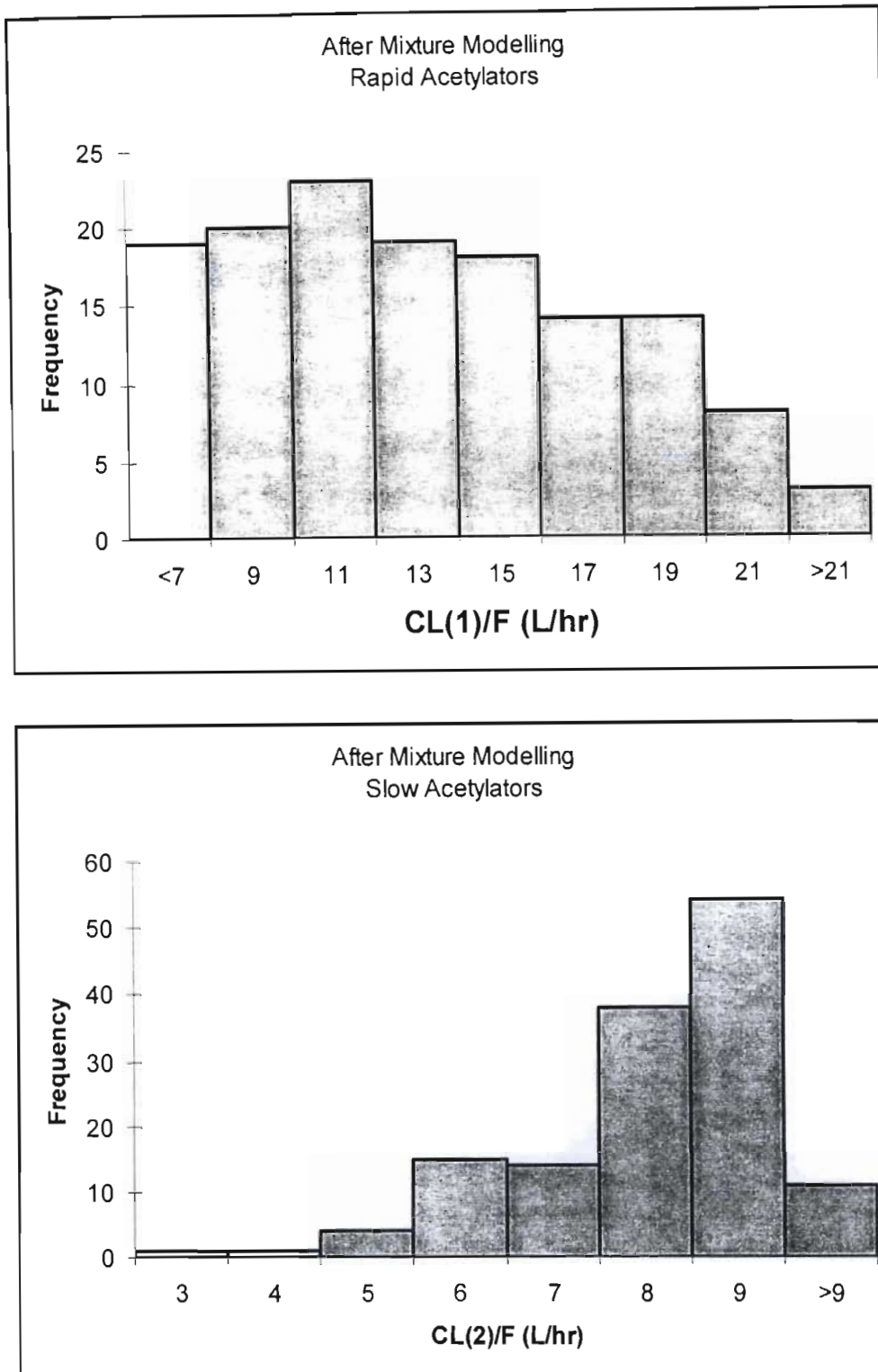
Figure 5.6 Histogram of Bayesian posthoc individual clearance values for fast (top) and slow (bottom) acetylators of isoniazid in patients with pulmonary tuberculosis *after* implementation of NONMEM's mixture modelling.

Covariate Model Development

A total of 28 covariates were tested in the univariate evaluation of potentially important covariate effects on INH CL/F and V/F. These covariates have been described under the section labelled Data in this Chapter and their effects on the OBF are recorded in Appendix M. Of these covariates, 16 were identified for possible inclusion in the final model. They are listed in the first column of Table 5.14 in rank order of the decrease in OBF caused by their univariate addition into the model.

Table 5.14 - Development of the Covariate Pharmacokinetic Model for the Population Pharmacokinetic Analysis of Isoniazid in Pulmonary Tuberculosis Patients (n=138) – Univariate Analyses and Step-wise Build-up to the Full Model

Significant Covariate Effects Identified During the Univariate Analysis in rank order of DOBF[1]	DOBF[1] during Step-wise Addition of covariates	Covariate Retained in Full Model
Globulin on CL/F	11.6	Yes
Enzyme inhibitors on CL/F	3.2	No
Antihistamines on CL/F	4.4	Yes
Log Viral Load on CL/F	+0.2	No
Penicillins on V/F	5.7	Yes
Hypoglycaemic agents/Diabetes mellitus on CL/F	1.1	No
Severity score for extent of lung involvement on CL/F	6.7	Yes
Severity score for extent of lung involvement on V/F	0.9	No
Drug resistance severity score on CL/F	0.2	No
Penicillins on CL/F	0	No
Weight on V/F	+0.7	No
Globulin on V/F	0.3	No
Diuretics/hypertension on CL/F	7.2	Yes
Total Bilirubin on V/F	5.0	Yes
Sex on CL/F	+10.1	No
Severity score for extent of lung cavitation on V/F	+11.0	No

[1]DOBF – difference in the minimum value of the objective function between 2 NONMEM runs. Chi square distributed – DOBF ≤ 3.84, p ≤ 0.05 df=1, CL/F – apparent clearance, V/F – apparent volume of distribution

In the stepwise buildup to the full model, only 6 covariates were retained. These were the effect of serum globulin, concurrent antihistamine treatment; concurrent anti-hypertensive treatment and extent of lung involvement on CL/F, and the effect of total serum bilirubin concentration and concurrent penicillin treatment on V/F.

These covariates were removed from the full model one-at-a-time. The consequent effect on the OBF formed the basis of developing the rank order for their removal from the full model (Table 5.15). During this backward deletion procedure, none of the covariates caused an increase in DOBF of ≥ 11. Although globulin caused a marginally significant increase in DOBF of 10.9, it was retained in the final model.

Table 5.15 - Development of the Covariate Pharmacokinetic Model for the Population Pharmacokinetic Analysis of Isoniazid in Pulmonary Tuberculosis Patients (n=138) – Step-wise Deletion to the Final Model

Model	DOBF[1]	Comments
CL/F = f(GLOB;H1B;EXT;HPT) V/F = f(PEN;BILI)	-	Full Model
CL/F = f(GLOB;EXT;HPT) V/F = f(PEN;BILI)	1.5	Concomitant antihistamine therapy does not influence isoniazid clearance
CL/F = f(GLOB;HPT) V/F = f(PEN;BILI)	3.3	Severity score for extent of lung involvement does not influence CL/F
CL/F = f(HPT) V/F = f(PEN;BILI)	10.9	Serum globulin concentration has a marginally significant influence on CL/F
CL/F = f(GLOB;HPT) V/F = f(PEN)	0.1	Total serum bilirubin concentration does not significantly influence V/F
CL/F = f(GLOB) V/F = f(PEN)	7.2	Concomitant antihypertensive therapy does not influence isoniazid CL/F
CL/F = f(GLOB)	6.3	Concomitant penicillin therapy does not influence isoniazid V/F

[1]DOBF – difference in the minimum value of the objective function between 2 NONMEM runs. Chi square distributed – DOBF ≥ 11, p ≤ 0.001 df=1, CL/F – apparent clearance, V/F – apparent volume of distribution, f – 'a function of ...', GLOB – serum globulin concentration centred on the median of 52 g/L, H1B – ingestion of concomitant antihistamine therapy, EXT – severity score for extent of lung involvement centred on the median of 5, HPT – ingestion of concomitant anti-hypertensive drug therapy, PEN - ingestion of concomitant penicillin therapy, BILI – total serum bilirubin concentration centred on the median of 10 μmol/L

Figure 5.7 compares plots of weighted residuals for the basic model with that for the final model. The fit of the final model is shown to be marginally better than the basic model. In particular, the absence of a clustering of data points at a predicted concentration of 7.5 μg/ml is noted.

Isoniazid: Basic Model

Isoniazid: Final Model
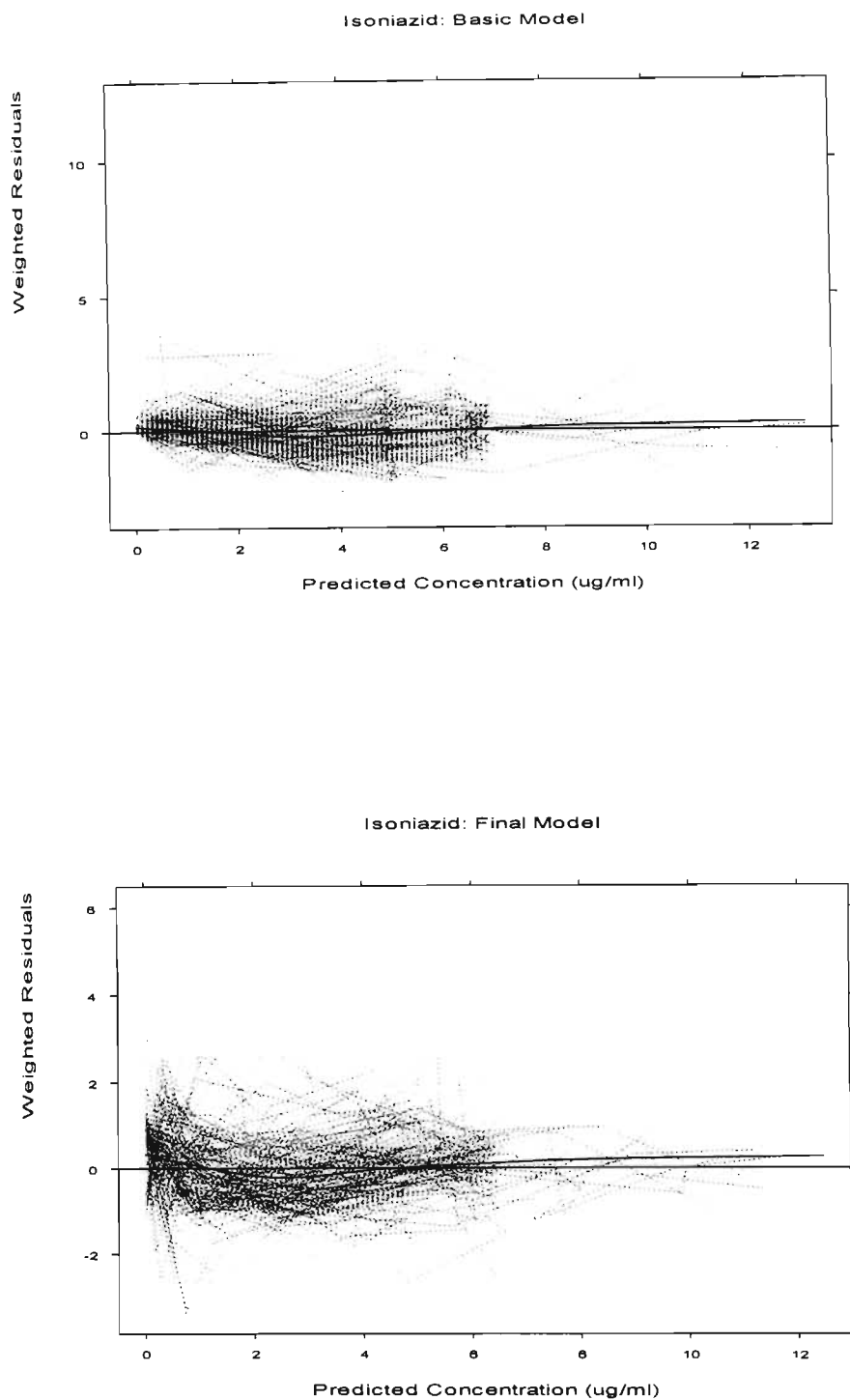
Figure 5.7 – Assessment of goodness of fit: Plots of weighted residuals versus predicted isoniazid concentrations for the basic (top) and the final (bottom) NONMEM models. The individual lines connect the concentration points measured in 138 patients with pulmonary tuberculosis.

Table 5.16 records the final parameter estimates for the INH population pharmacokinetic analysis.

The estimate of the mixing proportion indicates that this population comprised primarily of fast acetylators (85%). Among the fast acetylators there were 50 (43%) patients with MDR-TB and 66 (57%) with drug-sensitive TB. The number of MDR-TB and drug-sensitive TB patients among the slow acetylator group was 12 (60%) and 8 (40%) respectively. There was no significant difference between fast and slow acetylators with respect to the proportions of MDR-TB and drug-sensitive TB patients (p=0.16).

The slow acetylators had a 35% lower rate of metabolism than the fast acetylators. The time course of INH serum concentrations for the slow and fast acetylators is shown in Figure 5.8.

The small 95% confidence interval for error model parameters and the low RSE confirmed that the combined additive and CCV error model was appropriate for this data.

Table 5.16 – Population Pharmacokinetic Parameters for Isoniazid in Pulmonary Tuberculosis Patients (n=138).

Parameter	Acetylator Status	Population Parameter Estimate mean (CI)	Relative Standard Error (%)
Mixing Proportion	Fast	0.85 (0.78, 0.91)	4
CL/F (litre/hour)			
	Slow	4.73 (2.78, 6.98)	18
	Fast	13.00 (11.88, 14.12)	4
Slope for globulin – 52		0.09 (0.04, 0.13)	26
Inter-individual variability in CL/F (%)	Slow	32 (11, 44)	18
	Fast	41 (35, 46)	44
V/F (litre)		50.00 (45.52, 54.48)	5
Inter-individual variability in V/F (%)		41 (33, 48)	18
KA (hour^{-1})			
Inter-individual variability in KA (%)		152 (61, 207)	42
Residual Variability			
Proportional error (%)		28 (24, 31)	6
Additive error (µg/ml)		0.02 (0.018, 0.029)	12
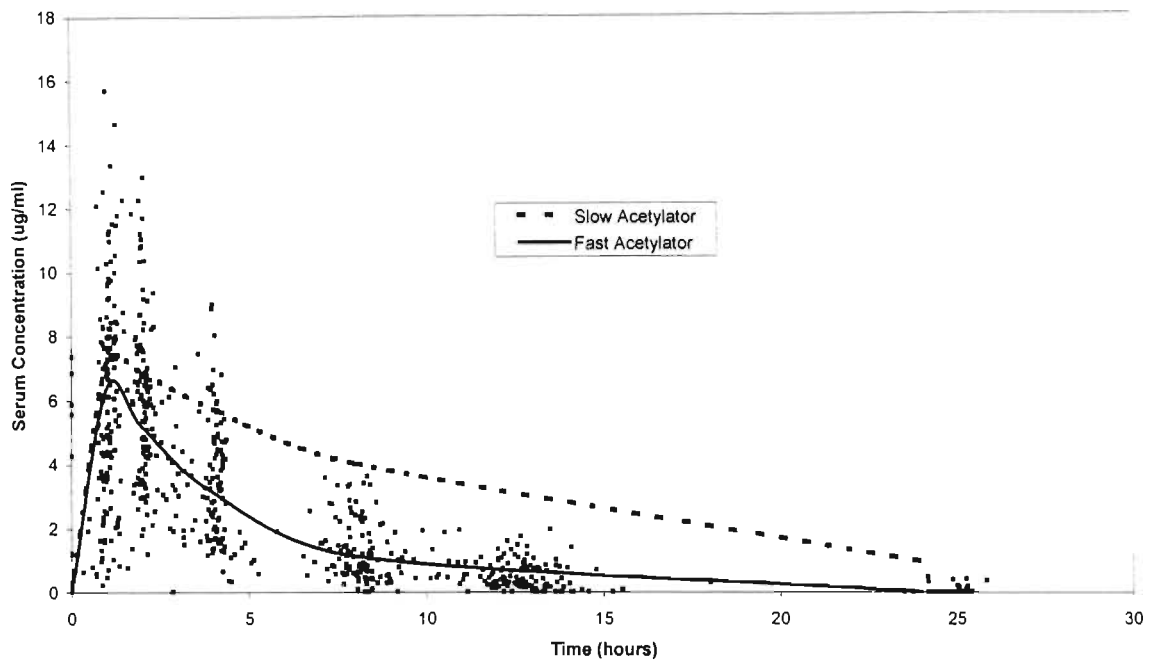
Figure 5.8 – Time course of isoniazid serum concentrations for slow (dashed line) and fast (solid line) acetylators. The scatter of points shows the isoniazid concentrations measured in 138 pulmonary tuberculosis patients. All serum concentrations have been normalised to a 400mg dose.

Table 5.17 - Population Pharmacodynamic Parameters for Isoniazid in Multi-drug Resistant and Drug-Sensitive Pulmonary TB patients.

Pharmacodynamic Parameter	ALL PATIENTS (n=86)[1]	MDR-TB		DRUG-SENSITIVE TB	
		HIV+ (n=12)	HIV– (n=27)	HIV+ (n=21)	HIV– (n=24)
Cmax : MIC					
Fast Acetylator	n=73	*n=8*	*n=23*	*n=18*	*n=22*
Geometric Mean	7.42	0.73	0.66	42.93	52.28
Median	34.03	0.71	0.49	51.53	55.78
Range	0.26 – 96.48	0.38 – 2.08	0.26 – 34.03	5.43 – 86.47	5.16 – 96.48
Slow Acetylator	n=13	*n=4*	*n=4*	*n=3*	*n=2*
Geometric Mean	3.95	1.04	0.59	51.32	54.01
Median	1.36	1.24	0.58	61.23	55.35
Range	0.31 – 67.46	0.37 – 2.03	0.31 – 1.31	35.45 – 62.29	43.24 – 67.46
Time > MIC (hours)					
Fast Acetylator	n = 73	*n=8*	*n=23*	*n=18*	*n=22*
Mean	9.53	0.58	1.20	15.81	16.13
Median	12.88	0	0	15.32	15.71
Range	0 – 23.99	0 – 4.6	0 – 13.65	5.97 – 23.00	8.45 – 22.12
Slow Acetylator	n=13	*n=4*	*n=4*	*n=3*	*n=2*
Mean	10.33	2.48	1.10	24.00	24.00
Median	4.37	2.28	0	24.00	24.00
Range	0 – 24.00	0 – 5.29	0 – 4.37	24.00 – 24.00	24.00 – 24.00
AUC > MIC (µg.hr/ml)					
Fast Acetylator	n=73	*n=8*	*n=23*	*n=18*	*n=22*
Geometric Mean	0	0	0	22.88	24.66
Median	15.75	0	0	22.33	21.63
Range	0 – 55.72	0 – 10.84	0 – 14.08	11.46 – 39.26	11.97 – 55.72
Slow Acetylator	n=13	*n=4*	*n=4*	*n=3*	*n=2*
Geometric Mean	0	0	0	41.10	45.29
Median	3.17	1.66	0	44.88	46.04
Range	0 – 54.30	0 – 12.66	0 – 3.17	30.52 – 50.66	37.79 – 54.30

Cmax = maximum serum concentration; MIC = minimum inhibitory concentration; AUC = area under the serum concentration time curve

[1]The total number of MDR-TB and Drug-sensitive TB patients does not add up to total number of patients included in the study. This is because the drug susceptibility status of 2 patients (1 HIV+ and 1 HIV-) could not be classified as they were found to be resistant to INH alone. The HIV status of 1 drug-sensitive patient could not be determined as the patient withdrew consent for HIV testing after recruitment into the study.

Table 5.17 shows the derived population pharmacodynamic parameters for INH. The drug-sensitive TB patients experienced greater exposure to serum concentrations of INH in excess of the MIC. This is reflected in the higher pharmacodynamic parameters compared to the MDR-TB patients.

For purposes of clinical utility, all subsequent statistical comparisons of these parameters were conducted within the **drug-sensitive TB group** only.

Among the drug-sensitive TB patients, there was no significant difference between HIV+ and HIV- patients with regards to any of the 3 parameters for INH. The fast and slow acetylators did not display any difference with respect to the Cmax : MIC ratios. However, slow acetylators experienced serum drug concentrations above the MIC for the entire dosing interval compared to only approximately 16 hours for the fast acetylators ($p < 0.001$). Their AUCs were also significantly higher ($p=0.004$).

The influence of altered pharmacokinetics on the pharmacodynamic parameters is illustrated in Figures 5.9 to 5.11 which show the frequency histograms of the 3 pharmacodynamic parameters for INH in drug-sensitive patients.
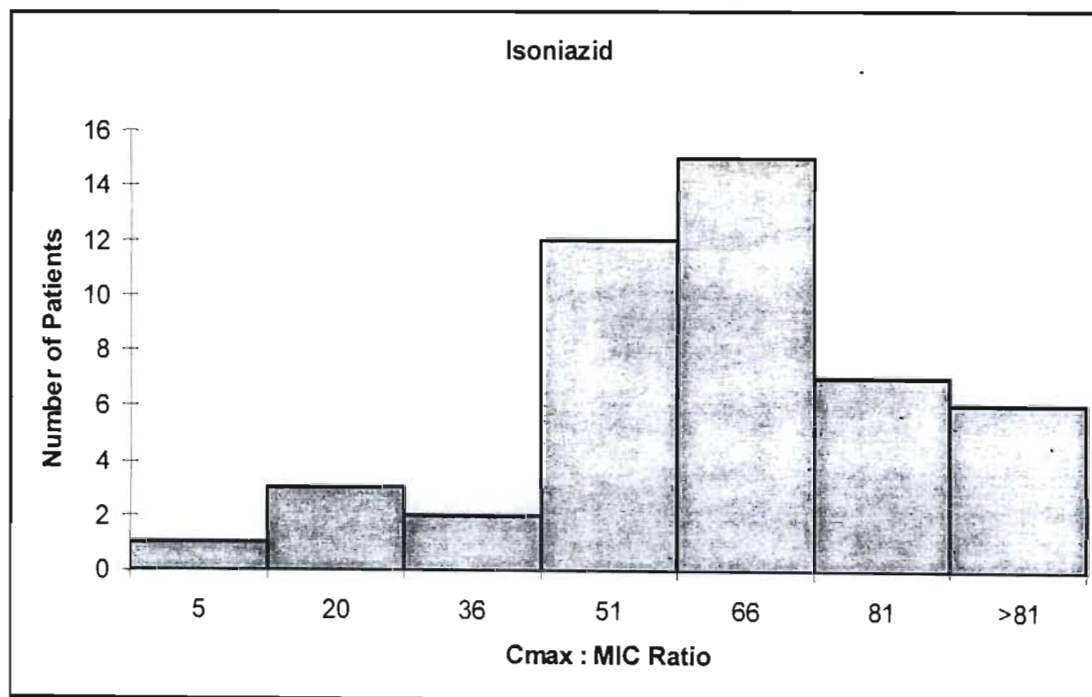
Figure 5.9 – Frequency histogram of Cmax: MIC ratio for isoniazid in drug-sensitive pulmonary tuberculosis patients (n = 86).

The majority of patients had Cmax:MIC ratios for INH above 50, with only 6 patients having values less than 40 (Figure 5.9).
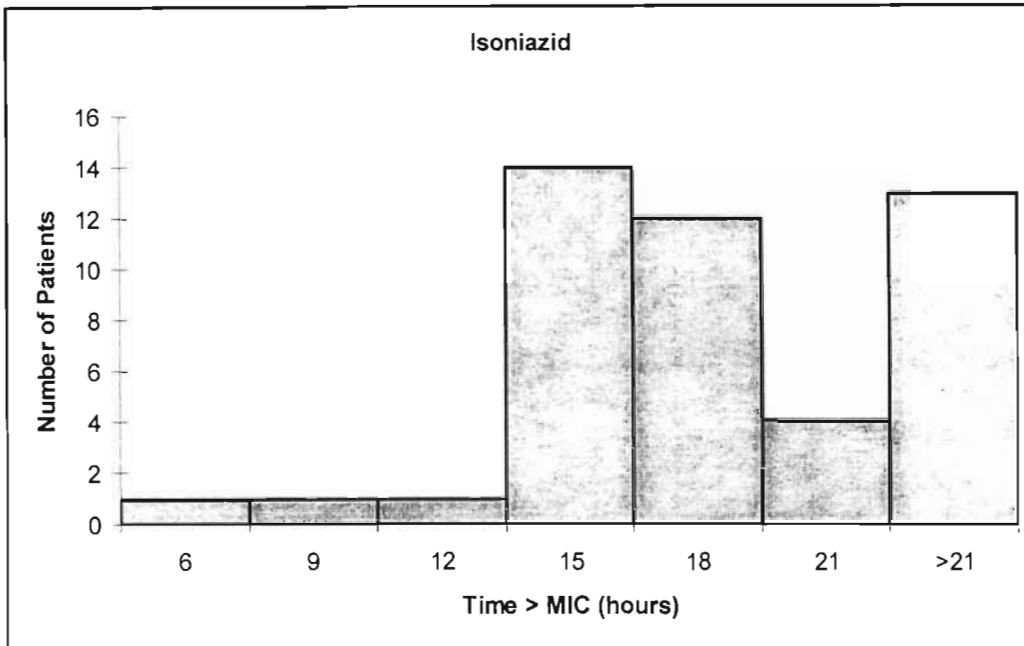
Figure 5.10 – Frequency histogram of time > MIC for isoniazid in drug-sensitive pulmonary tuberculosis patients (n = 86).

In the majority of patients, serum concentrations were maintained above the MIC for more than 15 hours of the dosing interval. There were 3 patients who had concentrations above the MIC for less than 12 hours of the dosing interval (Figure 5.10).
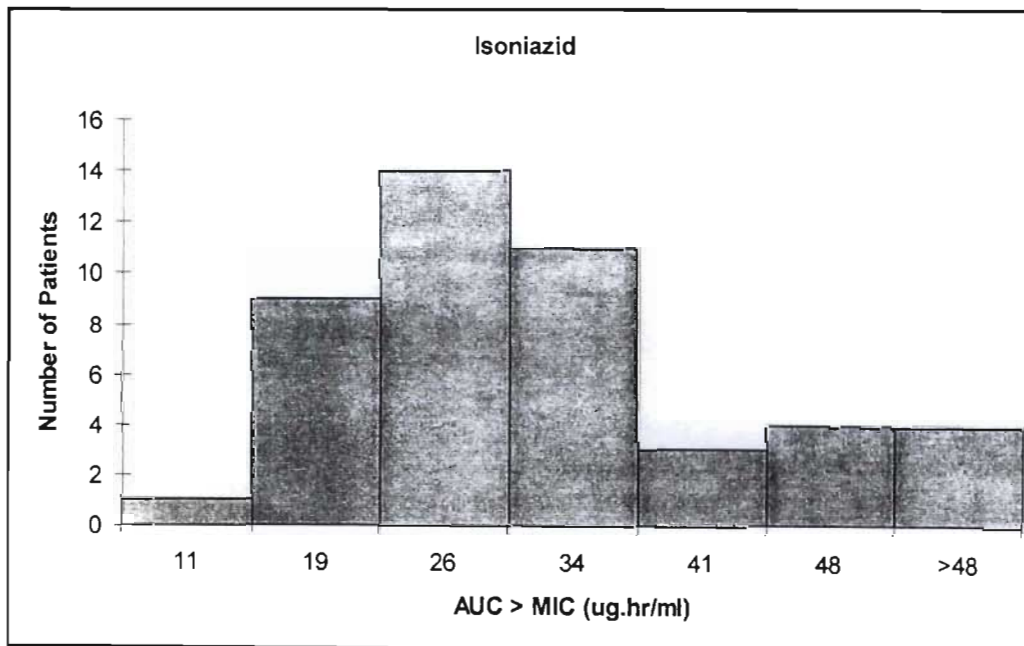
Figure 5.11 – Frequency histogram of AUC > MIC (µg.hr/ml) for isoniazid in drug-sensitive pulmonary tuberculosis patients (n = 86).

Most of the drug-sensitive patients achieved an AUC > MIC for INH of greater than 19 µg.hr/ml. There were no patients with a value of less than 11 µg.hr/ml for this parameter (Figure 5.11).

Table 5.18 - Population Pharmacodynamic Parameters for Rifampicin in Multi-drug Resistant and Drug-Sensitive Pulmonary TB patients

Pharmacodynamic Parameter	ALL PATIENTS (n=96)[1]	MDR-TB		DRUG-SENSITIVE TB	
		HIV+ (n=13)	HIV– (n=35)	HIV+ (n=25)	HIV– (n=20)
Cmax : MIC					
Geometric Mean	3.42	0.68	1.00	13.08	12.83
Median	7.87	0.59	0.86	12.87	12.72
Range	0.25 – 23.28	0.25 – 3.43	0.38 – 17.83	7.54 – 23.28	10.05 – 18.63
Time > MIC (hours)					
Mean	5.28	0.63	1.31	9.44	9.37·
Median	7.46	0	0	8.89	8.78
Range	0 – 15.88	0 – 8.81	0 – 12.28	7.44 – 14.33	7.33 – 15.88
AUC > MIC (µg.hr/ml)					
Geometric Mean	0	0	0	26.72	25.31
Median	19.25	0	0	25.79	24.43
Range	0 – 55.82	0 – 23.17	0 – 42.31	16.18 – 55.82	18.46 – 44.39

Cmax = maximum serum concentration; MIC = minimum inhibitory concentration; AUC = area under the serum concentration time curve

[1]The total number of MDR-TB and Drug-sensitive TB patients does not add up to total number of patients included in the study. This is because the drug susceptibility status of 2 patients (1 HIV+ and 1 HIV-) could not be classified as they were found to be resistant to INH alone. The HIV status of 1 drug-sensitive patient could not be determined as the patient withdrew consent for HIV testing after recruitment into the study.

Table 5.18 shows the derived population pharmacodynamic parameters for RFA. As noted for INH, the drug-sensitive TB patients experienced greater exposure to serum concentrations of RFA in excess of the MIC. This is reflected in the higher values of the pharmacodynamic parameters compared to the MDR-TB patients. There were 6 MDR-TB patients who achieved AUC>MIC and t>MIC values similar to the drug-sensitive patients, but only 1 patient had a Cmax:MIC ratio within the range of values seen in the drug-sensitive patients. In these patients, the AUC>MIC ranged from 15.32 to 42.31 µg.hr/ml and serum concentrations were maintained above the MIC for approximately 6 and 12 hours.

Among the drug-sensitive patients, there was no significant difference between HIV+ and HIV- patients with regards to any of the 3 parameters for RFA. Figures 5.12 to 5.14 shows the frequency distributions for the 3 derived pharmacodynamic parameters for RFA.
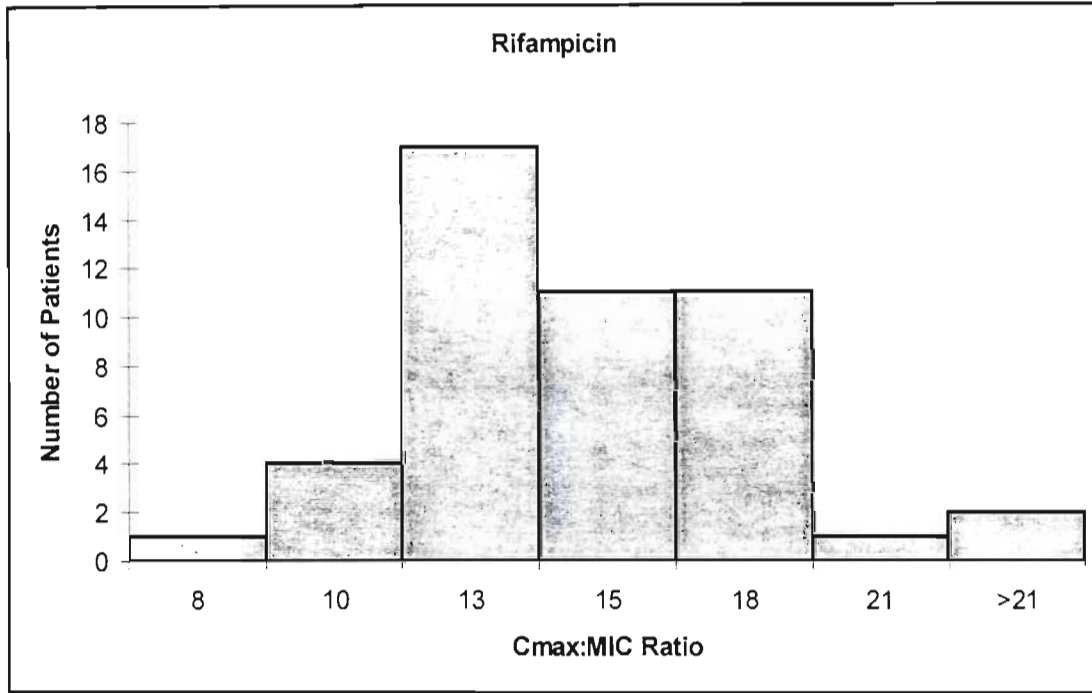
Figure 5.12 – Frequency Histogram of Cmax: MIC ratio for rifampicin in Drug-Sensitive Pulmonary Tuberculosis Patients (n = 96).

Figure 5.12 shows that the majority of patients had RFA concentrations more than 13 times that of the MIC for RFA. In 5 patients the Cmax:MIC ratio was less than 10, the lowest value being 7.54.
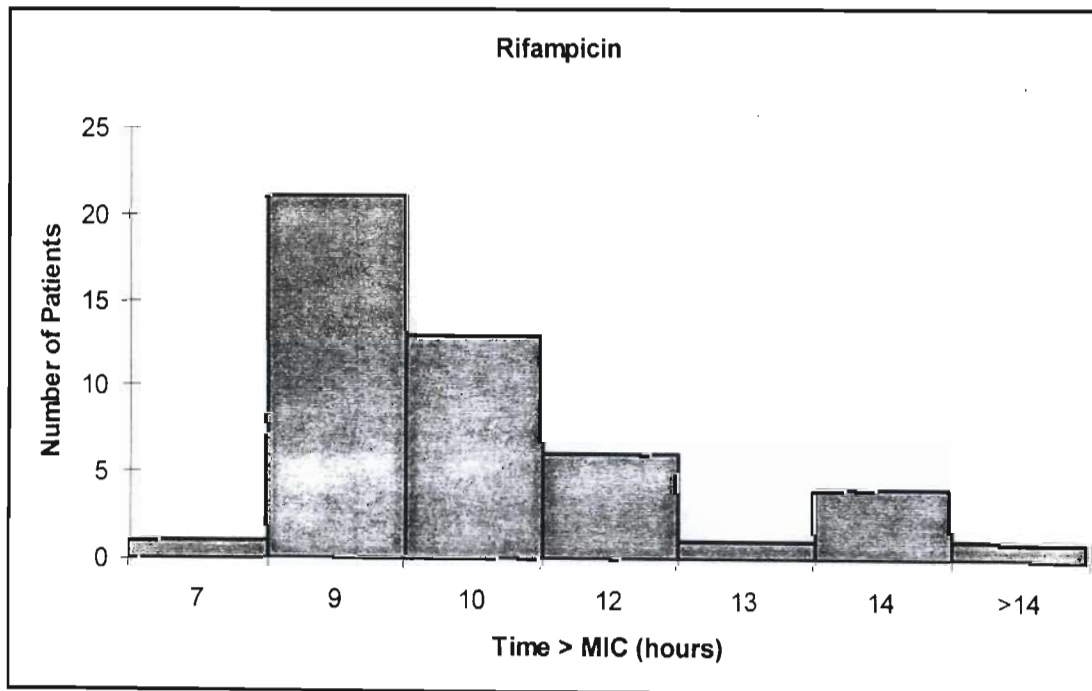
Figure 5.13 – Frequency Histogram of time > MIC (hour) for rifampicin in Drug-Sensitive Pulmonary Tuberculosis Patients (n = 96).

Serum concentrations were maintained above the MIC for more than 9 hours of the 24 hour dosing interval in all except 1 patient (Figure 5.13).
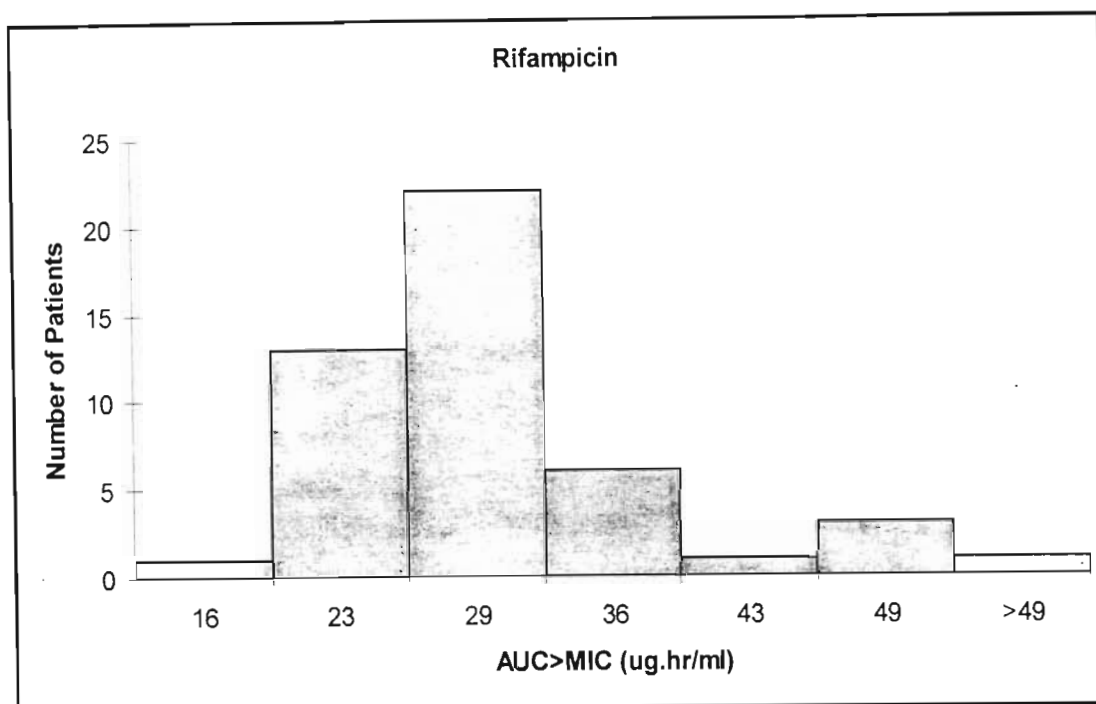
Figure 5.14 – Frequency Histogram of AUC > MIC (ug.hr/ml) for rifampicin in Drug-Sensitive Pulmonary Tuberculosis Patients (n = 96).

All except 1 drug-sensitive patient achieved an AUC>MIC for RFA of greater than 18 µg.hr/ml (Figure 5.14).

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Chapter 6

DISCUSSION

Overview

The rising prevalence of MDR-TB internationally *(Edlin et al. 1992; Iseman, 1993; Bloch et al. 1994; Neville et al. 1994; Cohn et al. 1997)* has been confirmed locally in this study for KwaZulu-Natal (Chapter 3) with an increase from 2.2% in 1983 to 3.7% in 1995 (p=0.01). MDR-TB renders TB virtually incurable *(Cole and Telenti, 1995b)*. The situation has been likened to that observed in the late 1960's prior to the introduction of RFA when an increase in resistance to the first line anti-TB drugs was noted *(Grassi and Peona, 1995)*. The major difference is however, that since 1970 no new anti-TB drug has been introduced by the pharmaceutical industry specifically to treat TB *(Cole and Telenti, 1995a)*. This emphasises the importance of searching for factors that counter the emergence of drug resistance. Further, we need to re-examine our rapidly declining armamentarium against TB with a view to finding better ways of utilising the available agents.

This study in 138 pulmonary TB patients found no clinically relevant or statistically significant difference between MDR-TB patients and drug-sensitive TB patients with regards to any of their pharmacokinetic characteristics for RFA and INH. If any differences were noted, they were shown to be due to factors other than MDR *per se.* Similarly, no statistically significant differences were noted between HIV+ and HIV-patients with regard to the pharmacokinetic or pharmacodynamic characteristics of INH and RFA.

These findings contradict the hypothesis of pharmacokinetic differences being implicated in the acquisition of MDR-TB as suggested by *(Bradford et al. 1996)* and several other authors *(Frieden et al. 1993; Patel et al. 1995)*. They proposed that malabsorption due to HIV infection, and consequent lower drug exposure contributes to the acquisition of MDR-TB.

The lack of differences between HIV+ and HIV- patients is in contrast to the reports suggesting malabsorption of the anti-TB drugs in HIV+ patients *(Berning et al. 1992; Peloquin et al. 1993; Patel et al. 1995; Peloquin et al. 1996; Sahai et al. 1997)* primarily emanating from researchers at the National Jewish Centre for Immunology and Respiratory Medicine in Denver, USA.

This is the first study to address the important and clinically relevant research question of whether there is an association between pharmacokinetics and MDR-TB in a group of patients stratified according to HIV status.

In general, conditions likely to influence the pharmacokinetics of drugs are those which affect normal functioning of the gastrointestinal tract (GIT), hepatic and renal systems. Patients with HIV infection and/or AIDS have been reported to have altered functioning of all 3 of these systems *(Unadkat and Agosti, 1990)*. These have been reviewed in Chapter 2.

Diarrhoea is the most common GIT symptom reported in AIDS. While this is caused by a wide variety of diarrhoeal pathogens, the human immunodeficiency virus itself has been found to infect the GIT mucosa and is associated with enteropathy. Opportunistic infections that affect immunocompromised hosts may also predispose to malabsorption *(Unadkat and Agosti, 1990)*.

Some of the opportunistic infections or the drugs used in their treatment may also result in aberrations in liver function that may alter the pharmacokinetics of the anti-TB drugs *(Unadkat and Agosti, 1990; Mandell and Petri, 1996)*.

A distinct form of HIV-associated renal disease with a broad spectrum of severity and sometimes associated with proteinuria has been reported *(Unadkat and Agosti, 1990)*. However, with the exception of severe renal disease, this is unlikely to cause alterations in the pharmacokinetics of INH and RFA, as the kidneys constitute a minor route of drug elimination for these drugs.

The studies that have addressed the role of altered pharmacokinetics of the anti-TB drugs in the presence of HIV infection have been comprehensively reviewed in Chapter 2. The salient issues are summarised hereunder.

The early studies consisted of case reports of patients with AIDS and TB in whom malabsorption of the anti-TB drugs was noted *(Berning et al. 1992; Peloquin et al. 1993; Peloquin et al. 1996)*. These studies were largely retrospective evaluations of a 2-hour post-dose serum drug concentration in relation to the authors' proposed normal range. The normal range was based on data from non-HIV infected patients and did not consider the possibility of delayed absorption. Further, a sample drawn at 2 hours post-dose corresponds to the most variable part of the pharmacokinetic profile and is very difficult to interpret.

Another report *(Patel et al. 1995)* highlighted the role of malabsorption in the acquisition of drug resistance. In this communication, 2 patients with HIV infection being treated for TB relapsed with drug-resistant isolates. In one of the 2 patients, the low drug bioavailability was confirmed using serial drug measurements over the entire pharmacokinetic profile. However it was the report by *Sahai et al 1997* that appeared to provide the definitive evidence for malabsorption in the presence of immune compromise. This study used a full prospective pharmacokinetic trial design and investigated patients at various stages of HIV infection as well as patients with diarrhoea.

More recently, 2 studies from Africa have failed to confirm malabsorption in the presence of HIV infection, AIDS or diarrhoea *(Choudhri et al. 1997; Taylor and Smith, 1998)*. It is interesting to note that the principal author of the *Sahai et al (1997)* paper that confirmed malabsorption was also a co-author on the first study *(Choudhri et al. 1997)* to contradict the hypothesis. The study by *Taylor and Smith (1998)* was noteworthy in that these authors observed better absorption (higher AUCs) for RFA among AIDS patients.

The present study adds to the current knowledge with respect to these contradictory results. It was conducted in a larger sample (n = 138) of patients than those used previously. The study was unique in that it was conducted in MDR-TB and drug-

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sensitive pulmonary TB patients stratified according to HIV status. The HIV+ patients were at various stages of HIV disease as determined using HIV viral loads.

Detailed Discussion

PHARMACOKINETIC PARAMETERS

The pharmacokinetic parameters obtained from the non-compartmental method of data analysis did not differ in any marked or systematic way from the NONMEM results. The non-compartmental analysis constituted part of the exploratory data analysis and helped to provide initial estimates and guides for the population analysis. The population approach was the primary data analytical method used. In addition to several advantages of this approach that will be highlighted in the ensuing discussion, the NONMEM analysis quantifies the size of the inter-individual variability for each pharmacokinetic parameter, together with identification of the covariates that cause the variability. Thus in this study, it was possible to search for the influence of covariates on the pharmacokinetic parameters other than those associated with the primary research questions of MDR-TB and HIV status.

Rifampicin

The absorption of RFA was relatively rapid with a KA of 0.83 hr^{-1}. This is in agreement with other reports indicating that in general, the absorption of RFA is rapid and complete. However, several studies have observed unexplained delays in drug absorption *(Kenny and Strates, 1981)*. In the present study, the use of the absorption lag time parameter appears to reduce the inter-individual variability in KA to the relatively low value of 52%. However, like others*(Kenny and Strates, 1981)*, a wide range in shape and AUC was noted for the RFA serum concentration versus time curves.

The effect of type and quality of food on RFA absorption has been widely studied and reviewed *(Kenny and Strates, 1981; Zent and Smith, 1995)*. While most authors have shown a reduction in the rate of absorption, some have suggested that the extent may also be reduced *(Kenny and Strates, 1981)*. In this study, the ward routine of drug administration at 05h00 with breakfast being served 3 to 4 hours later at approximately 08h30 eliminated the confounding influence of food effects on absorption.

This study found that upon initiation of treatment, the typical value of CL/F for RFA was 7.7 L/hr (Table 5.12). Other authors have reported similar values of between 4.6 and 9.4 L/hr after initiation of RFA treatment with 1 study reporting a CL of 17.8 L/hr (Table 2.1). Daily treatment thereafter results in an increase in the rate of drug metabolism. This occurs as a result of the induction of hepatic endoplasmic reticular enzyme system (P450IIIA) i.e. RFA auto-induction *(Kenny and Strates 1981; Dollery 1991b)*. In the present study, maximal enzyme induction was reached at approximately 10 days after treatment at which time the CL/F was 15.6 L/hr. This post enzyme auto-induction CL/F value is similar to that calculated from data reported by others viz 6.8 to 15.0 L/hr (Table 2.1).

The mean population V/F for RFA was 26.4 L at initiation of treatment and was 42.1 L after 10 days of therapy. A review of the literature suggested a V for RFA of 0.9 to 1.0 L/kg (Table 2.1). Using the median mass of 54kg for this population, the V/F obtained in this study was 0.53 L/kg (pre-induction) and 0.74 L/kg after induction. There was therefore reasonably good agreement between this study and the literature.

The dependence of V/F on auto-induction appears strange but has a sound mechanistic basis. After metabolism in the liver, RFA and its main metabolite desacetylrifampicin are excreted in the bile. The RFA is then reabsorbed into the blood while the desacetylrifampicin is not i.e. RFA undergoes enterohepatic recycling (Reynolds, 1993). When a drug is reabsorbed after biliary secretion during enterohepatic recycling, the biliary secretion is not a route of elimination but rather it constitutes a component of distribution (Zeind et al. 1996).

During the 10 day period during which enzyme auto-induction increases to a maximum, the conversion of RFA to its metabolites increases and thus the amount of RFA contributed by biliary secretion decreases. Since the total amount of drug being introduced into the body from the dose remains the same during this time, the decreased concentration in plasma reflects as an increased V/F.

Isoniazid

The one compartment model was also chosen to describe the pharmacokinetics of INH. During the NONMEM analysis of INH, the estimate of KA was constrained to a value of 3.5 hour^{-1} since it was found that there was insufficient data to characterise the absorption of INH in this study. The value of 3.5 hour^{-1} was determined using the sensitivity analysis procedure. This strategy was in accordance with the recommendations of Wade et al (1993) that if the data contains minimal information about KA, then its value should be fixed and a parameter for inter-individual variability in KA should form part of the model. The results of a series of simulation studies conducted by these authors confirmed that this procedure prevented the variability in KA being manifested in V/F. This shifting of the variability between KA and V/F was noted in the present study during the structural pharmacokinetic model building stage before fixing KA (Appendix J). The authors noted further that mis-specification of KA had no effect on the estimation of CL/F (Wade et al. 1993).

The pharmacokinetics of INH is widely reported to be characterised by a bimodal distribution based on genetic polymorphism of fast and slow acetylation (Dollery, 1991a). It was therefore decided to use the mixture modelling feature of NONMEM to determine the proportions of slow and fast acetylators. An examination of the distribution of the post hoc Bayesian individual CL/F values prior to implementation of mixture modelling (Figure 5.5) provided only a suggestion of the presence of a multi-modal distribution in CL/F values. It must be noted that in this data set, examination of this picture alone would not have been convincing evidence of the presence of multiple distinct populations. The very large decrease in OBF and associated better fit of the data upon implementation of NONMEM's mixture modelling option provided the necessary confirmation. This was corroborated by the distribution of half-life values for INH after the non-compartmental analysis.

The results of the non-compartmental analysis indicated that the HIV+, MDR-TB patients had higher AUC and Cmax values than patients in the other 3 groups (p≤0.03). These patients also received a higher mean dose of INH although this difference was not statistically significant. Upon correction for the patient's mass and dose the cAUC for the HIV+, MDR-TB patients was still higher but the difference in cCmax was no longer evident. Thus differences in weight and dose may offer a potential explanation. Neither of these differences were translated into effects on the pharmacokinetic parameters of CL/F and V/F determined by either the non-compartmental methods or the population analysis method.

In this study, using the NONMEM mixture-modelling feature, the proportion of fast acetylators in the population was found to be in the majority (85%). After the non-compartmental analysis on a subset of the NONMEM data set, and using a popular cut-off value of a half-life of 2 hours *(Gelman and Rumack 1998)* to distinguish between slow and fast acetylators, the proportion of fast acetylators was found to be very similar viz. 92%. The main population group in this study was Black Africans. Using the half-life to determine acetylator status, *Bach et al (1976)* found that 59% of Black South African patients were fast acetylators while *Buchanan et al (1976)* noted that 73% were fast acetylators. More recently, *Parkin et al (1997)* studied the phenotype and genotype of INH acetylation and noted that a larger proportion of South African patients was fast acetylators. These latter authors *(Parkin et al. 1997)* also noted a tri-modal distribution in INH acetylator elimination behaviour rather than the bimodal distribution previously described.

Confirmation of these proportions using an independent marker for INH acetylation is the subject of a separate follow-up study. This latter study will use restriction-fragment-length chain polymorphism (RFLP) to characterise the acetylase genotype. When complete, this should provide a valuable validation of NONMEM mixture models.

Some reports suggest that the clinical relevance of INH acetylator status is that the slow acetylators comprise a group at risk of various INH-related toxicities including peripheral neuropathy and various drug interactions. Thus it would appear that Black South African patients would be at lower risk of these toxicities since they are usually fast acetylators. On the other hand it has been postulated that hepatoxicity may be more common in fast acetylators, owing to the production of larger amounts of the metabolite acetylhydrazine which is thought to be involved in the development of this side-effect. However *Ellard (1984)* evaluated the literature and concluded that clinically important hepatic toxicity is unrelated to acetylator status.

The mean population CL/F for slow acetylators was found to be 4.7 L/hr while fast acetylators had a CL/F of 13.0 L/hr (Table 5.16). The CL/F for slow acetylators calculated from data or parameters reported in previous studies was 2.7 to 11.3 L/hr while that in fast acetylators was 7.2 to 27.2 L/hr (Table 2.4). Thus in this study, CL/F values were within the range of values noted previously in the literature.

The V/F obtained in this study of 50.0 L (equivalent to 0.93 L/kg in a 54-kg patient) compares well with that reported in the literature of 0.6 to 0.8 L/kg (Table 2.4).

There was no significant difference between the number of fast and slow acetylators in the MDR-TB and drugs-sensitive groups. This argues against acetylator status being implicated in the acquisition of drug resistance.

PHARMACODYNAMIC PARAMETERS

Tables 5.17 and 5.18 show the derived population pharmacodynamic parameters for INH and RFA respectively. These parameters attempt to integrate the individual patient's pharmacokinetics with the MIC of INH and RFA determined from organisms isolated from that patient's sputum.

The pharmacodynamic parameters calculated in this study are an important contribution to new knowledge as they have been calculated using pharmacokinetic and pharmacodynamic information within the same patient. In a previous study, *Peloquin and Berning (1994)* calculated the parameters using published MIC values and pharmacokinetic data obtained from the literature. Direct comparison of the parameters obtained in this study with those obtained by *Peloquin and Berning (1994)* is difficult due to the absence of dosing information as well as the range in MIC values that were recorded in their report. Both of these will have large influences on the value of the derived pharmacodynamic parameters. However, there is broad general agreement as noted below.

Studies conducted with bactericidal drugs against aerobic bacteria suggest that for the cell wall active drugs (e.g. beta-lactam antimicrobials), maintaining the serum concentration above the MIC for the entire dosing interval (t>MIC) is the most important parameter for eradicating the organism *(Peloquin, 1996)*. INH acts primarily against the cell wall and thus t>MIC would be an important parameter to optimise when dosing with this drug.

On the other hand, in the case of drugs that exert their effect on intra-cellular targets (e.g. aminoglycosides) the Cmax:MIC ratio is considered important as this ensures adequate penetration into the site of action. RFA would fall into this category as it acts on RNA polymerase within the cell *(Peloquin, 1996)*.

The AUC>MIC parameter provides an overall impression of drug exposure that incorporates information on how high the serum concentrations increased above the MIC as well as the duration of exposure to these concentrations.

While the t>MIC parameter is easy to conceptualise, it is more difficult to appreciate the impact of the parameters of Cmax:MIC and AUC>MIC given the absence of comparative data that is corroborated with clinical evidence of success or failure with regimens.

It is to be expected that (by definition) the drug-sensitive patients will experience greater exposure to serum concentrations of INH and RFA in excess of the MIC. Thus the values of their pharmacodynamic parameters were higher compared to those of the MDR-TB patients.

The possibility of TDM as an option *(Peloquin, 1996)* for the treatment of MDR-TB cannot be totally excluded based on the results of this study. However, the MIC

results that showed that the majority of isolates were either very resistant or very sensitive to INH and RFA are not very encouraging. This is typical of *M tuberculosis* and unlike other species of mycobacterium e.g. *M avium* where moderate resistance is frequently noted. The range of breakpoint concentrations tested in this study was based on an evaluation of the concentrations that were likely to be achievable in the clinical setting without producing unacceptable toxicity. This suggests that the dose required to produce drug exposure in excess of the MIC may be toxic to the patient and argues against using aggressively large doses of INH and RFA to treat MDR-TB.

Among the drug-sensitive patients, there was no significant difference between HIV+ and HIV- patients with regards to any of the pharmacodynamic parameters for both INH and RFA. This reflects the absence of pharmacokinetic differences between HIV+ and HIV- patients seen in this study.

Rifampicin

Among the drug-sensitive patients, there was no significant difference between HIV+ and HIV- patients with regards to any of the 3 pharmacodynamic parameters for RFA (Table 5.18).

As mentioned earlier, the Cmax:MIC would be important for RFA as this drug affects intracellular bacterial targets. The majority of patients had ratios in excess of 13 (Figure 5.12), while 5 patients had lower ratios of ≤ 10. When compared to the value of 24 reported by *Peloquin and Berning (1994)*, there is the suggestion that these patients are at risk of being under-dosed with RFA.

The AUC>MIC for RFA was greater than 23 μg.hr/ml in the majority of patients. Only 1 patient experienced a low AUC>MIC of 16 μg.hr/ml. *Peloquin and Berning (1994)* reported a value of 39.9 μg.hr/ml for this parameter. As mentioned above, the absence of information regarding the range in MIC values and dosing information in the study from which the pharmacokinetic parameters were obtained makes it difficult to ascertain the clinical significance of the lower AUC>MIC seen in the present study

Serum concentrations were maintained above the MIC for approximately 9 hours of the 24 hour dosing interval after RFA treatment in drug-sensitive patients.

Isoniazid

A mean Cmax:MIC ratio for INH of approximately 55 was obtained in both fast and slow acetylators as compared to a value of 40 reported by *Peloquin and Berning (1994)*. These authors did not note any difference due to acetylator status either. This is not surprising since the numerator (Cmax) in the ratio is dependent on the absorption process rather than drug elimination, the latter being responsible for the distinction between slow and fast acetylators.

The frequency histograms of the pharmacodynamic parameters for INH (Figures 5.9 to 5.11) shows the effect of inter-individual variability in pharmacokinetics on the pharmacodynamic parameters. While the majority of patients have Cmax:MIC ratios of greater than 50 for INH, there were 6 patients who had values of less than 36 for this parameter. Similarly, there were a small number of patients who had relatively

lower t>MIC and AUC>MIC compared to the majority of the drug-sensitive patients. The exact clinical relevance of this is uncertain. A follow-up study is currently being conducted to examine these pharmacodynamic parameters in relation to patient outcome variables such as acquisition of drug resistance and response to treatment.

The 5 drug-sensitive slow acetylators experienced serum drug concentrations of INH above the MIC for the entire dosing interval compared to only approximately 16 hours for the fast acetylators (p<0.001). Their AUCs were also significantly higher (p=0.004). Comparative values for slow and fast acetylators obtained *by Peloquin and Berning (1994)* were 19.2 and 11.6 hours.

There are clear implications for the derivation of integrated pharmacokinetic and pharmacodynamic indices of anti-TB drug activity and their use to establish individualised antibiotic dosage regimens in special high-risk groups. Such high-risk groups would include patients with MDR-TB or patients with high rates of drug clearance among the drug-sensitive patients. Whether the parameters determined in this study could be regarded as target breakpoint values requires correlation with information relating to clinical outcome in these patients. The parameters thus hold promise as a benchmark for further comparative evaluation.

The discussion hereafter is presented with a view to assist in generalising these results.

Race

Almost all the patients (135/138) recruited into this study were Black Africans. Although the author does not subscribe to divisions of people according to ethnic or racial lines, this is necessary in studies involving diseases that are distributed according to socio-economic status. Thus the large number of patients of African race highlights TB as a socio-economic disease affecting mainly the poor and the disadvantaged. In South Africa, poor and disadvantaged is synonymous with being Black.

Age

The present study also noted a significantly lower mean age in the HIV+ patients compared to the HIV- patients (31.0 ± 8.5 vs. 39.4 ± 12.1 years; p<0.0001). This highlights the crisis posed by the HIV epidemic in South Africa and sub-Saharan Africa as it affects the economically active age groups. This observation and warning that young adults comprise the age group most often affected by the dual epidemics of TB and HIV, particularly in developing countries, has been recorded by several authors *(De Cock et al. 1991; Nunn et al. 1992; Houston et al. 1994)*. More recently, this has been confirmed in KwaZulu-Natal *(Wilkinson and Moore, 1996b)*.

History of Prior treatment for TB

The greatest predictor for drug resistant TB is a history of prior treatment for TB *(Frieden et al. 1993)*. This has also been our experience in a previous study conducted at KGV *(Anastasis et al. 1997)*. It is not surprising therefore that this was again corroborated in this study. There were significantly more MDR-TB patients who had a history of previous treatment for TB (79%) compared to new cases (21%) (odds ratio

7.85, CI 3.34 to 18.77; p<0.0001). Among the re-treatment cases that had developed MDR-TB, there was a larger proportion of patients whose previous treatment had been interrupted or failed (odds ratio 2.34, CI 0.74 to 7.48, p=0.08).

Clearly, the greatest challenge in TB continues to be the issue of ensuring adherence (compliance) to treatment. Experts increasingly acknowledge adherence as a behavioural problem and recommend behavioural and social research efforts into the problem *(Sumartojo, 1993)*. In South Africa, with its diverse cultural heritage there has been little to no research into socio-behavioural factors leading to poor compliance.

The success of directly observed treatment (DOT) regimens in New York *(Frieden et al. 1995)* and in some centres in South Africa *(Wilkinson et al. 1996a)* demands wider investigation of its implementation according to modifications to suit local needs. Simultaneously with these research endeavours, tuberculosis control programs and research units need to evaluate the pharmacodynamic response to such regimens. The present study may help to provide the basis for such population pharmacokinetic-pharmacodynamic studies.

HIV status and Viral Loads

Although patients were recruited into the study with due regard of their MDR-TB and HIV status, no conscious effort was made to balance the groups with regard to sex. It is thus interesting to note that the sample consisted of a significantly larger proportion of HIV+ females than HIV+ males (53% vs. 31% p = 0.01). In the early 1980s in South Africa, HIV infection predominantly affected gay white men. There was a slower spread of infection to the Black heterosexual population. In the last decade however, the pattern of the HIV epidemic in South Africa has become similar to that seen in other African countries *(McIntyre, 1996)*. In heterosexually transmitted HIV infection as occurs currently in South Africa, women are at greater risk of infection than men are. This observation is reflected in the results of a seroprevalence study conducted in KwaZulu-Natal that noted a 3.2-fold higher prevalence of HIV-1 infection in females than males *(Abdool Karim et al. 1992)*. A study in Hlabisa, KwaZulu-Natal noted a higher prevalence of HIV-related TB among women as a consequence of the high baseline prevalence of HIV in the province *(Wilkinson and Moore, 1996b)*.

During recruitment of patients into this study, the group that was most difficult to find was that of patients who had MDR-TB and who were HIV+. This group comprised of 21 patients while the other 3 groups had more than 35 patients each. This observation corroborates our previous report that there is currently no association between drug resistant TB and HIV status in KwaZulu-Natal *(Anastasis et al. 1997)*.

In this study, HIV viral loads were used as the method of grading the patient's level of immune compromise. Most researchers report CD4+ lymphocyte counts for this purpose *(Choudhri et al. 1997; Sahai et al. 1997)*. However, CD4+ lymphocyte counts were not measured in this study. This was because funds were not available to perform this when the study was conducted. Measurement of CD4+ lymphocytes requires the use of fresh whole blood, drawn according to a strict protocol. Thus samples which were stored frozen could not be used for analysis at a later stage when the financial situation had improved.

Consequently, the more reliable (and more expensive) HIV viral loads were measured towards the end of the data collection phase of the study using serum that had been frozen at $-84^{\circ}C$. The International AIDS Society guidelines for the treatment of HIV infection recommend viral RNA measurements to CD4+ counts *(Carpenter et al. 1996)*. They indicate that HIV viral loads are a more accurate indication of prognosis and treatment benefit especially in asymptomatic patients with CD4+ counts of > 350 cells/μl. In recent reports, the HIV viral load was the most significant virological predictor of clinical progression of HIV infection *(Mellors et al. 1996; Brun-Vezinet et al. 1997)*. While baseline CD4+ counts were also an independent predictor, its predictive power was not sustained throughout the clinical course of infection especially in the milieu of drug therapy *(Brun-Vezinet et al. 1997)*.

There is a negative correlation between HIV viral load and CD4+ cell counts i.e. as the viral load increases, the CD4+ count decreases. At the local Virology laboratory, patients with viral load less than 10 000 copies per millilitre are classified as having mild immune compromise, those with levels up to 70 000 as moderate and those with levels greater than 70 000 as having severe immune compromise (personal communication S Singh, Medical Virology, University of Natal). This classification is in keeping with the observations of *Mellors et al 1996* regarding viral load as a prognostic indicator. Thus the patients in this study with a median viral load of 69 000 copies per millilitre are classified as having a moderate to severe level of immune compromise. These viral loads must be interpreted in the context of the reports of an accelerated course of immune deficiency seen in patients with concurrent TB and HIV *(Whalen et al. 1995)*.

In general, the methodology used, PCR nucleic acid amplification, performed well under the operating conditions used. There were 3 samples that were considered problematic. Two of these had inadequate internal controls and 1 sample persistently recorded low RNA levels. There was little doubt that these patients were in fact HIV+. The definition of HIV seropositivity used in this study was stringent and followed internationally accepted criteria which requires at least 2 positive immunoassay tests. The majority of the HIV+ patients in this study had at least 3 tests conducted – the first done during their routine clinical care and the latter 2 confirmatory tests done for purposes of this study as per the protocol. The discrepant HIV viral load results may have been due to the absence of free virus in the patient's blood or because of a level undetectable by the assay system.

The median HIV viral load observed in this study among the HIV+ patients was 6.9×10^4 copies per millilitre (range 200 to 2.7×10^6 copies per millilitre). No significant differences were noted between MDR-TB and drug-sensitive TB patients with regard to their viral loads.

Thus the groups were comparable with regards to HIV disease severity. *Peloquin et al 1993* suggested that severity of HIV disease (using CD4+ counts) might be a marker for anti-TB drug malabsorption. The results of the present study showed that irrespective of the degree of immune compromise, there was no difference in pharmacokinetics of INH and RFA.

Concomitant Diseases

While many of the patients in this study had oral candidiasis (14 of 59 HIV+ patients), there were few other opportunistic infections in the HIV+ patients. Two patients had genital herpes and 1 patient had Karposi's sarcoma. The absence of widespread opportunistic infections corroborates the opinion that TB occurs early in the spectrum of opportunistic infections in a patient infected with HIV. Further since opportunistic infections have been implicated in the aetiology of drug malabsorption and liver function aberrations *(Unadkat and Agosti, 1990)*, this may also partially explain the absence of differences in the pharmacokinetics of INH and RFA in HIV+ and HIV- patients seen in this study.

Liver Function

Hepatotoxicity induced by anti-TB drugs is well described in the literature. In addition, minor temporary elevations in liver function tests (LFT) are a common occurrence early in the course of treatment. The elevations are considered by most TB physicians to be clinically unimportant as the elevated values often return to normal levels with continued treatment *(Brausch and Bass, 1993; Zeind et al. 1996)*. A baseline LFT is generally recommended at the commencement of TB treatment and this is standard policy at KGV. This is repeated if clinical symptoms suggest hepatotoxicity.

In this study, the median values of the LFTs were within the laboratory's normal range. However, individual values in excess of the upper limit of normal (ULN) were noted. Several patients had GGT levels in excess of the ULN. This enzyme is frequently elevated in patients with alcohol abuse and is often used for screening purposes *(Sherlock, 1981)*. In the context of drug induced liver injury however, the 2 enzymes of particular interest *(Vial et al. 1997)* are ALT values in excess of 2 x ULN and AST values in excess of 5 x ULN. Only 1 patient had an ALT value > 2 x ULN viz. 112 u/l while no patient had an AST value > 5 x ULN.

One may argue that those patients with elevated liver enzymes should have been excluded from this study. However, the relevance of population pharmacokinetic studies is again highlighted as their inclusion allows one to search for potential covariate markers that influence drug metabolism.

In this study, there was no relationship between any liver function measurement and the pharmacokinetics of INH and RFA.

Albumin and globulin together comprise the measure of total protein according to the assay system used by the KGV laboratory. These show an inverse relationship to each other *(Sherlock, 1981)*. The HIV–, MDR-TB patients had significantly higher albumin levels than patients from the other groups (p < 0.001). This observation could not be extended into a statistically significant difference between either the MDR-TB and drug-sensitive patients or the HIV+ and HIV- patients. It was also noted that HIV- patients had lower globulin levels than the HIV+ patients (p<0.001).
These may have implications for drug binding. However, during the NONMEM analysis, the data did not unequivocally support the inclusion of globulin as a covariate affecting pharmacokinetics. In the case of INH, a marginally significant influence of serum globulin on CL/F was noted. Although globulin was included in the final INH model, further investigation of this covariate is considered necessary to

exclude a potentially spurious effect. This is especially important since it is known that binding of INH to plasma proteins is low i.e. between 4 and 30% (*Gelman and Rumack 1998*).

Radiology

The majority of the patients included in the study had extensive lung field involvement with multiple cavities as noted from the radiological assessments. This was to be expected in this select population from a specialist referral hospital where one is likely to encounter patients with more severe disease. There was no statistically significant difference between MDR-TB and drug-sensitive patients with respect to the severity scores. However, the HIV+ patients had lower radiographic severity scores than the HIV- patients (extent 4.8 ± 1.1 vs. 5.2 ± 1.0 p = 0.02; cavitation 3.8 ± 1.6 vs. 4.3 ± 1.4 p = 0.06).

This apparent incongruity is well described in the literature. As the HIV disease progresses, the clinical and pathological picture of pulmonary TB changes. There is less necrosis and cavitation, bacilli become abundant and the chest radiograph shows infiltration (*Zeind et al. 1996; Wilkinson and Moore, 1996b*). It should be noted that necrosis and cavitation occurs as the immune response to TB infection.

The method upon which the radiographic classification system was based dates back to 1966 (*Simon, 1966*). However, its relevance and utility is emphasised by this method being frequently quoted and used by relatively modern studies after minor modifications. One of the difficulties with evaluation of radiographs for research purposes is that of observer-error (*Simon, 1966*). In this study, a specialist radiologist with several decades of experience in TB was responsible for all radiographic evaluations. In addition, free discussion of the score for each radiograph was conducted with the principal investigator.

The purpose of the radiographic classification in the present analysis was to ensure that there was no bias in terms of distribution of patients according to severity of disease. In some disease states patients with more severe disease tend to have altered pharmacokinetics e.g. quinine in acute malaria (*Mandell and Petri, 1996*). In this study there was no effect of severity of pulmonary involvement on the pharmacokinetics of INH and RFA.

Of necessity, certain aspects of the scoring system will be debatable; e.g. a score of 3 for a patient with a single large cavity occupying almost an entire lung field vs. a score of 4 for someone with multiple small cavities may not correlate with clinical severity. Despite this limitation, the scoring system provides a quantitative tool for the assessment of pulmonary involvement using chest radiographs.

Study Site

This study was conducted at the TB unit of a large specialist referral unit for patients primarily from KwaZulu-Natal. The patients recruited into the study were thus likely to be those with more severe disease. The case-control design of the study reduced the effect of this bias on the results relating to the primary research questions of the study. However, any differences between TB patients in general and this study population need to be borne in mind when generalising and interpreting the results.

Missing Data

This study included several patients in whom some data were missing e.g. LFT results or microbiology results. These missing results did not represent a source of bias because the missing values were evenly distributed among the groups – hence retaining comparability of the groups. The missing values were highlighted during the presentation of the results and included several patients with incomplete pharmacokinetic profiles. These latter patients had to be excluded from the non-compartmental analysis procedure since a representative AUC could not be calculated. Their inclusion in the NONMEM analysis highlights another advantage of the population approach to pharmacokinetic parameter estimation i.e. the use of data from protocols that have sparse sampling schedules.

During population pharmacokinetic data analysis, there are often concerns about assuming that missing indicator variables are negative or about setting missing data to the group median value. In this study, 1 patient who withdrew her consent for HIV testing after recruitment into the study was assumed to be HIV- and 2 patients who were resistant to INH alone were assumed to be drug sensitive. This low incidence of missing indicator variables was considered to be unlikely to influence the data analysis. As regards the 15 patients with missing liver function test results, the absence of clinical features of liver function abnormality was confirmed before setting these patient's missing data to the group median value. In addition, NONMEM runs in which these patient's data was excluded were compared to runs where the data was included as the group median value. In all cases, there was no change to the decision regarding the covariate model development, thus confirming the validity of this procedure.

Sampling Strategy

There were several reasons for employing the unusual sampling strategy of drawing the pharmacokinetic profiles over two days. The main reason was to minimise disruption of a ward with 30 to 50 sleeping patients at 05h00 – the usual time of dose administration so as to attempt venipuncture on a large number of patients. It was more convenient to insert the in-dwelling venous cannula and commence sampling the day before. An alternative would have been to alter the time of dose administration, but this was considered unacceptable since the study protocol required that there be minimal changes to the usual clinical scenario. It was the intention to study drug usage under operational conditions – an important advantage and requirement of the population approach.

One advantage of using this strategy was that it provided more information on intra-individual variability since blood samples were collected over 2 dosing intervals. It did however, require the assumption that sampling was done over a single dosing interval when conducting the non-compartmental pharmacokinetic analysis.

Protocol Deviations

The conscious and constant attempts to minimise disruption of the normal ward routine were important for the population approach protocol and was appreciated by both the medical and nursing personnel. This was also reflected in the high quality of

the data and the small number of protocol deviations. When these occurred, the principal investigator was promptly made aware of the breach. An unavoidable change to the ward routine was the instruction to withhold drug administration on the second study day until the pre-dose sample had been collected. On only 1 of the 13 days over which the study was conducted was this instruction disregarded and 9 patients inadvertently received a second dose of drug.

Failure to dose with RFA for a period of time sufficient to attain maximal enzyme auto-induction presented a confounder for MDR-TB since these patients were in the majority in this group. However, this offered an opportunity to investigate RFA auto-induction from a NONMEM perspective. The exploratory data analytical tools such as the GAM analysis were especially useful in deciding on the best estimate of the time to maximal enzyme auto-induction.

Concurrent Medications

Ethical considerations and minimal disruption of normal ward routine also dictated that there were no restrictions on the use of occasional medication prescribed for temporary relief of symptom e.g. analgesics and antacids. The population approach allows for such possible drug interactions to be investigated although cell sizes are often not large enough to make definitive conclusions. These observations of potential interactions are usually hypothesis generating. There was a low incidence of concurrent drug therapy in this study.

Drug Assay

RFA and INH are both reported to be unstable compounds in biological fluids *(Weber et al. 1983; Hutchings et al. 1983)*. Anti-TB drug assays were conducted at a WHO recognised analytical laboratory that adheres to stringent Good Laboratory Practice (GLP) standards. A strict protocol was employed during sampling preparation, storage and transport of samples at –85°C. Some analysts recommend the inclusion of anti-oxidants (e.g. ascorbic acid) in samples containing RFA to prevent its degradation prior to assay *(Weber et al. 1983)*. The method employed in the present study was to employ short runs of not more than 10 samples per run. The laboratory has not observed any significant degradation in sample or difficulties with regard to stability of samples in their on-going quality control procedures.

Population Pharmacokinetic Model Building

The procedure used for model building during the NONMEM analysis was the traditional and tedious method. Several authors have recommended the use of newer exploratory data analysis techniques such as the GAM analysis and tree based modelling techniques *(Mandema et al. 1992; Verotta, 1997)*. These greatly expedite the process of identification of important covariates. These were used in the present analysis on an *ad hoc* basis. It is acknowledged that the path to the final model (see Appendix G and J) could have been accelerated if there had been wider implementation of these techniques.

Definitions

In this study, strict definitions for the primary research outcome parameters of HIV and drug resistance confirmation were used. A patient was defined as drug resistant only if confirmed with drug susceptibility tests conducted according to strict laboratory procedures. This was invariably corroborated with a poor clinical response to standard chemotherapy. In a similar manner, the majority of drug sensitive patients were confirmed with susceptibility tests. However, since drug sensitive patients frequently have rapid clearance of AFB from the sputum, microbiological confirmation of susceptibility was not always possible. In 10 cases, the classification was based on the presence of at least 3 consecutive monthly sputum smears that were negative for acid fast bacilli. This definition is in keeping with accepted clinical practice *(Anonymous1996)*.

HIV status was confirmed using 2 and in most cases 3 immunoassay tests. The HIV viral load provided further confirmation of the HIV status. A potential but unavoidable difficulty was the possibility that some of the HIV – patients were in the so-called 'window period' during which they have immunologically undetectable infection *(Fauci and Lane, 1991)*.

The definition of MDR-TB as being resistant to at least both INH and RFA used in this study is that suggested by the WHO *(Kochi et al. 1993)*. Further, for purposes of this study, patients with drug-sensitive TB were those who did not have resistance to INH or RFA. However, this group included 23 patients (32%) with resistance to 1 or more second-line anti-TB drugs other than INH and/or RFA. This consisted of 3 patients with resistance to streptomycin, 17 with resistance to ethionamide, 14 with resistance to cycloserine and 5 with resistance to thiacetazone. These patients also complied with the clinical definition of drug-sensitive viz. at least 3 consecutive monthly sputum smears that were negative for acid fast bacilli.
The inclusion of these patients in the drug-sensitive group does not minimise the importance of the resistance they displayed – rather the intention was to emphasise the importance of resistance to INH and RFA. These 2 drugs are essential components for the rapid bactericidal effect required in the WHO's short course chemotherapy regimen.
However, the possibility of methodological difficulties with the susceptibility testing such as the definition and interpretation of breakpoints for susceptibility for the second line anti-TB drugs may have contributed to detection of drug resistance in the drug-sensitive group *(Johnson, 1991)*.

Among the MDR-TB patients, only 1 patient had resistance to 2 drugs i.e. INH and RFA only. The majority of patients, 52 of 62 (84%) patients, displayed resistance to $\geq$ 5 drugs. This highlights the dismal and depressing scenario faced by the clinician that attempts to treat MDR-TB. Resistance to the first line anti-TB drugs leaves one with having to choose from less effective and usually more toxic second and third line agents. This corroborates Mitchison's hypothesis that INH and RFA are resistance protective agents i.e. they prevent the emergence of resistance to their companion drugs *(Mitchison, 1992)*. The removal of INH and RFA from the regimen due to drug resistance results in the rapid acquisition of resistance to the other drugs in the regimen.

The therapeutic dilemma posed by this scenario highlights the urgent need for research into new TB drugs or treatments and the better utilisation of available

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agents. Recent reports *(Grassi and Peona, 1995)* cast little hope about the former – hence our attention defaults to the latter option.

Treatment Protocols

The mainstay of treatment for the MDR-TB patients in this study was a quinolone and an aminoglycoside. It was noteworthy that this study showed a low level of resistance to these drugs among all patients tested i.e. 3-4% for the quinolones and 8% for kanamycin. Pyrazinamide continues to be used despite a lack of knowledge of its susceptibility patterns. Due to methodological difficulties pyrazinamide resistance is not routinely assessed at KGV. However, the need for ongoing surveillance of microbiological trends as highlighted in Chapter 3 cannot be ignored.

Summation

In summary therefore, this study found no association between the pharmacokinetics of INH and RFA and MDR-TB. Neither was there any association between HIV status or degree of immune compromise and the pharmacokinetics of INH and RFA. Using the population approach, the pharmacokinetics of INH and RFA were described in a population of pulmonary TB patients stratified according to HIV status. The importance of enzyme auto-induction on CL/F and V/F for RFA and the higher proportion of fast acetylators for INH in Black South Africans were noted. Population pharmacodynamic parameters for INH and RFA were described and represent potential benchmarks for future prospective clinical evaluation.

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Chapter 7

CONCLUSIONS

No significant difference between MDR-TB patients and drug-sensitive TB patients was found with regards to the pharmacokinetic characteristics of INH and RFA.

Similarly, no difference was noted between HIV+ and HIV- patients with regard to the pharmacokinetic characteristics of INH and RFA.

This study found that upon initiation of treatment, the average 54-kg patient has a CL/F for RFA of 7.7 L/hr. After continuous daily treatment, maximal enzyme auto-induction was reached at approximately 10 days at which time the CL/F was 15.6 L/hr. The mean population V/F for RFA was 26.4 L at initiation of treatment and 42.1 L after 10 days of therapy. The inter-individual variability expressed as a % coefficient of variation (CV) for RFA was 39% for CL/F and 26% for V/F. Residual or intra-individual variability was described with a proportional component of 39% and an additive component of 0.05 μg/ml.

The population pharmacokinetic parameters for RFA obtained in this study agree well with that reported in the literature.

In this study, the proportion of INH fast acetylators in the population was found to be in the majority (85%). This proportion is in agreement with reports in the literature that fast acetylators are in the majority in South African Black patients – the main population group studied.

The mean population CL/F for slow acetylators of INH was found to be 4.7 L/hr while fast acetylators had a CL/F of 13.0 L/hr. The V/F for INH obtained in this study was 50.0 L. These values are similar to those reported in the literature. The inter-individual variability in INH CL/F was 32% for slow acetylators and 41% for fast acetylators while there was a 41 % variability in V/F. Intra-individual variability was described with a proportional component of 28% and an additive component of 0.02 μg/ml.

Drug-sensitive TB patients displayed higher and more prolonged drug concentrations in excess of the MIC than MDR-TB patients. This is reflected in the higher values for their pharmacodynamic parameters.

The majority of drug-sensitive patients had Cmax concentrations in excess of 50 x the MIC for INH and 13 x the MIC for RFA.

Slow acetylators of INH experienced drug concentrations above the MIC for the entire dosing interval compared to only approximately 16 hours for fast acetylators. In the case of RFA, serum concentrations were maintained above the MIC for greater than 9 hours of the dosing interval in the majority of drug-sensitive patients.

A small group of drug-sensitive patients displayed low drug exposure pharmacokinetic parameters (AUC>MIC) for INH. The clinical relevance of this is unknown.

There was no association between INH acetylator status and drug resistance.

There were no differences between HIV+ and HIV- patients with respect to their pharmacodynamic parameters.

Appendix A   PATIENT/VOLUNTEER INFORMATION SHEET

Appendix A

TUBERCULOSIS DRUG RESISTANCE STUDY

PATIENT/VOLUNTEER INFORMATION SHEET

Thank you for your interest in this study.

Objective

This purpose of this study is to determine why particular patients with tuberculosis (TB) develop resistance to their anti-TB drug treatment.

Importance of your participation

Although highly effective treatment against TB is available, the TB bacillus is constantly developing resistance to the drugs used. Resistance to anti-TB drug treatment is therefore a problem of great importance to medical science and research in this area is a major priority.

As a patient with TB, this study may have immediate implications for your treatment. In addition, on the long term, it will benefit other TB patients.

Instructions

All volunteers must ensure that they take their anti-TB treatment regularly for at least 1-week before the day of the study. If any doses are omitted, this must be reported to the clinic staff.

An overnight fast (no food or drink) from 22h00 (10pm) on the evening before the study day will be imposed. One glass of water will be permitted upon awakening on the morning of the study day.

DO NOT TAKE YOUR ANTI-TB MEDICATION ON THE MORNING OF THE STUDY DAY, HOWEVER, IT **MUST** BE CARRIED WITH YOU TO THE STUDY CENTRE, AS THIS WILL BE ADMINISTERED TO YOU AFTER THE FIRST BLOOD SAMPLES HAVE BEEN WITHDRAWN.

We require your permission to collect information from your clinic and/or hospital records. The following tests will be conducted if your doctor has not already done them:

HIV status
Anti-TB drug levels
Sputum examinations

Procedure

On the morning of the study day, patients will report to the study centre at 07h00 after an overnight fast of at least 10 hours and after rectal and bladder emptying.

Appendix A

Upon entry into the study centre, a blood sample (8 ml) will be withdrawn prior to you taking your medications. Patients will then be asked to take their anti-TB medication under supervision and the time will be recorded.
Thereafter, further blood samples (8ml each) will be withdrawn at 1, 2, 4, 8 and 12 hours after medication administration.

Patients will be required to return to the study centre 1 week later at which time the results of the above tests will be discussed with you.

Hospital in-patients may have the above schedule adjusted to follow the normal ward routine.

Contacts

If there are any further questions that you may wish to ask, please contact the people mentioned below.

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Appendix B   DEMOGRAPHIC CHARACTERISTICS

Appendix B

DEMOGRAPHIC CHARACTERISTICS

ID	Date of Birth yy/mm/dd	SEX	Date of Admission yy/mm/dd	History of TB treatment	MDR	HIV	Group	Date of Study yy/mm/dd	Weight (kg)	Height (cm)	AGE (years)	RACE
1	58/09/24	1	96/10/15	5	1	1	1	97/03/19	51.8	170	39	
2	52/05/15	1	96/12/18	3	0	1	3	97/03/19	48.7	171.5	45	
3	65/09/15	1	96/08/29	0	0	1	3	97/03/19	70	178	32	
4	61/02/26	1	97/02/24	4	1	1	1	97/03/19	43.3	169	36	
5	58/11/19	1	96/11/26	0	0	1	3	97/03/19	58	175	39	
6	55/04/20	1	96/09/27	0	1	0	2	97/03/19	55.7	170	42	
7	54/11/25	1	97/02/10	5	1	0	2	97/03/19	99.5	176	43	
8	57/07/01	1	96/10/07	5	1	0	2	97/04/09	57.25	173	40	
9	53/04/23	1	97/01/28	5	0	0	4	97/04/09	65.5	163	44	
10	71/05/15	1	97/02/20	0	0	1	3	97/04/09	62	176.5	26	
11	58/06/25	1	97/01/20	5	1	0	2	97/04/09	55.5	172.5	39	
12	60/11/26	1	96/11/11	5	1	0	2	97/04/09	48.75	169	37	
13	57/06/24	1	96/08/26	5	1	0	2	97/04/09	57.25	167	40	
14	65/08/18	1	97/03/24	5	1	0	2	97/04/16	49	167.5	32	
15	61/12/02	1	96/07/11	0	1	0	2	97/04/16	65	168	36	
16	52/09/17	1	97/03/13	5	1	0	2	97/04/16	57	175.5	45	
17	47/12/25	1	97/04/01	5	1	0	2	97/04/16	41	167	50	
18	69/02/16	1	97/02/21	1	1	0	2	97/04/16	55	171	28	
19	64/05/05	1	96/11/13	4	1	1	1	97/04/16	45	163	33	
20	60/07/21	1	96/11/21	5	1	1	1	97/04/16	40	169	37	
21	67/12/09	1	96/11/27	5	1	0	2	97/04/16	62	183	30	
22	54/10/02	1	96/12/24	4	1	0	2	97/04/16	62	169	43	
23	58/09/15	1	97/04/07	5	1	0	2	97/04/16	60	176	39	
24	59/07/03	1	97/02/20	5	1	0	2	97/04/16	60	173	38	
25	44/01/03	1	97/01/28	0	0	0	4	97/04/23	86.4	184	53	
26	41/04/30	1	97/04/08	3	0	0	4	97/04/23	82.2	174	56	
27	53/10/15	1	97/01/21	4	0	0	4	97/04/23	63.3	171	44	
28	45/03/02	1	97/03/12	5	1	0	2	97/04/23	53.8	165	52	
29	69/04/04	1	96/11/11	5	1	0	2	97/04/23	57	182	28	
30	47/06/04	1	97/01/16	0	0	1	3	97/04/23	65.2	165	50	
31	62/11/02	1	97/01/16	3	0	0	4	97/04/23	48.5	154	35	
32	46/03/06	1	97/01/29	5	2	1		97/04/23	45	161	51	
33	53/05/08	1	96/12/10	0	1	0	2	97/04/23	43.5	156.5	44	
34	49/01/25	1	97/02/06	4	0	0	4	97/04/23	59	173	48	
35	64/02/10	1	97/03/25	3	0	1	3	97/04/23	61	177	33	
36	50/03/11	1	97/01/06	0	0	0	4	97/04/23	40	163	47	
37	67/07/01	1	97/04/04	0	0	1	3	97/04/23	56	171	30	
38	58/10/07	1	97/03/26	0	0	0	4	97/04/23	60	166.5	39	
39	71/05/01	1	97/03/03	0	0	0	4	97/04/23	59	173.5	26	
40	70/10/23	1	96/12/31	0	0	0	4	97/04/23	56	159.5	27	
41	69/02/28	1	97/03/26	2	0	0	4	97/05/07	46	134.5	28	
42	56/07/01	1	97/04/30	0	0	0	4	97/05/07	46.7	174	41	
43	54/03/01	1	97/02/24	4	0	0	4	97/05/07	75.3	172	43	
44	65/07/01	1	97/02/06	0	0	0	4	97/05/07	60.1	173	32	
45	66/04/01	1	97/03/27	0	0	1	3	97/05/07	49	135	31	
46	66/07/01	1	97/03/11	0	0	1	3	97/05/07	62.8	183	31	
47	60/07/01	1	97/03/11	3	0	0	4	97/05/07	58.5	169	37	

SEX – 0=female, 1=male; History of TB treatment – 0=new case, 1=re-treatment case, 2&3=re-treatment after previous cure or treatment completion; 4&5=re-treatment after previous treatment interuption or failure; MDR – 0=no, 1=yes, GROUP – 1=MDR+ & HIV+, 2=MDR+ & HIV-, 3=MDR- & HIV+, 4=MDR- & HIV-; RACE – Blank = African, 2 = "Coloured", 3 = White.

Appendix B

DEMOGRAPHIC CHARACTERISTICS

ID	Date of Birth yy/mm/dd	SEX	Date of Admission yy/mm/dd	History of TB treatment	MDR	HIV	Group	Date of Study yy/mm/dd	Weight (kg)	Height (cm)	AGE (years)	RACE
48	49/12/09	1	97/04/15	3	0	0	4	97/05/07	65.5	178	48	
49	55/04/14	1	97/02/12	3	0	0	4	97/05/07	76.5	178	42	
50	69/09/19	1	97/01/07	0	0	1	3	97/05/07	73	174.5	28	
51	79/12/24	0	97/04/11	0	2	0		97/05/14	46.3	160	18	
52	79/02/07	0	97/01/06	0	0	0	4	97/05/14	60.5	164.5	18	
53	69/12/21	0	97/02/14	0	0	1	3	97/05/14	65	163	28	
54	75/11/18	0	97/04/02	0	0	1	3	97/05/14	52	152	22	
55	80/09/05	0	97/03/24	3	0	0	4	97/05/14	43.6	152	18	
56	66/02/18	0	97/01/21	4	0	1	3	97/05/14	59.9	162.5	31	2
57	46/12/30	0	97/04/15	0	0	0	4	97/05/14	64.5	164.5	51	
58	75/07/30	0	97/02/02	0	0	1	3	97/05/14	46.5	152	22	
59	69/12/09	0	97/04/14	0	0	1	3	97/05/14	52	154	28	
60	47/12/26	0	97/02/12	0	0	0	4	97/05/14	45.1	155	50	
61	76/07/10	0	97/02/06	4	0	0	4	97/05/14	71.3	171.5	21	
62	37/10/08	0	97/03/10	0	0	0	4	97/05/14	78.3	152	60	
63	73/10/28	0	96/07/26	4	1	1	1	97/05/21	52.3	158	24	
64	60/12/15	0	97/02/14	0	0	1	3	97/05/21	54.2	153.4	37	
65	77/06/02	0	97/02/04	0	0	1	3	97/05/21	54.3	161	20	
66	43/12/24	0	97/03/14	0	1	0	2	97/05/21	56.4	159.5	54	
67	74/07/01	0	97/03/12	0	0	1	3	97/05/21	43.9	155.1	23	
68	71/08/17	0	96/08/13	0	1	1	1	97/05/21	51	166.4	26	
69	72/04/12	0	96/09/16	0	1	0	2	97/05/21	45.7	156.5	25	
70	57/04/20	0	96/10/07	4	1	0	2	97/05/21	73.5	160.5	40	
71	65/07/01	0	97/02/07	0	0	1	3	97/05/21	52.4	148.8	32	
72	32/02/02	0	97/04/25	0	0	0	4	97/05/21	48	163	65	2
73	52/07/01	0	97/04/29	0	0	1	3	97/05/21	39.5	149.3	45	
74	47/03/06	0	97/02/25	4	1	0	2	97/05/21	41.7	150.3	50	3
75	50/01/11	0	96/11/15	0	1	1	1	97/05/21	81.8	161	47 ·	
76	65/03/21	0	97/01/29	0	1	1	1	97/05/21	44	158	32	
77	73/05/23	0	97/04/08	5	1	0	2	97/05/21	45.2	155.5	24	
78	49/06/25	0	96/09/02	4	1	0	2	97/05/21	70.8	159	48	
79	60/04/21	1	97/02/25	3	1	1	1	97/05/28	46	164	37	
80	32/07/01	1	96/12/19	2	1	0	2	97/05/28	52	140	65	
81	75/02/03	1	97/04/21	4	1	1	1	97/05/28	57	150	22	
82	71/08/08	1	97/02/11	4	1	0	2	97/05/28	52	171.5	26	
83	58/09/20	1	97/04/29	2	1	0	2	97/05/28	52	166	39	
84	47/11/25	1	96/10/22	4	1	0	2	97/05/28	50	175	50	
85	47/07/01	1	96/10/24	4	1	0	2	97/05/28	51	166	50	
86	32/04/12	1	97/04/18	0	0	0	4	97/05/28	62.5	162	65	
87	38/06/11	1	97/01/08	2	1	0	2	97/05/28	52.5	172	59	
88	49/07/27	0	97/04/17	0	0	0	4	97/06/11	43.3	149	48	
89	72/01/18	0	97/06/10	5	1	1	1	97/06/11	66	171	25	
90	73/01/22	0	96/12/24	3	0	0	4	97/06/11	49.2	150	24	
91	74/11/25	0	97/04/14	0	0	1	3	97/06/11	34.8	146	23	
92	66/08/07	0	97/03/03	0	0	1	3	97/06/11	44.1	161	31	
93	63/01/07	0	96/12/09	4	1	0	2	97/06/11	44	152	34	
94	69/06/06	0	97/01/21	5	1	1	1	97/06/11	47.5	165	28	

SEX – 0=female, 1=male; History of TB treatment – 0=new case, 1=re-treatment case, 2&3=re-treatment after previous cure or treatment completion; 4&5=re-treatment after previous treatment interuption or failure; MDR – 0=no, 1=yes, GROUP – 1=MDR+ & HIV+, 2=MDR+ & HIV-, 3=MDR- & HIV+, 4=MDR- & HIV-; RACE – Blank = African, 2 = "Coloured", 3 = White.

DEMOGRAPHIC CHARACTERISTICS

ID	Date of Birth yy/mm/dd	SEX	Date of Admission yy/mm/dd	History of TB treatment	MDR	HIV	Group	Date of Study yy/mm/dd	Weight (kg)	Height (cm)	AGE (years)	RACE
95	62/12/13	0	97/03/19	3	1	0	2	97/06/11	67.1	164	35	
96	77/02/07	0	97/02/10	3	1	0	2	97/06/11	55	155.5	20	
97	61/11/18	0	97/01/24	0	1	1	1	97/07/09	47.3	152	36	
98	67/03/27	0	97/02/24	4	0	1	3	97/07/09	77	174	30	
99	72/08/15	0	97/06/17	0	0	1	3	97/07/09	48.1	153	25	
100	62/05/10	0	97/02/13	4	0	0	4	97/07/09	54.8	166.5	35	
101	70/04/30	0	97/06/04	0	0	1	3	97/07/09	45.9	149	27	
102	65/01/30	0	97/05/27	0	0	0	4	97/07/09	44.4	160	32	
103	68/12/06	0	97/05/13	0	0	0	4	97/07/09	41	159	29	
104	78/06/28	0	97/06/03	0	0	1	3	97/07/09	42.3	160	19	
105	46/01/08	0	96/10/31	3	1	0	2	97/07/09	73.2	152	51	
106	64/12/04	0	97/05/03	4	0	1	3	97/07/09	48.6	156	33	
107	68/09/16	0	97/06/04	4	0	1	3	97/07/23	47.2	152	29	
108	78/12/27	0	97/05/02	4	0	1	3	97/07/23	62	163	19	
109	76/06/20	0	96/10/28	4	1	1	1	97/07/23	51.5	154	21	
110	69/09/16	0	97/04/25	0	0	1	3	97/07/23	53	154.5	28	
111	71/11/23	0	96/12/06	4	1	0	2	97/07/23	38.5	157	26	
112	71/04/06	0	96/10/29	2	1	0	2	97/07/23	54	165	26	
113	44/10/28	0	96/11/12	0	1	0	2	97/07/23	79	168	53	
114	66/12/17	0	97/04/22	4	1	0	2	97/08/06	54	120.5	31	
115	68/11/17	0	97/02/05	2	1	0	2	97/08/06	46.5	120.5	29	
116	72/05/27	0	97/07/30	4	0			97/08/06	46	120.5	25	
117	65/07/01	0	97/07/22	0	0	1	3	97/08/06	59	120.1	32	
118	73/10/24	0	97/06/09	0	0	1	3	97/08/06	45	120.1	24	
119	79/07/01	0	97/04/17	0	0	0	4	97/08/06	46	110.5	18	
120	65/12/12	0	97/05/29	4	1	0	2	97/08/06	47	120.2	32	
121	61/07/01	0	97/03/13	4	1	0	2	97/08/06	58	120.4	36	
122	72/06/30	0	97/08/01	0	0	1	3	97/08/06	63	158	25	
123	65/11/15	0	97/04/29	0	1	1	1	97/08/06	61	156	32	
124	69/05/23	0	97/04/01	0	1	1	1	97/08/06	45.8	157	28	
125	37/10/21	0	97/07/24	3	1	1	1	97/08/13	56.5	157.5	60	
126	51/01/15	1	97/07/04	3	1	1	1	97/08/13	61.5	165	46	
127	67/04/25	1	97/04/10	4	1	1	1	97/08/13	48.5	159.5	30	
128	68/04/23	0	97/07/08	5	0	1	3	97/08/13	41.75	150	29	
129	38/06/25	0	97/06/30	0	0	0	4	97/08/13	64	155.5	59	
130	70/09/12	0	97/05/14	0	0	1	3	97/08/13	65	164.5	27	
131	78/03/24	0	97/08/01	0	0	1	3	97/08/13	55.8	154	19	
132	71/12/12	0	97/07/22	0	0	1	3	97/08/13	50.2	165.5	26	
133	68/08/28	0	97/07/23	3	0	0	4	97/08/13	41.7	161	29	
134	69/10/07	0	97/08/04	2	1	1	1	97/08/13	41.2	154	28	
135	38/07/01	0	97/07/23	0	0	0	4	97/08/13	51	161.5	59	
136	64/08/02	0	97/06/23	0	0	0	4	97/08/13	53	160.5	33	
137	67/10/10	0	96/12/04	0	1	1	1	97/08/13	78.8	156.5	30	
138	52/01/06	0	97/06/17	0	0	0	4	97/08/13	47.7	151	45	

SEX – 0=female, 1=male; History of TB treatment – 0=new case, 1=re-treatment case, 2&3=re-treatment after previous cure or treatment completion; 4&5=re-treatment after previous treatment interuption or failure; MDR – 0=no, 1=yes, GROUP – 1=MDR+ & HIV+, 2=MDR+ & HIV-, 3=MDR- & HIV+, 4=MDR- & HIV-; RACE – Blank = African, 2 = "Coloured", 3 = White.

Appendix C   LIVER FUNCTION TEST RESULTS AND HIV VIRAL LOAD

Appendix C

LIVER FUNCTION TEST RESULTS & HIV VIRAL LOAD

ID	ALK u/L	BILI μmol/L	ALB g/L	GLOB g/L	GGT u/L	ALT u/L	AST u/L	VIRAL LOAD copies/ml
1	65	9	24	73	21	10	27	30598
2	108	12	16	77	134	15	27	17857
3								272483
4	84	15	16	49	39	14	50	587185
5		21	25	64	60	18		95640
6	70	9	27	57	60	31	50	
7	57	14	40	43	74	27	35	
8	88	15	28	44	78	34	29	
9	128	14	38	37	100	13	21	
10								9442
11	49	8	30	48	26	12	28	
12	110	14	19	51	29	15	23	
13	64	11	35	52	35	23	41	
14	65	9	28	43	21	5	18	
15	87	17	35	39	31	15		
16	54	10	31	51	34	15	22	
17	130	33	20	87	34	27	94	
18	100	16	20	57	42	11	23	
19	261	13	14	67	105	7	28	289655
20	135	9	18	77	150	28	47	120072
21								
22	74	13	29	52	35	7	26	
23	55	8	37	38	27	16	19	
24	58	18	32	46	20	27	25	
25	211	14	24	39	110	17	31	
26	85	10	36	42	31	14	23	
27	91	7	25	49	19	10	29	
28								
29	79	21	36	63	31	19	30	
30	86	8	27	51	30	15	40	
31	232	22	26	54	233	21	75	
32	88	13	21	50	43	10	24	1891
33	79	8	24	53	23	12	22	
34	59	11	19	46	28	20	40	
35	73	15	33	52	23	12	24	13747
36	75	10	20	44	32	18	24	
37	110	11	23	55	80	19	42	304267
38	67	18	23	44	25	16	25	
39	45	12	23	53	44	12	17	
40								
41	51	12	19	42	29	16	32	
42	139	9	26	42	34	26	32	
43	102	13	27	51	47	20	38	
44	61	10	30	54	34	16	26	
45								544444
46	188	11	28	50	99	55	55	726686
47	118	17	22	50	101	17	23	
48	87	6	23	53	23	15	33	
49	84	9	37	36	169	32	28	
50	107	15	36	37	116	31	33	19916

LIVER FUNCTION TEST RESULTS & HIV VIRAL LOAD

ID	ALK u/L	BILI μmol/L	ALB g/L	GLOB g/L	GGT u/L	ALT u/L	AST u/L	VIRAL LOAD copies/ml
51	96	10	22	47	32	11	27	
52	70	9	23	53	19	13	38	
53	68	14	26	47	33	18	36	11392
54	100	14	30	55	46	17	37	23210
55	74	11	31	57	25	8	15	
56	89	2	30	57	77	25	22	2721348
57								
58	128	10	16	57	53	14	35	1026087
59	83	12	18	59	19	12	40	170317
60	194	13	28	43	171	24	42	
61	55	8	26	49	34	13	25	
62	78	12	23	54	97	19	22	
63	114	9	36	60	43	26	56	49101
64	133	10	15	65	22	19	32	102211
65	68	7	35	44	97	112	87	200
66	74	10	30	36	21	17	21	
67	103	8	24	56	55	14	33	222790
68	83	18	31	48	48	37	41	11900
69	65	5	34	52	28	23	43	
70	47	8	35	43	24	11	18	
71	71	7	29	56	92	15	34	172330
72	52	4	29	56	50	16	23	
73	84	23	23	54	65	43	27	665761
74	69	6	39	48	22	16	27	
75	105	4	24	68	47	22	37	4550
76	47	11	19	79	38	11	28	623558
77	48	11	36	42	18	9	23	
78	119	4	26	47	67	24	43	
79	152	8	22	71	64	17	34	16567
80								
81	71	10	34	60	77	9	25	1493
82	112	9	20	55	26	9	22	
83								
84								
85	110	14	38	46	65	44	61	
86	55	20	46	43	27	52	46	
87	73	11	36	41	18	11	15	
88	72	13	24	47	86	9	31	
89	74	21	35	65	54	17	33	25478
90	78	10	24	50	32	10	39	
91	165	23	16	51	71	59	71	6408
92	51	2	26	44	45	15	23	31907
93	65	5	32	53	23	5	21	
94	74	9	32	61	56	17	24	150698
95	65	6	37	41	30	13	23	
96	129	11	30	54	156	23	28	
97	79	12	25	60	75	30	66	2382183
98	100	9	17	60	25	11	34	72273
99								46895
100	184	18	17	48	46	38	52	

Appendix C

LIVER FUNCTION TEST RESULTS & HIV VIRAL LOAD

ID	ALK u/L	BILI µmol/L	ALB g/L	GLOB g/L	GGT u/L	ALT u/L	AST u/L	VIRAL LOAD copies/ml
101	61	7	22	77	21	13	33	936691
102	192	10	16	58	25	21	38	
103	221	17	20	48	39	18	62	
104	97	8	21	73	23	18	32	18197
105	100	6	40	38	52	17	20	
106	202	19	17	60	86	14	47	
107	58	15	17	107	37	13	35	704947
108	97	11	24	72	12	20	52	58831
109	152	9	29	62	44	18	31	26949
110	100	17	21	46	31	16	48	1012308
111	78	7	19	50	37	20	30	
112	100	17	25	61	102	66	24	
113	54	10	30	31	18	11	20	
114	76	16	27	44	34	16	29	
115	124	7	30	52	95	27	38	
116	45	7	18	66	18	7	33	18546
117	59	6	25	52	19	13	17	4575
118	85	9	18	58	37	13	30	18473
119	231	16	17	52	46	10	27	
120	131	6	26	48	85	8	18	
121	102	11	23	50	73	45	93	
122	47	9	19	51	32	29	58	93225
123								65183
124	43	7	26	54	37	10	16	
125	95	3	17	75	36	18	36	337319
126	66	8	23	77	30	10	30	1135294
127	64	8	19	82	56	8	27	144325
128								13310
129	61	9	27	49	19	16	31	
130	117	15	15	58	54	23	53	156800
131	85	11	29	97	94	29	29	468
132	77	9	21	66	31	9	21	21388
133	69	8	24	46	43	14	19	
134								658537
135	54	11	29	80	36	30	37	
136	99	9	20	42	37	45	104	
137	46	14	23	64	30	12	34	2649
138	99	16	27	47	59	11	33	

Appendix D   DRUG SUSCEPTIBILITY RESULTS

Appendix D

DRUG SUSCEPTIBILITY RESULTS

ID	Isoniazid 0.2 µg/ml	Isoniazid 1.0 µg/ml	Rifampicin	Streptomycin 2.0 µg/ml	Streptomycin 10.0 µg/ml	Ethambutol	Capreomycin	Ethionamide	Cycloserine	Kanamycin	Ciprofloxacin	Ofloxacin	Thiacetazone	MIC-inh µg/ml	MIC-rfa µg/ml
1		1	1	1		1	0	0	1	0	0	0	0		
2		0	0			0									
3									.						
4	1	1	1	0	0	1	0	1	0	0	0	0	1	>16	>12
5		0	0			0									
6	1	1	1	0	0	1	0	1	0	0	0	0	1	>16	4
7	1	1	1	1	1	1	0	1	0	0	0	0	0	8	>12
8	1	1	1	1	1	1	0	1	0	1	0	0	1	16	>12
9	0	0	0	1	1	0	0	1	0	0	0	0	0	1	<0.5
10	0	0	0	0	0	0	0	1	1	0	0	0	1	1	<0.5
11	1	1	1	0	0	1	0	0	1	1	0	0	0	>16	>12
12	1	1	1	1	1	1	0	0	0	0	0	0	1	4	>12
13	1	1	1	1	1	1	0	1	0	0	0	0	1	8	>12
14	1	1	1	1	1	1	0	0	1	0	0	0	1	>16	8
15	1	1	1	1	1	1	1	1	1	1	0	0	0	16	>12
16	1	1	1	1	1	1	0	0	0	0	0	0	1	16	>12
17	1	1	1	1	1	1	0	1	0	0	0	0	0	>16	>12
18	1	1	1	1	1	1	0	1	1	0	0	0	1	4	12
19	1	1	1	1	1	1	0	1	1	0	0	0	1	8	>12
20	1	1	1	1	1	1	0	1	0	0	0	0	1	4	>12
21	1	1	1	0	0	1	0	1	0	0	0	0	1	>16	8
22	1	1	1	0	0	0	0	0	0	0	0	0	0	8	>12
23		1	1	1		1		1	0	0	0	0	1		
24	1	1	1	1	1	1	0	1	0	1	0	0	1	>16	>12
25	0	0	0	0	0	0	0	1	0	0	0	0	0	<0.1	<0.5
26	0	0	0	0	0	0	0	0	0	0	0	0	0	<0.1	<0.5
27		0	0			0									
28		1	1	1		1	1	1	1	1	1	1	1		
29	1	1	1	0	0	1	1	1	0	1	0	0	1	>16	2
30															
31		0	0			0									
32		1	0	0	0	0	0	0	1	0	0	0	0		
33	1	1	1	0	0	1	1	1	1	0	0	0	1	>16	4
34	0	0	0	0	0	0	0	0	0	0	0	0	0	<0.1	<0.5
35	0	0	0	0	0	0	0	0	0	0	0	0	0	<0.1	<0.5
36															
37	0	0	0	0	0	0	0	0	0	0	0	0	0	<0.1	<0.5
38	0	0	0	0	0	0	0	0	0	0	0	0	0	<0.1	<0.5
39	0	0	0	0	0	0	0	0	0	0	0	0	0	<0.1	<0.5
40	0	0	0	0	0	0	0	0	0	0	0	0	0	<0.1	<0.5
41	0	0	0	0	0	0	0	0	0	0	0	0	0	<0.1	<0.5

0 = sensitive, 1 = resistant, Blank = not known.

DRUG SUSCEPTIBILITY RESULTS

ID	Isoniazid 0.2 μg/ml	Isoniazid 1.0 μg/ml	Rifampicin	Streptomycin 2.0 μg/ml	Streptomycin 10.0 μg/ml	Ethambutol	Capreomycin	Ethionamide	Cycloserine	Kanamycin	Ciprofloxacin	Ofloxacin	Thiacetazone	MIC-inh μg/ml	MIC-rfa μg/ml
42															
43	0	0	0	1	1	0	0	1	0	0	0	0	1	<0.1	<0.5
44	0	0	0	0	0	0	0	0	0	0	0	0	0	<0.1	<0.5
45	0	0	0	0	0	0	0	0	0	0	0	0	0	<0.1	<0.5
46	0	0	0	0	0	0	0	0	0	0	0	0	0	<0.1	<0.5
47	0	0	0	0	0	0	0	0	0	0	0	0	0	<0.1	<0.5
48	0	0	0	0	0	0	0	0	0	0	0	0	0	<0.1	<0.5
49	0	0	0	0	0	0	0	0	0	0	0	0	0	<0.1	<0.5
50	0	0	0	0	0	0	0	0	0	0	0	0	0	<0.1	<0.5
51	1	1	0	0	0	0	0	1	0	0	0	0	0	4	<0.5
52	1	1	0	0	0	0	0	0	0	0	0	0	0		<0.5
53	0	0	0	0	0	0	0	0	0	0	0	0	0		
54	0	0	0	0	0	0	0	0	0	0	0	0	0	<0.1	<0.5
55	0	0	0	0	0	0	0	0	0	0	0	0	0	<0.1	<0.5
56	0	0	0	0	0	0	0	0	1	0	0	0	0	<0.1	<0.5
57	0	0	0	0	0	0	0	0	0	0	0	0	0	<0.1	<0.5
58	0	0	0	0	0	0	0	0	0	0	0	0	0		
59	0	0	0	0	0	0	0	0	0	0	0	0	0	<1	<0.5
60	0	0	0	0	0	0	0	0	1	0	0	0	0	<0.1	<0.5
61		0	0			0									
62	0	0	0	0	0	0	0	0	1	0	0	0	0	<0.1	<0.5
63	1	1	1	1	1	1	1	1	0	1	0	0	0	>16	12
64	0	0	0	0	0	0	0	0	0	0	0	0	0	<0.1	<0.5
65	0	0	0	0	0	0	0	1	0	0	0	0	0	1	<0.5
66	1	1	1	1	1	0	0	1	0	0	0	0	1	4	>12
67	0	0	0	0	0	0	0	0	0	0	0	0	0	<0.1	<0.5
68	1	1	1	1	1	1	0	0	0	0	0	0	0	8	>12
69	1	1	1	1	1	1	0	1	0	0	0	0	0	8	>12
70	1	1	1	1	0	1	0	1	0	0	0	0	1	>16	>12
71															
72	0	0	0	0	0	0	0	0	0	0	0	0	0	<0.1	<0.5
73	0	0	0	0	0	0	0	1	0	0	0	0	0	<0.1	<0.5
74	1	1	1	1	1	1	0	1	1	0	0	0	1	16	4
75		1	1	1		0	0	1	1	0	0	0	1		
76	1	1	1	1	1	0	0	1	0	0	0	0	0	4	>12
77	1	1	1	1	1	1	0	1	1	0	0	0	1	>16	>12
78	1	1	1	1	1	1	0	1	1	0	1	0	1	16	>12
79	1	1	1	1	1	1	0	1	1	0	0	0	1	>16	2
80	1	1	1	1	1	0	0	0	0	0	0	0	0	16	>12
81	1	1	1	1	1	1	0	1	0	0	0	0	0	4	>12
82	1	1	1	1	1	1	0	0	1	0	0	0	0	4	>12

0 = sensitive, 1 = resistant, Blank = not known.

Appendix D

DRUG SUSCEPTIBILITY RESULTS

ID	Isoniazid 0.2 µg/ml	Isoniazid 1.0 µg/ml	Rifampicin	Streptomycin 2.0 µg/ml	Streptomycin 10.0 µg/ml	Ethambutol	Capreomycin	Ethionamide	Cycloserine	Kanamycin	Ciprofloxacin	Ofloxacin	Thiacetazone	MIC-inh µg/ml	MIC-rfa µg/ml
83	1	1	1	0	0	1	0	1	0	0	0	0	0	4	>12
84	1	1	1	1	1	1	1	1	1	1	1	1	1	>16	>12
85	1	1	1	0	0	1	0	1	1	0	0	0	0	4	>12
86															
87	1	1	1	0	0	0	0	0	0	0	0	0	0	8	>12
88															
89	1	1	1	1	1	1	0	1	0	0	0	0	1	8	>12
90	0	0	0	1	1	0	0	0	1	0	0	0	0	<0.1	<0.5
91	0	0	0	0	0	0	0	1	1	0	0	0	0	<0.1	<0.5
92	0	0	0	0	0	0	0	0	0	0	0	0	0	<0.1	<0.5
93	1	1	1	1	1	1	0	1	0	0	0	0	0	4	>12
94		1	1	1		1	0	1		0	0	0	1		
95	1	1	1	0	0	0	1	0	0	0	1	1	0	>16	>12
96	1	1	1	1	1	1	0	1	0	0	0	0	1	8	>12
97	1	1	1	1	1	0	0	0	1	0	0	0	0	4	>12
98	0	0	0	0	0	0	0	1	1	0	0	0	1	<0.1	<0.5
99	0	0	0	0	0	0	0	0	0	0	0	0	0	<0.1	<0.5
100	0	0	0	0	0	0	0	1	1	0	0	0	0	<0.1	<0.5
101	0	0	0	0	0	0	0	0	0	0	0	0	0	<0.1	<0.5
102	0	0	0	0	0	0	0	1	0	0	0	0	0	<0.1	<0.5
103	0	0	0	0	0	0	0	1	1	0	0	0	0	<0.1	<0.5
104	0	0	0	0	0	0	0	0	0	0	0	0	0	<0.1	<0.5
105	1	1	1	0	0	0	0	1	0	0	0	0	1	16	4
106															
107															
108	0	0	0	0	0	0	0	0	1	0	0	0	0	<0.1	<0.5
109	1	1	1	1	1	1	0	1	0	0	0	0	1	>16	>12
110	0	0	0	0	0	0	0	1	1	0	0	0	0		
111	1	1	1	1	1	0	0	1	1	0	0	0	1	4	2
112		1	1	1		0	0	0	0	0	0	0	0		
113	1	1	1	1	1	1	0	1	0	0	0	0	1		
114	1	1	1	0	0	0	0	1	0	0	0	0	0	16	>12
115		1	1	1		1	0	1	0	0	0	0	1		
116	0	0	0	0	0	0								<0.1	<0.5
117	0	0	0	0	0	0	0	0	0	0	0	0	0		
118	0	0	0	0	0	0	0	0	0	0	0	0	0	<0.1	<0.5
119	0	0	0	0	0	0	0	1	0	0	0	0	1	<0.1	<0.5
120	1	1	1	0	0	0	0	0	0	0	0	0	0	<0.1	<0.5
121	0	0	1	0	0	1	0	1	0	0	0	0	1	8	2
122	0	0	0	0	0	0									
123	1	1	1	1	0	0	0	0	0	0	0	0	0	>16	>12

0 = sensitive, 1 = resistant, Blank = not known.

Appendix D

DRUG SUSCEPTIBILITY RESULTS

ID	Isoniazid 0.2 µg/ml	Isoniazid 1.0 µg/ml	Rifampicin	Streptomycin 2.0 µg/ml	Streptomycin 10.0 µg/ml	Ethambutol	Capreomycin	Ethionamide	Cycloserine	Kanamycin	Ciprofloxacin	Ofloxacin	Thiacetazone	MIC-inh µg/ml	MIC-rfa µg/ml
124	1	1	1	1	1	1	0	1	0	0	0	0	0	8	>12
125		1	1			1									>12
126	1	1	1	1	1	0	0	1	0	0	0	0	0		
127	1	1	0	0	0	0	0	0	0	0	0	0	0		
128	0	0	0	0	0	0									
129	0	0	0	0	0	0									
130	0	0	0	0	0	0	0	1	1	0	0	0	0	1	<0.5
131	0	0	0	0	0	0	0	1	1	0	0	0		1	<0.5
132	0	0	0	0	0	0	0	0	0	0	0	0	0	<0.1	<0.5
133	0	0	0	0	0	0	0	0	0	0	0	0	0		
134	1	1	1	0	0	0	0	0	0	0	0	0	0	>16	>12
135	0	0	0	0	0	0	0	0	0	0	0	0	0	1	<0.5
136	0	0	0	0	0	0	0	1	0	0	0	0	1	<0.1	<0.5
137	1	1	1	1	0	1	0	0	0	0	0	0	0		
138	0	0	0	0	0	0	0	1	1	0	0	0	0	<0.1	<0.5

0 = sensitive, 1 = resistant, Blank = not known.

Appendix E   RIFAMPICIN DOSE AND CONCENTRATION DATA

Appendix E

RIFAMPICIN DOSE AND CONCENTRATION DATA

ID	DATE (mm/dd/yy)	TIME	DOSE mg	CONC µg/ml
1	3/19/97	05:27	450	.
1	3/19/97	15:34	.	2.78
1	3/20/97	05:00	450	.
1	3/20/97	05:19	.	0.03
1	3/20/97	06:14	.	8.23
1	3/20/97	07:20	.	6.71
1	3/20/97	09:25	.	4.63
1	3/20/97	13:30	.	1.19
2	3/19/97	05:30	450	.
2	3/19/97	15:50	.	0.94
2	3/20/97	05:00	450	.
2	3/20/97	05:50	.	0.15
2	3/20/97	06:40	.	3.59
2	3/20/97	07:34	.	6
2	3/20/97	09:50	.	4.8
2	3/20/97	13:21	.	2.51
3	3/19/97	05:30	600	.
3	3/19/97	15:46	.	0.24
3	3/20/97	05:00	600	.
3	3/20/97	05:49	.	5.13
3	3/20/97	06:35	.	7.16
3	3/20/97	07:41	.	5.13
3	3/20/97	09:58	.	1.62
3	3/20/97	13:45	.	1.21
4	3/19/97	05:25	450	.
4	3/19/97	15:55	.	0.12
4	3/20/97	05:00	450	.
4	3/20/97	05:35	.	1.26
4	3/20/97	06:30	.	5.09
4	3/20/97	07:46	.	2.22
4	3/20/97	14:02	.	0.33
5	3/19/97	05:30	600	.
5	3/19/97	15:39	.	0.06
5	3/20/97	05:00	600	.
5	3/20/97	05:30	.	8.44
5	3/20/97	06:23	.	7.58
5	3/20/97	07:28	.	3.69
5	3/20/97	09:45	.	0.92
5	3/20/97	13:53	.	0.58
6	3/19/97	05:37	600	.
6	3/19/97	15:43	.	0.61
6	3/20/97	05:00	600	.
6	3/20/97	05:25	.	0.03

ID	DATE (mm/dd/yy)	TIME	DOSE mg	CONC µg/ml
6	3/20/97	06:18	.	0.03
6	3/20/97	07:25	.	2.04
6	3/20/97	09:35	.	2.44
6	3/20/97	13:47	.	0.77
7	3/19/97	05:25	600	.
7	3/19/97	15:59	.	1.09
8	4/9/97	05:00	600	.
8	4/9/97	12:50	.	3.25
8	4/9/97	16:05	.	1.2
9	4/9/97	05:00	600	.
9	4/9/97	12:44	.	0.4
9	4/9/97	16:08	.	0.18
9	4/10/97	05:20	600	.
9	4/10/97	05:20	.	0.03
9	4/10/97	06:20	.	0.03
9	4/10/97	07:27	.	12.5
9	4/10/97	09:22	.	7.14
10	4/9/97	05:00	600	.
10	4/9/97	12:39	.	0.89
10	4/9/97	15:37	.	0.2
10	4/10/97	05:34	.	0.03
10	4/10/97	05:35	600	.
10	4/10/97	06:28	.	7.51
10	4/10/97	07:37	.	8.09
10	4/10/97	09:43	.	3.63
11	4/9/97	05:10	600	.
11	4/9/97	12:30	.	4.02
11	4/9/97	16:11	.	1.74
11	4/10/97	05:29	.	0.08
11	4/10/97	05:30	600	.
11	4/10/97	06:24	.	1.9
11	4/10/97	07:30	.	12.7
11	4/10/97	09:38	.	8.03
12	4/9/97	05:00	600	.
12	4/9/97	12:22	.	5.4
12	4/9/97	15:45	.	2.56
12	4/10/97	05:50	.	0.03
12	4/10/97	05:51	600	.
12	4/10/97	06:37	.	0.03
12	4/10/97	07:55	.	5.44
12	4/10/97	09:51	.	6.98
13	4/9/97	05:00	600	.
13	4/9/97	12:15	.	4.03

Appendix E

RIFAMPICIN DOSE AND CONCENTRATION DATA

ID	DATE (mm/dd/yy)	TIME	DOSE mg	CONC µg/ml
13	4/9/97	15:55	.	1.73
13	4/10/97	05:11	.	0.03
13	4/10/97	05:12	600	.
13	4/10/97	06:13	.	3.96
13	4/10/97	07:21	.	12.5
13	4/10/97	09:27	.	10.3
14	4/16/97	04:35	450	.
14	4/16/97	18:00	.	0.42
14	4/17/97	05:30	.	0.03
14	4/17/97	05:34	450	.
14	4/17/97	06:30	.	2.7
14	4/17/97	07:32	.	12.4
14	4/17/97	09:43	.	6.64
15	4/16/97	05:09	600	.
15	4/16/97	12:50	.	1.77
15	4/16/97	17:31	.	0.25
15	4/17/97	06:04	.	0.03
15	4/17/97	06:06	600	.
15	4/17/97	06:58	.	12.1
15	4/17/97	08:07	.	12.5
15	4/17/97	10:15	.	5.62
16	4/16/97	04:45	600	.
16	4/16/97	13:11	.	5.68
16	4/16/97	17:27	.	1.26
16	4/17/97	05:43	.	0.08
16	4/17/97	05:45	600	.
16	4/17/97	06:39	.	3.39
16	4/17/97	07:43	.	13.4
16	4/17/97	09:56	.	7
18	4/16/97	04:38	600	.
18	4/16/97	12:22	.	4.97
18	4/16/97	17:15	.	0.78
18	4/17/97	06:22	600	.
18	4/17/97	06:22	.	0.03
18	4/17/97	07:19	.	0.06
18	4/17/97	08:20	.	4.37
18	4/17/97	10:03	.	5.44
19	4/16/97	05:00	450	.
19	4/16/97	12:29	.	4.52
19	4/16/97	17:00	.	1.44
19	4/17/97	05:36	450	.
19	4/17/97	05:36	.	0.02
19	4/17/97	06:35	.	3.13

ID	DATE (mm/dd/yy)	TIME	DOSE mg	CONC µg/ml
19	4/17/97	07:38	.	7.3
19	4/17/97	09:50	.	5.08
20	4/16/97	04:30	450	.
20	4/16/97	13:42	.	0.97
20	4/16/97	17:15	.	0.25
20	4/17/97	07:28	450	.
20	4/17/97	08:20	.	0.03
21	4/16/97	05:10	600	.
21	4/16/97	12:43	.	0.39
21	4/16/97	17:50	.	0.03
21	4/17/97	06:19	600	.
21	4/17/97	06:19	.	0.03
21	4/17/97	07:03	.	2.4
21	4/17/97	08:15	.	7.25
21	4/17/97	10:26	.	3.7
22	4/16/97	05:13	600	.
22	4/16/97	12:56	.	1.61
22	4/16/97	18:18	.	0.18
22	4/17/97	06:15	600	.
22	4/17/97	06:15	.	0.03
22	4/17/97	08:15	.	9.3
22	4/17/97	10:21	.	4.52
23	4/16/97	05:07	600	.
23	4/16/97	12:38	.	1.07
23	4/17/97	05:55	600	.
23	4/17/97	07:52	.	5.56
23	4/17/97	08:30	.	9.42
24	4/16/97	05:05	600	.
24	4/16/97	13:27	.	1.03
24	4/16/97	18:12	.	0.26
24	4/17/97	05:57	.	0.03
24	4/17/97	05:59	600	.
24	4/17/97	06:47	.	0.51
24	4/17/97	07:55	.	6.94
24	4/17/97	10:10	.	3.68
25	4/23/97	05:00	600	.
25	4/23/97	12:40	.	0.72
25	4/23/97	17:02	.	0.03
25	4/24/97	06:16	.	0.03
25	4/24/97	06:19	600	.
25	4/24/97	07:27	.	6.45
25	4/24/97	08:40	.	3.97
25	4/24/97	09:43	.	3.32

Appendix E

RIFAMPICIN DOSE AND CONCENTRATION DATA

ID	DATE (mm/dd/yy)	TIME	DOSE mg	CONC µg/ml
26	4/23/97	05:00	600	.
26	4/23/97	13:06	.	3.15
26	4/23/97	16:38	.	1.23
26	4/24/97	05:19	.	0.03
26	4/24/97	05:21	600	.
26	4/24/97	06:30	.	10.9
26	4/24/97	07:38	.	10.4
26	4/24/97	09:41	.	5.23
27	4/23/97	05:00	600	.
27	4/23/97	12:48	.	1.3
27	4/23/97	16:55	.	0.15
27	4/24/97	06:10	.	0.03
27	4/24/97	06:11	600	.
27	4/24/97	07:23	.	4.68
27	4/24/97	08:35	.	7.09
27	4/24/97	10:02	.	3.85
28	4/23/97	05:00	600	.
28	4/23/97	13:00	.	1.71
28	4/23/97	16:42	.	0.38
28	4/24/97	05:25	.	0.03
28	4/24/97	05:28	600	.
28	4/24/97	06:40	.	7.39
28	4/24/97	07:46	.	5.42
28	4/24/97	09:46	.	3.33
29	4/23/97	05:34	600	.
29	4/24/97	05:32	.	0.03
29	4/24/97	05:34	600	.
29	4/24/97	07:03	.	6.72
29	4/24/97	08:30	.	8.55
29	4/24/97	09:56	.	4.4
30	4/23/97	05:00	450	.
30	4/23/97	12:50	.	0.69
30	4/23/97	17:06	.	0.03
30	4/24/97	06:00	450	.
30	4/24/97	06:00	.	0.03
30	4/24/97	07:15	.	3.26
30	4/24/97	08:19	.	4.93
30	4/24/97	09:51	.	3.05
31	4/23/97	05:00	450	.
31	4/23/97	12:57	.	3.08
31	4/23/97	16:35	.	1.07
31	4/24/97	05:50	.	0.03
31	4/24/97	05:52	450	.

ID	DATE (mm/dd/yy)	TIME	DOSE mg	CONC µg/ml
31	4/24/97	06:55	.	2.66
31	4/24/97	08:08	.	8.15
31	4/24/97	10:05	.	5.08
32	4/23/97	05:00	450	.
32	4/23/97	13:23	.	1.29
32	4/23/97	16:52	.	0.03
32	4/24/97	05:43	450	.
32	4/24/97	06:50	.	0.13
32	4/24/97	07:55	.	1.63
32	4/24/97	09:59	.	2.74
33	4/23/97	05:14	450	.
33	4/23/97	13:13	.	1.59
34	4/23/97	05:12	600	.
34	4/23/97	13:34	.	0.91
34	4/23/97	16:15	.	0.26
34	4/24/97	05:27	.	0.03
34	4/24/97	05:31	600	.
34	4/24/97	06:27	.	0.03
34	4/24/97	07:13	.	1.25
34	4/24/97	08:23	.	3.48
35	4/23/97	04:55	600	.
35	4/23/97	13:23	.	0.26
35	4/23/97	16:44	.	0.09
35	4/24/97	05:18	.	0.03
36	4/23/97	04:50	450	.
36	4/23/97	12:46	.	4.96
36	4/23/97	16:20	.	2.01
36	4/24/97	06:05	.	0.03
36	4/24/97	06:10	450	.
36	4/24/97	07:00	.	0.03
36	4/24/97	08:00	.	0.75
36	4/24/97	09:05	.	6.13
37	4/23/97	04:52	450	.
37	4/23/97	14:10	.	1.02
37	4/23/97	16:53	.	0.45
38	4/23/97	04:53	600	.
38	4/23/97	13:45	.	1.27
38	4/23/97	16:50	.	0.18
38	4/24/97	05:37	.	0.03
38	4/24/97	05:42	600	.
38	4/24/97	06:50	.	0.32
38	4/24/97	07:48	.	3.51
38	4/24/97	08:51	.	3.1

Appendix E

RIFAMPICIN DOSE AND CONCENTRATION DATA

ID	DATE (mm/dd/yy)	TIME	DOSE mg	CONC µg/ml	ID	DATE (mm/dd/yy)	TIME	DOSE mg	CONC µg/ml
39	4/23/97	05:15	600	.	45	5/7/97	04:55	600	.
39	4/23/97	13:03	.	2.38	45	5/7/97	13:06	.	0.91
39	4/23/97	16:37	.	0.54	45	5/7/97	18:18	.	1.03
39	4/24/97	06:15	.	0.03	46	5/7/97	04:55	600	.
39	4/24/97	06:19	600	.	46	5/7/97	11:45	.	1.42
39	4/24/97	07:08	.	10.8	46	5/7/97	17:30	.	0.13
39	4/24/97	08:10	.	12.4	47	5/7/97	06:04	450	.
39	4/24/97	09:12	.	7.35	47	5/8/97	06:02	.	0.03
40	4/23/97	05:10	600	.	47	5/8/97	06:04	450	.
40	4/23/97	13:28	.	0.64	47	5/8/97	07:11	.	5.79
40	4/23/97	17:00	.	0.15	47	5/8/97	08:05	.	7.13
40	4/24/97	05:50	.	0.03	47	5/8/97	09:40	.	5.28
40	4/24/97	05:55	600	.	48	5/7/97	06:12	600	.
40	4/24/97	06:47	.	5.89	48	5/8/97	06:11	.	0.03
40	4/24/97	07:41	.	6.96	48	5/8/97	06:12	600	.
40	4/24/97	08:41	.	7.43	48	5/8/97	07:16	.	0.33
41	5/7/97	04:55	600	.	48	5/8/97	08:11	.	4.7
41	5/7/97	13:11	.	0.96	48	5/8/97	09:35	.	4.15
41	5/7/97	17:19	.	.0.17	49	5/7/97	04:55	600	.
41	5/8/97	05:57	.	0.03	49	5/7/97	11:27	.	3.47
41	5/8/97	05:59	600	.	49	5/7/97	17:40	.	0.28
41	5/8/97	06:59	.	0.03	49	5/8/97	05:18	.	0.03
41	5/8/97	07:53	.	8.47	49	5/8/97	05:20	600	.
41	5/8/97	09:15	.	6.13	49	5/8/97	06:25	.	7.73
42	5/7/97	05:54	450	.	49	5/8/97	07:24	.	6.97
42	5/8/97	05:53	.	0.03	49	5/8/97	09:09	.	4.18
42	5/8/97	05:54	450	.	50	5/7/97	04:55	600	.
42	5/8/97	06:56	.	5.78	50	5/7/97	11:32	.	1.28
42	5/8/97	07:41	.	6.4	50	5/7/97	17:56	.	0.07
43	5/7/97	04:55	600	.	50	5/8/97	05:30	.	0.03
43	5/7/97	12:08	.	1.08	50	5/8/97	05:32	600	.
43	5/7/97	18:00	.	0.03	50	5/8/97	06:31	.	5.03
43	5/8/97	05:34	.	0.03	50	5/8/97	07:30	.	4.57
43	5/8/97	05:38	600	.	50	5/8/97	09:22	.	3.1
43	5/8/97	06:41	.	9	51	5/14/97	04:58	450	.
43	5/8/97	07:35	.	7.82	51	5/14/97	13:39	.	2.64
43	5/8/97	09:28	.	3.98	51	5/14/97	17:46	.	0.63
44	5/7/97	04:55	600	.	51	5/15/97	05:43	.	0.03
44	5/7/97	11:55	.	0.47	51	5/15/97	05:45	450	.
44	5/7/97	18:11	.	0.03	51	5/15/97	07:02	.	0.2
44	5/8/97	05:46	600	.	51	5/15/97	08:25	.	2.54
44	5/8/97	06:48	.	9.14	52	5/14/97	04:52	450	.
44	5/8/97	08:36	.	7.25	52	5/14/97	12:55	.	1.13

Appendix E

RIFAMPICIN DOSE AND CONCENTRATION DATA

ID	DATE (mm/dd/yy)	TIME	DOSE mg	CONC µg/ml
52	5/14/97	17:23	.	0.28
52	5/15/97	05:58	450	.
52	5/15/97	08:49	.	7.41
53	5/14/97	04:53	600	.
53	5/14/97	13:48	.	0.66
53	5/15/97	05:50	.	0.03
53	5/15/97	05:53	600	.
53	5/15/97	07:08	.	8.4
53	5/15/97	08:49	.	5.24
54	5/14/97	04:55	450	.
54	5/14/97	13:22	.	0.68
54	5/14/97	17:28	.	0.14
54	5/15/97	06:11	.	0.03
54	5/15/97	06:13	450	.
54	5/15/97	07:26	.	4.76
54	5/15/97	08:12	.	4.79
54	5/15/97	09:41	.	2.7
55	5/14/97	04:59	450	.
55	5/14/97	13:12	.	0.72
55	5/14/97	18:03	.	0.03
55	5/15/97	06:16	.	0.03
55	5/15/97	06:17	450	.
55	5/15/97	07:19	.	0.03
55	5/15/97	08:10	.	0.09
56	5/14/97	05:00	600	.
56	5/14/97	13:52	.	0.58
56	5/14/97	18:20	.	0.03
56	5/15/97	05:36	.	0.03
56	5/15/97	05:37	600	.
56	5/15/97	06:52	.	2.42
56	5/15/97	07:42	.	2.19
56	5/15/97	09:35	.	1.21
57	5/14/97	04:56	600	.
57	5/14/97	13:00	.	0.44
57	5/14/97	18:15	.	0.03
58	5/14/97	04:57	450	.
58	5/14/97	13:05	.	0.97
58	5/15/97	05:28	450	.
58	5/15/97	05:28	.	0.13
58	5/15/97	08:00	.	2.89
59	5/14/97	04:50	450	.
59	5/14/97	12:35	.	0.82
59	5/14/97	17:06	.	0.13

ID	DATE (mm/dd/yy)	TIME	DOSE mg	CONC µg/ml
59	5/15/97	06:25	450	.
59	5/15/97	09:25	.	3.01
60	5/14/97	04:51	450	.
60	5/14/97	12:42	.	0.66
60	5/14/97	18:10	.	0.1
60	5/15/97	05:17	.	0.03
60	5/15/97	05:18	450	.
60	5/15/97	06:34	.	6.05
60	5/15/97	07:32	.	3.87
60	5/15/97	09:20	.	2.5
61	5/14/97	04:54	600	.
61	5/14/97	14:05	.	0.26
61	5/14/97	17:33	.	0.16
61	5/15/97	06:08	600	.
61	5/15/97	09:18	.	7.56
62	5/14/97	05:02	600	.
62	5/14/97	13:17	.	0.54
62	5/14/97	17:42	.	0.09
62	5/15/97	05:33	.	0.03
62	5/15/97	05:34	600	.
62	5/15/97	06:46	.	0.07
62	5/15/97	07:37	.	0.19
62	5/15/97	09:28	.	2.09
63	5/21/97	04:33	600	.
63	5/21/97	13:22	.	0.74
63	5/21/97	17:32	.	0.3
63	5/22/97	05:15	600	.
63	5/22/97	05:15	.	0.03
63	5/22/97	06:20	.	4.62
63	5/22/97	07:24	.	13.6
63	5/22/97	09:19	.	8.9
64	5/21/97	04:30	450	.
64	5/21/97	13:15	.	0.2
64	5/21/97	17:51	.	0.06
64	5/22/97	05:50	.	0.06
64	5/22/97	05:51	450	.
64	5/22/97	06:55	.	5.31
64	5/22/97	07:40	.	5.73
64	5/22/97	09:56	.	1.89
65	5/21/97	04:34	450	.
65	5/21/97	12:39	.	1.5
65	5/21/97	17:40	.	0.15
65	5/22/97	05:39	450	.

Appendix E

RIFAMPICIN DOSE AND CONCENTRATION DATA

ID	DATE (mm/dd/yy)	TIME	DOSE mg	CONC μg/ml
65	5/22/97	06:32	.	3.1
65	5/22/97	07:31	.	8.64
65	5/22/97	09:40	.	5.2
66	5/21/97	04:35	600	.
66	5/21/97	12:45	.	0.49
66	5/21/97	17:10	.	0.03
66	5/22/97	05:27	.	0.03
66	5/22/97	05:29	600	.
66	5/22/97	06:30	.	0.03
66	5/22/97	07:30	.	5.36
66	5/22/97	09:35	.	5.95
67	5/21/97	04:31	450	.
67	5/21/97	13:37	.	1.17
67	5/21/97	17:20	.	0.15
67	5/22/97	05:40	.	0.03
67	5/22/97	05:41	450	.
67	5/22/97	06:35	.	0.03
67	5/22/97	07:36	.	6.03
67	5/22/97	09:49	.	4.02
68	5/21/97	04:32	450	.
68	5/21/97	13:29	.	2.09
68	5/21/97	17:29	.	0.67
68	5/22/97	06:02	450	.
68	5/22/97	06:52	.	3.91
68	5/22/97	07:53	.	11.1
68	5/22/97	10:10	.	5.69
69	5/21/97	04:40	450	.
69	5/21/97	13:33	.	2.94
69	5/21/97	17:35	.	0.71
69	5/22/97	05:48	.	0.03
69	5/22/97	05:49	450	.
69	5/22/97	06:43	.	0.03
69	5/22/97	07:49	.	5.98
69	5/22/97	10:00	.	9.76
70	5/21/97	04:41	600	.
70	5/21/97	12:52	.	1.69
70	5/21/97	17:42	.	0.28
70	5/22/97	05:44	.	0.03
70	5/22/97	05:45	600	.
70	5/22/97	06:45	.	0.97
70	5/22/97	07:43	.	3.31
70	5/22/97	09:31	.	2.59
71	5/21/97	04:37	450	.

ID	DATE (mm/dd/yy)	TIME	DOSE mg	CONC μg/ml
71	5/21/97	13:12	.	2.94
71	5/21/97	17:23	.	0.44
71	5/22/97	05:33	.	0.03
71	5/22/97	05:34	450	.
71	5/22/97	06:39	.	3.63
71	5/22/97	07:37	.	9.35
71	5/22/97	09:53	.	4.19
72	5/21/97	04:38	450	.
72	5/21/97	12:57	.	1.49
72	5/21/97	17:17	.	0.19
72	5/22/97	05:53	.	0.03
72	5/22/97	05:54	450	.
72	5/22/97	07:04	.	7.96
72	5/22/97	07:58	.	10.1
72	5/22/97	09:45	.	5.67
73	5/21/97	04:39	450	.
73	5/21/97	13:06	.	0.06
73	5/21/97	17:46	.	0.03
73	5/22/97	06:04	.	0.03
73	5/22/97	06:05	450	.
73	5/22/97	06:50	.	0.03
73	5/22/97	07:47	.	4.39
73	5/22/97	10:10	.	7.19
74	5/21/97	04:15	450	.
74	5/21/97	13:56	.	1.08
74	5/21/97	18:06	.	0.19
74	5/22/97	06:17	450	.
74	5/22/97	07:03	.	9.48
74	5/22/97	08:01	.	9.77
74	5/22/97	10:26	.	5.94
75	5/21/97	04:25	600	.
75	5/21/97	14:03	.	0.22
75	5/21/97	18:02	.	0.06
75	5/22/97	06:25	600	.
75	5/22/97	07:18	.	2.19
75	5/22/97	08:12	.	2.91
75	5/22/97	10:19	.	1.15
76	5/21/97	04:20	450	.
76	5/21/97	14:12	.	2.08
76	5/21/97	17:59	.	0.78
76	5/22/97	06:10	.	0.27
76	5/22/97	06:13	450	.
76	5/22/97	07:08	.	3.48

Appendix E

RIFAMPICIN DOSE AND CONCENTRATION DATA

ID	DATE (mm/dd/yy)	TIME	DOSE mg	CONC µg/ml
76	5/22/97	08:10	.	10.5
76	5/22/97	10:31	.	6.55
77	5/21/97	04:15	450	.
77	5/21/97	13:53	.	1.83
77	5/21/97	17:55	.	0.53
77	5/22/97	06:10	450	.
77	5/22/97	07:01	.	0.06
77	5/22/97	08:06	.	6.13
77	5/22/97	10:23	.	4.76
78	5/21/97	04:42	600	.
78	5/21/97	13:41	.	1.37
78	5/21/97	17:14	.	0.52
78	5/22/97	05:22	.	0.03
78	5/22/97	05:25	600	.
78	5/22/97	06:26	.	0.31
78	5/22/97	07:26	.	2.65
78	5/22/97	09:25	.	6.99
79	5/28/97	04:56	450	.
79	5/28/97	12:35	.	3.13
79	5/28/97	16:05	.	1.46
79	5/29/97	05:37	450	.
79	5/29/97	05:37	.	0.03
79	5/29/97	06:41	.	5.73
79	5/29/97	07:42	.	7.16
79	5/29/97	09:55	.	4
80	5/28/97	04:58	600	.
80	5/28/97	12:20	.	5.29
80	5/28/97	15:52	.	2.48
80	5/29/97	05:42	600	.
80	5/29/97	05:42	.	0.02
80	5/29/97	06:45	.	11.3
80	5/29/97	07:49	.	11.3
81	5/28/97	04:51	600	.
81	5/28/97	12:40	.	4.36
81	5/28/97	16:22	.	2.9
81	5/29/97	05:49	.	0.1
81	5/29/97	05:50	600	.
81	5/29/97	06:51	.	10.2
81	5/29/97	07:55	.	10.6
81	5/29/97	10:04	.	4.37
82	5/28/97	04:55	600	.
82	5/28/97	12:30	.	0.03
82	5/29/97	05:44	600	.

ID	DATE (mm/dd/yy)	TIME	DOSE mg	CONC µg/ml
82	5/29/97	07:04	.	1.4
82	5/29/97	08:01	.	5.28
82	5/29/97	10:00	.	4.24
83	5/28/97	05:00	600	.
83	5/28/97	12:25	.	2.62
83	5/28/97	15:56	.	0.75
83	5/29/97	05:32	600	.
83	5/29/97	05:32	.	0.03
83	5/29/97	06:37	.	3.44
83	5/29/97	07:37	.	4.89
84	5/28/97	04:50	600	.
84	5/28/97	12:58	.	2.76
84	5/28/97	15:43	.	1
84	5/29/97	05:55	600	.
84	5/29/97	08:45	.	8.34
85	5/28/97	04:50	450	.
85	5/28/97	12:44	.	2.23
85	5/28/97	17:52	.	0.26
85	5/29/97	05:22	450	.
85	5/29/97	05:22	.	0.03
85	5/29/97	06:28	.	5.74
85	5/29/97	07:29	.	7.46
85	5/29/97	09:32	.	3.42
86	5/28/97	05:15	600	.
86	5/28/97	12:36	.	3.02
86	5/28/97	17:48	.	0.64
86	5/29/97	05:11	600	.
86	5/29/97	05:11	.	0.03
86	5/29/97	06:16	.	5.93
86	5/29/97	07:16	.	5.67
86	5/29/97	09:16	.	5.52
87	5/28/97	05:15	450	.
87	5/28/97	12:26	.	0.48
87	5/28/97	17:43	.	0.03
87	5/29/97	05:15	450	.
87	5/29/97	05:15	.	0.03
87	5/29/97	06:22	.	1.88
87	5/29/97	07:23	.	3.06
87	5/29/97	09:23	.	4.24
88	6/11/97	05:22	450	.
88	6/11/97	12:48	.	1.76
88	6/11/97	17:15	.	0.66
88	6/12/97	05:50	450	.

Appendix E

RIFAMPICIN DOSE AND CONCENTRATION DATA

ID	DATE (mm/dd/yy)	TIME	DOSE mg	CONC μg/ml
88	6/12/97	05:50	.	0.03
88	6/12/97	06:58	.	7.83
88	6/12/97	07:46	.	7.66
88	6/12/97	09:50	.	4.56
89	6/11/97	05:10	600	.
89	6/11/97	13:03	.	1.86
89	6/11/97	17:10	.	0.82
89	6/12/97	05:30	600	.
89	6/12/97	05:30	.	0.03
89	6/12/97	06:31	.	1.36
89	6/12/97	07:32	.	7.62
89	6/12/97	09:43	.	6.11
90	6/11/97	05:17	450	.
90	6/11/97	12:37	.	0.46
90	6/11/97	17:25	.	0.03
90	6/12/97	05:51	450	.
90	6/12/97	06:35	.	0.17
90	6/12/97	07:48	.	7.27
90	6/12/97	09:53	.	4.29
91	6/11/97	05:15	450	.
91	6/11/97	13:09	.	1.5
91	6/11/97	17:38	.	0.26
91	6/12/97	05:27	450	.
91	6/12/97	05:27	.	0.03
91	6/12/97	06:27	.	3.8
91	6/12/97	07:29	.	6.53
91	6/12/97	09:25	.	4.56
92	6/11/97	05:20	600	.
92	6/11/97	12:29	.	0.91
92	6/11/97	17:07	.	0.18
92	6/12/97	05:18	600	.
92	6/12/97	05:18	.	0.03
92	6/12/97	06:18	.	1.52
92	6/12/97	07:20	.	12.1
92	6/12/97	09:14	.	5.49
93	6/11/97	05:21	450	.
93	6/11/97	12:55	.	2.04
93	6/11/97	17:30	.	0.36
93	6/12/97	05:36	450	.
93	6/12/97	05:36	.	0.03
93	6/12/97	06:44	.	7.52
93	6/12/97	07:37	.	12.2
93	6/12/97	09:38	.	5.33

ID	DATE (mm/dd/yy)	TIME	DOSE mg	CONC μg/ml
94	6/11/97	05:16	450	.
94	6/11/97	13:02	.	0.15
94	6/11/97	17:32	.	0.38
94	6/12/97	05:44	450	.
94 .	6/12/97	05:44	.	0.14
94	6/12/97	06:46	.	2.5
94	6/12/97	07:41	.	9.3
94	6/12/97	09:45	.	4.3
95	6/11/97	05:20	600	.
95	6/11/97	13:12	.	1.9
95	6/11/97	17:20	.	0.4
95	6/12/97	05:22	600	.
95	6/12/97	05:22	.	0.03
95	6/12/97	06:21	.	0.03
95	6/12/97	07:25	.	13.9
95	6/12/97	09:19	.	9.9
96	6/11/97	05:00	450	.
96	6/11/97	13:24	.	3.3
96	6/11/97	17:52	.	1.1
96	6/12/97	06:03	450	.
96	6/12/97	06:03	.	0.03
96	6/12/97	07:08	.	8.7
96	6/12/97	08:05	.	5.4
96	6/12/97	10:05	.	5.1
97	7/9/97	05:15	450	.
97	7/9/97	13:56	.	0.45
97	7/9/97	17:21	.	0.41
97	7/10/97	05:42	.	0.26
97	7/10/97	05:43	450	.
97	7/10/97	06:48	.	2.9
97	7/10/97	07:42	.	6.6
97	7/10/97	09:39	.	3.3
98	7/9/97	05:19	600	.
98	7/9/97	13:37	.	0.5
98	7/9/97	17:12	.	0.1
98	7/10/97	05:40	600	.
98	7/10/97	05:40	.	0.03
98	7/10/97	06:45	.	2.1
98	7/10/97	07:45	.	7.4
98	7/10/97	09:43	.	4.9
99	7/9/97	05:10	450	.
99	7/9/97	14:33	.	2.9
99	7/9/97	17:31	.	0.6

Appendix E

RIFAMPICIN DOSE AND CONCENTRATION DATA

ID	DATE (mm/dd/yy)	TIME	DOSE mg	CONC µg/ml
99	7/10/97	05:37	450	.
99	7/10/97	05:37	.	0.03
99	7/10/97	06:44	.	8
99	7/10/97	07:39	.	8.9
99	7/10/97	09:35	.	4.8
100	7/9/97	05:11	600	.
100	7/9/97	14:04	.	0.03
100	7/9/97	17:28	.	0.03
100	7/10/97	05:52	600	.
100	7/10/97	05:52	.	0.03
100	7/10/97	06:55	.	7.2
100	7/10/97	07:49	.	8
100	7/10/97	09:50	.	3.5
101	7/9/97	05:14	450	.
101	7/9/97	14:30	.	0.4
101	7/9/97	17:25	.	0.14
101	7/10/97	05:47	450	.
101	7/10/97	08:31	.	7.3
102	7/9/97	05:10	450	.
102	7/9/97	13:25	.	1.8
102	7/10/97	05:25	450	.
102	7/10/97	08:34	.	4.1
103	7/9/97	05:18	450	.
103	7/9/97	13:45	.	3.9
103	7/9/97	17:37	.	1.6
103	7/10/97	05:28	450	.
103	7/10/97	05:28	.	0.07
103	7/10/97	06:33	.	0.14
103	7/10/97	08:25	.	1.7
104	7/9/97	05:22	450	.
104	7/9/97	13:50	.	1.1
104	7/9/97	17:19	.	0.2
104	7/10/97	05:34	450	.
104	7/10/97	05:34	.	0.03
104	7/10/97	06:38	.	5.6
105	7/9/97	05:20	600	.
105	7/9/97	13:30	.	1.3
105	7/9/97	17:08	.	0.3
105	7/10/97	05:23	.	0.03
105	7/10/97	05:24	600	.
105	7/10/97	06:26	.	0.7
105	7/10/97	08:23	.	8.1
106	7/9/97	05:15	450	.

ID	DATE (mm/dd/yy)	TIME	DOSE mg	CONC µg/ml
106	7/9/97	14:15	.	3
106	7/10/97	05:30	450	.
106	7/10/97	08:38	.	5.2
107	7/23/97	04:15	450	.
107	7/23/97	13:50	.	0.4
107	7/23/97	17:52	.	0.03
107	7/24/97	05:15	450	.
107	7/24/97	05:15	.	0.03
107	7/24/97	06:30	.	1.4
107	7/24/97	07:20	.	6.8
107	7/24/97	09:25	.	4.1
108	7/23/97	04:16	600	.
108	7/23/97	13:03	.	0.2
108	7/23/97	17:28	.	0.03
108	7/24/97	05:10	.	0.03
108	7/24/97	05:11	600	.
108	7/24/97	07:19	.	5
108	7/24/97	09:55	.	1.3
109	7/23/97	04:57	600	.
109	7/23/97	12:43	.	6
109	7/23/97	17:25	.	2.7
109	7/24/97	05:27	600	.
109	7/24/97	05:27	.	0.03
109	7/24/97	06:42	.	7.4
110	7/23/97	04:55	450	.
110	7/23/97	12:53	.	1.2
110	7/23/97	17:34	.	0.2
110	7/24/97	05:19	450	.
110	7/24/97	05:19	.	0.03
110	7/24/97	06:23	.	5.5
110	7/24/97	07:15	.	6.4
110	7/24/97	09:16	.	4.5
111	7/23/97	04:56	450	.
111	7/23/97	14:04	.	0.9
111	7/23/97	17:47	.	0.2
111	7/24/97	05:34	450	.
111	7/24/97	05:34	.	0.03
111	7/24/97	06:49	.	5.9
111	7/24/97	07:35	.	11.2
111	7/24/97	09:47	.	6.4
112	7/23/97	04:59	600	.
112	7/23/97	13:38	.	8.4
112	7/23/97	17:37	.	4.1

Appendix E

RIFAMPICIN DOSE AND CONCENTRATION DATA

ID	DATE (mm/dd/yy)	TIME	DOSE mg	CONC μg/ml
112	7/24/97	05:22	600	.
112	7/24/97	05:22	.	0.04
112	7/24/97	06:35	.	0.8
112	7/24/97	07:26	.	22.5
112	7/24/97	09:30	.	16.5
113	7/23/97	04:58	600	.
113	7/23/97	13:23	.	5.1
113	7/23/97	17:41	.	2.4
113	7/24/97	05:25	600	.
113	7/24/97	05:25	.	0.03 .
113	7/24/97	06:38	.	9.4
113	7/24/97	07:28	.	25
113	7/24/97	09:37	.	14.3
114	8/5/97	05:15	600	.
114	8/5/97	09:05	.	6.6
114	8/6/97	05:15	600	.
114	8/6/97	13:04	.	1.9
114	8/6/97	17:57	.	0.2
114	8/7/97	05:26	.	0.03
114	8/7/97	05:27	600	.
114	8/7/97	06:27	.	7.9
114	8/7/97	07:27	.	13.6
114	8/7/97	09:25	.	7.4
115	8/6/97	05:11	450	.
115	8/6/97	12:45	.	5.8
115	8/7/97	05:41	.	0.1
115	8/7/97	05:42	450	.
115	8/7/97	06:41	.	11.1
115	8/7/97	07:48	.	20.8
115	8/7/97	09:48	.	11.3
116	8/6/97	05:05	450	.
116	8/6/97	13:17	.	3.3
116	8/7/97	05:44	.	0.03
116	8/7/97	05:45	450	.
116	8/7/97	06:47	.	3.1
116	8/7/97	07:55	.	9.2
116	8/7/97	09:55	.	5
117	8/6/97	05:06	600	.
117	8/6/97	13:10	.	0.36
117	8/6/97	17:52	.	0.04
117	8/7/97	05:35	.	0.03
117	8/7/97	05:36	600	.
117	8/7/97	06:35	.	6.3

ID	DATE (mm/dd/yy)	TIME	DOSE mg	CONC μg/ml
117	8/7/97	07:41	.	6.6
117	8/7/97	09:41	.	3.8
118	8/6/97	05:12	450	.
118	8/6/97	12:49	.	1.1
118	8/6/97	17:50	.	0.3
118	8/7/97	05:36	.	0.03
118	8/7/97	05:37	450	.
118	8/7/97	06:31	.	9.8
118	8/7/97	07:32	.	9.2
118	8/7/97	09:39	.	5
119	8/6/97	05:10	450	.
119	8/6/97	13:01	.	1.2
119	8/6/97	17:39	.	0.3
119	8/7/97	05:23	.	0.03
119	8/7/97	05:24	450	.
119	8/7/97	06:24	.	0.3
119	8/7/97	07:23	.	8.4
119	8/7/97	09:22	.	7
120	8/6/97	05:10	450	.
120	8/6/97	12:53	.	2.8
120	8/7/97	05:50	450	.
120	8/7/97	05:50	.	0.03
120	8/7/97	06:51	.	4
120	8/7/97	08:00	.	15.2
120	8/7/97	10:02	.	5.7
121	8/6/97	05:08	450	.
121	8/6/97	13:08	.	1.9
121	8/6/97	17:30	.	0.2
121	8/7/97	05:38	450	.
121	8/7/97	05:38	.	0.03
121	8/7/97	06:39	.	8.1
121	8/7/97	07:47	.	12.7
121	8/7/97	09:45	.	5.6
122	8/6/97	05:20	600	.
122	8/6/97	13:36	.	0.03
122	8/6/97	18:02	.	0.03
122	8/7/97	06:02	600	.
122	8/7/97	07:04	.	0.03
122	8/7/97	08:09	.	2.9
122	8/7/97	10:09	.	3.1
123	8/6/97	04:55	600	.
123	8/6/97	13:31	.	1.1
123	8/6/97	18:18	.	0.25

Appendix E

RIFAMPICIN DOSE AND CONCENTRATION DATA

ID	DATE (mm/dd/yy)	TIME	DOSE mg	CONC µg/ml
123	8/7/97	06:05	.	0.07
123	8/7/97	06:06	600	.
123	8/7/97	07:08	.	11.2
123	8/7/97	10:13	.	5.2
124	8/6/97	05:05	450	.
124	8/6/97	10:13	.	7.4
124	8/6/97	13:41	.	4.3
124	8/6/97	18:08	.	0.9
125	8/13/97	05:00	600	.
125	8/13/97	11:55	.	3.3
125	8/13/97	17:30	.	0.4
125	8/14/97	06:09	.	0.03
125	8/14/97	06:10	600	.
125	8/14/97	07:30	.	5.6
125	8/14/97	08:21	.	9.6
125	8/14/97	10:03	.	7
126	8/13/97	05:00	450	.
126	8/13/97	12:15	.	2.9
126	8/13/97	17:42	.	0.9
126	8/14/97	06:25	.	0.03
126	8/14/97	06:26	450	.
126	8/14/97	07:42	.	4.3
126	8/14/97	08:27	.	7.2
126	8/14/97	10:13	.	4.6
127	8/13/97	05:25	450	.
127	8/13/97	13:10	.	6.4
127	8/13/97	17:10	.	2.7
127	8/14/97	06:18	.	0.18
127	8/14/97	06:19	450	.
127	8/14/97	07:36	.	7.9
127	8/14/97	08:31	.	11.8
127	8/14/97	10:20	.	8.5
128	8/13/97	05:00	450	.
128	8/13/97	13:05	.	3.3
128	8/13/97	17:57	.	1.5
128	8/14/97	05:53	.	0.03
128	8/14/97	05:57	450	.
128	8/14/97	07:10	.	10.6
128	8/14/97	08:11	.	13.3
128	8/14/97	09:52	.	7.2
129	8/13/97	04:30	600	.
129	8/13/97	12:34	.	1.12
129	8/13/97	18:08	.	0.03

ID	DATE (mm/dd/yy)	TIME	DOSE mg	CONC µg/ml
129	8/14/97	04:45	600	.
129	8/14/97	05:20	600	.
129	8/14/97	05:20	.	0.03
129	8/14/97	06:41	.	37.9
129	8/14/97	07:33	.	34.7
129	8/14/97	09:18	.	13.3
130	8/13/97	04:30	600	.
130	8/13/97	12:49	.	0.79
130	8/13/97	18:32	.	0.03
130	8/14/97	05:37	.	0.03
130	8/14/97	05:38	600	.
130	8/14/97	06:58	.	0.03
130	8/14/97	07:57	.	4.2
130	8/14/97	09:38	.	4.3
131	8/13/97	04:30	600	.
131	8/13/97	12:40	.	0.5
131	8/13/97	18:28	.	0.03
131	8/14/97	04:45	600	.
131	8/14/97	05:28	600	.
131	8/14/97	05:28	.	2.7
131	8/14/97	06:47	.	7.7
131	8/14/97	07:50	.	14.5
131	8/14/97	09:33	.	10.5
132	8/13/97	04:30	450	.
132	8/13/97	12:45	.	0.55
132	8/13/97	18:18	.	0.08
132	8/14/97	04:45	450	.
132	8/14/97	05:41	450	.
132	8/14/97	05:41	.	8.6
132	8/14/97	09:15	.	15.6
133	8/13/97	04:30	450	.
133	8/13/97	12:43	.	0.5
133	8/13/97	18:35	.	0.05
133	8/14/97	04:45	450	.
133	8/14/97	05:45	.	1.7
133	8/14/97	05:46	450	.
133	8/14/97	07:02	.	24.2
133	8/14/97	08:05	.	17.1
133	8/14/97	09:43	.	14.2
134	8/13/97	04:35	450	.
134	8/13/97	12:56	.	2.1
134	8/13/97	18:24	.	0.3
134	8/14/97	04:45	450	.

Appendix E

RIFAMPICIN DOSE AND CONCENTRATION DATA

ID	DATE (mm/dd/yy)	TIME	DOSE mg	CONC μg/ml
134	8/14/97	05:15	450	.
134	8/14/97	05:15	.	2.4
134	8/14/97	06:42	.	9.3
134	8/14/97	08:00	.	21.1
134	8/14/97	09:15	.	18.1
135	8/13/97	04:35	450	.
135	8/13/97	12:46	.	0.09
135	8/13/97	18:14	.	0.1
135	8/14/97	04:45	450	.
135	8/14/97	05:31	.	2.7
135	8/14/97	05:32	450	.
135	8/14/97	06:44	.	12
135	8/14/97	07:38	.	12.1
135	8/14/97	09:26	.	8.2
136	8/13/97	04:35	450	.
136	8/13/97	12:50	.	0.3
136	8/13/97	18:21	.	0.03
136	8/14/97	04:45	450	.
136	8/14/97	05:25	450	.
136	8/14/97	05:25	.	3
136	8/14/97	06:53	.	8
136	8/14/97	07:58	.	16.7
136	8/14/97	09:40	.	14.6
137	8/13/97	04:35	600	.
137	8/13/97	12:38	.	1
137	8/13/97	18:39	.	0.15
137	8/14/97	04:45	600	.
137	8/14/97	05:23	600	.
137	8/14/97	05:23	.	0.03
137	8/14/97	06:46	.	14.1
137	8/14/97	07:48	.	29.3
137	8/14/97	09:22	.	19.8
138	8/13/97	04:10	450	.
138	8/13/97	13:20	.	0.4
138	8/13/97	18:44	.	0.07
138	8/14/97	05:42	450	.
138	8/14/97	05:42	.	0.03
138	8/14/97	07:16	.	1.47
138	8/14/97	09:30	.	7.2

Appendix F   ISONIAZID DOSE AND CONCENTRATION DATA

Appendix F

ISONIAZID DOSE AND CONCENTRATION DATA

ID	DATE (mm/dd/yy)	TIME	DOSE mg	CONC µg/ml
1	3/19/97	05:27	400	.
1	3/19/97	08:15	133	.
1	3/19/97	14:00	133	.
1	3/19/97	15:34	.	1.56
1	3/19/97	17:00	133	.
1	3/20/97	05:19	400	.
1	3/20/97	05:19	.	0.96
1	3/20/97	06:14	.	6.49
1	3/20/97	07:20	.	3.97
1	3/20/97	08:15	133	.
1	3/20/97	09:25	.	2.08
1	3/20/97	13:30	.	1.17
2	3/19/97	05:30	400	.
2	3/19/97	15:50	.	0.57
2	3/20/97	05:50	.	0.025
2	3/20/97	05:51	400	.
2	3/20/97	06:40	.	8.54
2	3/20/97	07:34	.	5.99
2	3/20/97	09:50	.	3.86
2	3/20/97	13:21	.	1.48
3	3/19/97	05:30	400	.
3	3/19/97	15:46	.	0.48
3	3/20/97	05:49	400	.
3	3/20/97	05:49	.	0.025
3	3/20/97	06:35	.	6.22
3	3/20/97	07:41	.	3.6
3	3/20/97	09:58	.	1.61
3	3/20/97	13:45	.	0.61
4	3/19/97	05:25	400	.
4	3/19/97	15:55	.	1.88
4	3/20/97	05:35	.	0.5
4	3/20/97	05:38	400	.
4	3/20/97	06:30	.	6.01
4	3/20/97	07:46	.	7
4	3/20/97	14:02	.	3.36
5	3/19/97	05:30	400	.
5	3/19/97	15:39	.	1.35
5	3/20/97	05:30	400	.
5	3/20/97	05:30	.	0.025
5	3/20/97	06:23	.	7.78
5	3/20/97	07:28	.	6.18
5	3/20/97	09:45	.	2.95
5	3/20/97	13:53	.	1.59
6	3/19/97	05:37	400	.
6	3/19/97	15:43	.	0.45
6	3/20/97	05:25	400	.
6	3/20/97	05:25	.	0.025

ID	DATE (mm/dd/yy)	TIME	DOSE mg	CONC µg/ml
6	3/20/97	06:18	.	2.12
6	3/20/97	07:25	.	3.9
6	3/20/97	09:35	.	3.31
6	3/20/97	13:47	.	1.08
7	3/19/97	05:25	400	.
7	3/19/97	15:59	.	0.48
7	3/20/97	05:10	.	0.025
7	3/20/97	05:15	400	.
7	3/20/97	06:10	.	7.64
7	3/20/97	07:17	.	4.87
7	3/20/97	09:30	.	1.7
7	3/20/97	17:55	.	0.2
8	4/9/97	05:00	400	.
8	4/9/97	12:50	.	1.74
8	4/9/97	16:05	.	0.96
8	4/10/97	05:45	.	0.025
8	4/10/97	05:46	400	.
8	4/10/97	09:40	.	3.44
9	4/9/97	05:00	400	.
9	4/9/97	12:44	.	1.16
9	4/9/97	16:08	.	0.57
9	4/10/97	05:20	400	.
9	4/10/97	05:20	.	0.025
9	4/10/97	06:20	.	4.4
9	4/10/97	07:27	.	5.09
9	4/10/97	09:22	.	2.6
10	4/9/97	05:00	400	.
10	4/9/97	12:39	.	1.44
10	4/9/97	15:37	.	0.98
10	4/10/97	05:34	.	0.025
10	4/10/97	05:35	400	.
10	4/10/97	06:28	.	6.03
10	4/10/97	07:37	.	4.8
10	4/10/97	09:43	.	2.33
11	4/9/97	05:10	400	.
11	4/9/97	12:30	.	0.85
11	4/9/97	16:11	.	0.41
11	4/10/97	05:29	.	0.025
11	4/10/97	05:30	400	.
11	4/10/97	06:24	.	4.29
11	4/10/97	07:30	.	6.84
11	4/10/97	09:38	.	3.3
12	4/9/97	05:00	400	.
12	4/9/97	09:00	133	.
12	4/9/97	12:22	.	7.32
12	4/9/97	14:00	133	.
12	4/9/97	15:45	.	6.25

Appendix F

ISONIAZID DOSE AND CONCENTRATION DATA

ID	DATE (mm/dd/yy)	TIME	DOSE mg	CONC µg/ml
12	4/9/97	17:00	133	.
12	4/10/97	05:50	.	2.88
12	4/10/97	05:51	400	.
12	4/10/97	06:37	.	7.19
12	4/10/97	07:55	.	10.68
12	4/10/97	09:00	133	.
12	4/10/97	09:51	.	9.86
13	4/9/97	05:00	400	.
13	4/9/97	12:15	.	2.79
13	4/9/97	15:55	.	1.96
13	4/10/97	05:11	.	0.34
13	4/10/97	05:12	400	.
13	4/10/97	06:13	.	5.67
13	4/10/97	07:21	.	6.28
13	4/10/97	09:27	.	4.12
14	4/16/97	08:40	133	.
14	4/16/97	14:30	133	.
14	4/16/97	16:45	133	.
14	4/16/97	18:00	.	2.04
14	4/17/97	05:30	.	1.01
14	4/17/97	06:30	.	0.81
14	4/17/97	07:32	.	0.71
14	4/17/97	09:01	133	.
14	4/17/97	09:43	. .	0.52
15	4/16/97	08:35	133	.
15	4/16/97	12:50	.	0.66
15	4/16/97	14:47	133	.
15	4/16/97	16:35	133	.
15	4/16/97	17:31	.	2.55
15	4/17/97	06:04	.	0.18
15	4/17/97	06:58	.	0.08
15	4/17/97	08:07	.	0.1
15	4/17/97	09:15	133	.
15	4/17/97	10:15	.	0.48
16	4/16/97	04:45	400	.
16	4/16/97	08:45	133	.
16	4/16/97	13:11	.	0.76
16	4/16/97	14:30	133	.
16	4/16/97	16:45	133	.
16	4/16/97	17:27	.	1.17
16	4/17/97	05:43	.	0.08
16	4/17/97	05:45	400	.
16	4/17/97	06:39	.	7
16	4/17/97	07:43	.	3.6
16	4/17/97	09:05	133	.
16	4/17/97	09:56	.	2.64
17	4/16/97	08:30	133	.

ID	DATE (mm/dd/yy)	TIME	DOSE mg	CONC µg/ml
17	4/16/97	13:05	.	1.2
17	4/16/97	14:40	133	.
17	4/16/97	16:45	133	.
17	4/16/97	17:05	.	0.64
17	4/17/97	06:01	.	0.025
17	4/17/97	06:53	.	0.025
17	4/17/97	08:00	.	0.025
17	4/17/97	09:14	133	.
17	4/17/97	10:35	.	1.86
18	4/16/97	04:38	300	.
18	4/16/97	12:22	.	1.11
18	4/16/97	17:15	.	0.14
18	4/17/97	06:22	300	.
18	4/17/97	06:22	.	0.025
18	4/17/97	07:19	.	1.25
18	4/17/97	08:20	.	3.62
18	4/17/97	10:03	.	2.25
19	4/16/97	05:00	300	.
19	4/16/97	12:29	.	2.51
19	4/16/97	17:00	.	1.02
19	4/17/97	05:36	300	.
19	4/17/97	05:36	.	0.23
19	4/17/97	06:35	.	11.77
19	4/17/97	07:38	.	9.74
19	4/17/97	09:50	.	5.1
20	4/16/97	04:30	300	.
20	4/16/97	13:42	.	0.6
20	4/16/97	17:15	.	0.18
20	4/17/97	07:28	300	.
20	4/17/97	08:20	.	0.82
21	4/16/97	05:10	400	.
21	4/16/97	12:43	.	2.22
21	4/16/97	17:50	.	0.76
21	4/17/97	06:19	400	.
21	4/17/97	06:19	.	0.025
21	4/17/97	07:03	.	5.17
21	4/17/97	08:15	.	6.61
21	4/17/97	10:26	.	3.62
22	4/16/97	05:13	400	.
22	4/16/97	12:56	.	0.9
22	4/16/97	18:18	.	0.34
22	4/17/97	06:15	400	.
22	4/17/97	06:15	.	0.025
22	4/17/97	08:15	.	3.46
22	4/17/97	10:21	.	1.99
23	4/16/97	05:07	300	.
23	4/16/97	12:38	.	0.79

Appendix F

ISONIAZID DOSE AND CONCENTRATION DATA

ID	DATE (mm/dd/yy)	TIME	DOSE mg	CONC µg/ml		ID	DATE (mm/dd/yy)	TIME	DOSE mg	CONC µg/ml
23	4/17/97	05:55	300	.		30	4/23/97	12:50	.	2.53
23	4/17/97	07:52	.	2.22		30	4/23/97	17:06	.	0.51
23	4/17/97	08:30	.	2.3		30	4/24/97	06:00	300	.
24	4/16/97	05:05	300	.		30	4/24/97	06:00	.	0.16
24	4/16/97	13:27	.	1.39		30	4/24/97	07:15	.	6.34
24	4/16/97	18:12	.	0.58		30	4/24/97	08:19	.	6.23
25	4/23/97	05:00	400	.		30	4/24/97	09:51	.	4.05
25	4/23/97	12:40	.	0.56		31	4/23/97	05:00	300	.
25	4/23/97	17:02	.	0.19		31	4/23/97	12:57	.	1.01
25	4/24/97	06:16	.	0.025		31	4/23/97	16:35	.	0.53
25	4/24/97	06:19	400	.		31	4/24/97	05:50	.	0.025
25	4/24/97	07:27	.	4.24		31	4/24/97	05:52	300	.
25	4/24/97	08:40	.	2.54		31	4/24/97	06:55	.	8.41
25	4/24/97	09:43	.	1.97		31	4/24/97	08:08	.	6.17
26	4/23/97	05:00	400	.		31	4/24/97	10:05	.	3.34
26	4/23/97	13:06	.	0.9		32	4/23/97	05:00	300	.
26	4/23/97	16:38	.	0.36		32	4/23/97	13:23	.	0.99
26	4/24/97	05:19	.	0.025		32	4/23/97	16:52	.	0.48
26	4/24/97	05:21	400	.		32	4/24/97	05:43	300	.
26	4/24/97	06:30	.	7.3		32	4/24/97	06:50	.	4.96
26	4/24/97	07:38	.	5.12		32	4/24/97	07:55	.	4.54
26	4/24/97	09:41	.	2.8		32	4/24/97	09:59	.	2.95
27	4/23/97	05:00	400	.		33	4/23/97	05:14	300	.
27	4/23/97	12:48	.	0.73		33	4/23/97	13:13	.	1.81
27	4/23/97	16:55	.	0.34		34	4/23/97	05:12	300	.
27	4/24/97	06:10	.	0.025		34	4/23/97	13:34	.	0.91
27	4/24/97	06:11	400	.		34	4/23/97	16:15	.	0.42
27	4/24/97	07:23	.	6.06		34	4/24/97	05:27	.	0.025
27	4/24/97	08:35	.	4.29		34	4/24/97	05:31	300	.
27	4/24/97	10:02	.	2.6		34	4/24/97	06:27	.	1.19
28	4/23/97	05:00	400	.		34	4/24/97	07:13	.	1.03
28	4/23/97	13:00	.	0.54		34	4/24/97	08:23	.	1.44
28	4/23/97	16:42	.	0.25		35	4/23/97	04:55	400	.
28	4/24/97	05:25	.	0.025		35	4/23/97	13:23	.	1.39
28	4/24/97	05:28	400	.		35	4/23/97	16:44	.	0.56
28	4/24/97	06:40	.	7.93		35	4/24/97	05:18	.	0.025
28	4/24/97	07:46	.	5.79		36	4/23/97	04:50	300	.
28	4/24/97	09:46	.	1.44		36	4/23/97	12:46	.	0.85
29	4/23/97	05:00	400	.		36	4/23/97	16:20	.	0.37
29	4/23/97	13:17	.	0.35		36	4/24/97	06:05	.	0.09
29	4/23/97	17:10	.	0.025		36	4/24/97	06:10	300	.
29	4/24/97	05:32	.	0.025		36	4/24/97	07:00	.	0.73
29	4/24/97	05:34	400	.		36	4/24/97	08:00	.	2.56
29	4/24/97	07:03	.	8.16		36	4/24/97	09:05	.	2.41
29	4/24/97	08:30	.	7.03		37	4/23/97	04:52	300	.
29	4/24/97	09:56	.	4.77		37	4/23/97	14:10	.	0.82
30	4/23/97	05:00	300	.		37	4/23/97	16:53	.	0.76

Appendix F

ISONIAZID DOSE AND CONCENTRATION DATA

ID	DATE (mm/dd/yy)	TIME	DOSE mg	CONC µg/ml		ID	DATE (mm/dd/yy)	TIME	DOSE mg	CONC µg/ml
38	4/23/97	04:53	400	.		44	5/7/97	04:55	400	.
38	4/23/97	13:45	.	0.53		44	5/7/97	11:55	.	1.55
38	4/23/97	16:50	.	0.29		44	5/7/97	18:11	.	0.44
38	4/24/97	05:37	.	0.025		44	5/8/97	05:46	400	.
38	4/24/97	05:42	400	.		44	5/8/97	06:48	.	7.52
38	4/24/97	06:50	.	5.05		44	5/8/97	08:36	.	4.07
38	4/24/97	07:48	.	4.26		45	5/7/97	04:55	400	.
38	4/24/97	08:51	.	1.94		45	5/7/97	13:06	.	0.76
39	4/23/97	05:15	300	.		45	5/7/97	18:18	.	0.69
39	4/23/97	13:03	.	1.51		46	5/7/97	04:55	400	.
39	4/23/97	16:37	.	0.71		46	5/7/97	11:45	.	0.96
39	4/24/97	06:15	.	0.16		46	5/7/97	17:30	.	0.37
39	4/24/97	06:19	300	.		47	5/7/97	04:55	400	.
39	4/24/97	07:08	.	4.64		47	5/7/97	12:03	.	3.59
39	4/24/97	08:10	.	5.6		47	5/7/97	17:50	.	0.85
39	4/24/97	09:12	.	3.42		47	5/8/97	06:02	.	0.025
40	4/23/97	05:10	300	.		47	5/8/97	06:04	400	.
40	4/23/97	13:28	.	0.21		47	5/8/97	07:11	.	9.75
40	4/23/97	17:00	.	0.1		47	5/8/97	08:05	.	9.47
40	4/24/97	05:50	.	0.025		47	5/8/97	09:40	.	5.67
40	4/24/97	05:55	300	.		48	5/7/97	04:55	400	.
40	4/24/97	06:47	.	2.74		48	5/7/97	11:37	.	2.55
40	4/24/97	07:41	.	2.53		48	5/7/97	17:45	.	0.76
40	4/24/97	08:41	.	1.51		48	5/8/97	06:11	.	0.025
41	5/7/97	04:55	400	.		48	5/8/97	06:12	400	.
41	5/7/97	13:11	.	0.43		48	5/8/97	07:16	.	8.25
41	5/7/97	17:19	.	0.025		48	5/8/97	08:11	.	7.61
41	5/8/97	05:57	.	0.025		48	5/8/97	09:35	.	4.11
41	5/8/97	05:59	400	.		49	5/7/97	04:55	400	.
41	5/8/97	06:59	.	3.76		49	5/7/97	11:27	.	1.15
41	5/8/97	07:53	.	7.57		49	5/7/97	17:40	.	0.28
41	5/8/97	09:15	.	3.38		49	5/8/97	05:18	.	0.025
42	5/7/97	04:55	400	.		49	5/8/97	05:20	400	.
42	5/7/97	13:03	.	2.36		49	5/8/97	06:25	.	4.86
42	5/7/97	18:06	.	0.75		49	5/8/97	07:24	.	3.4
42	5/8/97	05:53	.	2.29		49	5/8/97	09:09	.	1.78
42	5/8/97	05:54	400	.		50	5/7/97	04:55	300	.
42	5/8/97	06:56	.	7.95		50	5/7/97	11:32	.	0.41
42	5/8/97	07:41	.	7.9		50	5/7/97	17:56	.	0.81
43	5/7/97	04:55	400	.		50	5/8/97	05:30	.	0.025
43	5/7/97	12:08	.	0.36		50	5/8/97	05:32	300	.
43	5/7/97	18:00	.	0.025		50	5/8/97	06:31	.	3.22
43	5/8/97	05:34	.	0.025		50	5/8/97	07:30	.	2.63
43	5/8/97	05:38	400	.		50	5/8/97	09:22	.	1.23
43	5/8/97	06:41	.	4.84		51	5/14/97	04:58	300	.
43	5/8/97	07:35	.	3.39		51	5/14/97	13:39	.	0.37
43	5/8/97	09:28	.	1.17		51	5/14/97	17:46	.	0.23

Appendix F

ISONIAZID DOSE AND CONCENTRATION DATA

ID	DATE (mm/dd/yy)	TIME	DOSE mg	CONC µg/ml
51	5/15/97	05:43	.	0.025
51	5/15/97	05:45	300	.
51	5/15/97	07:02	.	1.56
51	5/15/97	08:25	.	2.19
52	5/14/97	04:52	300	.
52	5/14/97	12:55	.	0.49
52	5/14/97	17:23	.	0.36
52	5/15/97	05:58	300	.
52	5/15/97	08:49	.	0.025
53	5/14/97	04:53	400	.
53	5/14/97	13:48	.	0.56
53	5/15/97	05:50	.	0.025
53	5/15/97	05:53	400	.
53	5/15/97	07:08	.	7.31
53	5/15/97	08:49	.	2.41
54	5/14/97	04:55	300	.
54	5/14/97	13:22	.	0.34
54	5/14/97	17:28	.	0.17
54	5/15/97	06:11	.	0.025
54	5/15/97	06:13	300	.
54	5/15/97	07:26	.	4.3
54	5/15/97	08:12	.	2.64
54	5/15/97	09:41	.	1.7
55	5/14/97	04:59	.300	.
55	5/14/97	13:12	.	0.6
55	5/14/97	18:03	.	0.08
55	5/15/97	06:16	.	0.025
55	5/15/97	06:17	300	.
55	5/15/97	07:19	.	5
55	5/15/97	08:10	.	4.87
56	5/14/97	05:00	400	.
56	5/14/97	13:52	.	0.31
56	5/14/97	18:20	.	0.12
56	5/15/97	05:36	.	0.025
56	5/15/97	05:37	400	.
56	5/15/97	06:52	.	5.39
56	5/15/97	07:42	.	3.27
56	5/15/97	09:35	.	1.37
57	5/14/97	04:56	400	.
57	5/14/97	13:00	.	0.4
57	5/14/97	18:15	.	0.21
58	5/14/97	04:57	300	.
58	5/14/97	13:05	.	0.48
58	5/15/97	05:28	300	.
58	5/15/97	05:28	.	0.025
58	5/15/97	08:00	.	3.53
59	5/14/97	04:50	300	.

ID	DATE (mm/dd/yy)	TIME	DOSE mg	CONC µg/ml
59	5/14/97	12:35	.	0.44
59	5/14/97	17:06	.	0.16
59	5/15/97	06:25	300	.
59	5/15/97	09:25	.	2.79
60	5/14/97	04:51	300	.
60	5/14/97	12:42	.	0.94
60	5/14/97	18:10	.	0.025
60	5/15/97	05:17	.	0.025
60	5/15/97	05:18	300	.
60	5/15/97	06:34	.	8.61
60	5/15/97	07:32	.	4.83
60	5/15/97	09:20	.	3.15
61	5/14/97	04:54	400	.
61	5/14/97	14:05	.	1.03
61	5/14/97	17:33	.	0.28
61	5/15/97	06:08	400	.
61	5/15/97	09:18	.	3.8
62	5/14/97	05:02	400	.
62	5/14/97	13:17	.	0.87
62	5/14/97	17:42	.	0.24
62	5/15/97	05:33	.	0.025
62	5/15/97	05:34	400	.
62	5/15/97	06:46	.	7.79
62	5/15/97	07:37	.	5.84
62	5/15/97	09:28	.	1.65
63	5/21/97	04:33	400	.
63	5/21/97	08:35	133	.
63	5/21/97	13:22	.	2.83
63	5/21/97	14:25	133	.
63	5/21/97	17:05	133	.
63	5/21/97	17:32	.	3.85
63	5/22/97	05:15	400	.
63	5/22/97	05:15	.	0.56
63	5/22/97	06:20	.	7.84
63	5/22/97	07:24	.	8.9
63	5/22/97	08:35	133	.
63	5/22/97	09:19	.	5.87
64	5/21/97	04:30	300	.
64	5/21/97	13:15	.	1.61
64	5/21/97	17:51	.	0.65
64	5/22/97	05:50	.	0.025
64	5/22/97	05:51	300	.
64	5/22/97	06:55	.	7.28
64	5/22/97	07:40	.	5.99
64	5/22/97	09:56	.	3.93
65	5/21/97	04:34	300	.
65	5/21/97	12:39	.	0.34

Appendix F

ISONIAZID DOSE AND CONCENTRATION DATA

ID	DATE (mm/dd/yy)	TIME	DOSE mg	CONC µg/ml
65	5/21/97	17:40	.	0.025
65	5/22/97	05:39	300	.
65	5/22/97	06:32	.	5.25
65	5/22/97	07:31	.	4.72
65	5/22/97	09:40	.	2.15
66	5/21/97	04:35	400	.
66	5/21/97	12:45	.	2.53
66	5/21/97	17:10	.	1.11
66	5/22/97	05:27	.	0.4
66	5/22/97	05:29	400	.
66	5/22/97	06:30	.	3.59
66	5/22/97	07:30	.	11.71
66	5/22/97	09:35	.	5.98
67	5/21/97	04:31	300	.
67	5/21/97	13:37	.	1.41
67	5/21/97	17:20	.	0.91
67	5/22/97	05:40	.	0.86
67	5/22/97	05:41	300	.
67	5/22/97	06:35	.	3.62
67	5/22/97	07:36	.	9.19
67	5/22/97	09:49	.	3.73
68	5/21/97	04:32	300	.
68	5/21/97	13:29	.	0.3
68	5/21/97	17:29	.	0.15
68	5/22/97	06:02	300	.
68	5/22/97	06:52	.	5.87
68	5/22/97	07:53	.	5.78
68	5/22/97	10:10	.	2.54
69	5/21/97	04:40	300	.
69	5/21/97	08:30	133	.
69	5/21/97	13:33	.	1.87
69	5/21/97	14:15	133	.
69	5/21/97	17:00	133	.
69	5/21/97	17:35	.	1.59
69	5/22/97	05:48	.	0.32
69	5/22/97	05:49	300	.
69	5/22/97	06:43	.	4.43
69	5/22/97	07:49	.	5.95
69	5/22/97	09:30	133	.
69	5/22/97	10:00	.	4.63
70	5/21/97	04:41	400	.
70	5/21/97	12:52	.	2.25
70	5/21/97	17:42	.	0.76
70	5/22/97	05:44	.	0.18
70	5/22/97	05:45	400	.
70	5/22/97	06:45	.	8.61
70	5/22/97	07:43	.	6.76

ID	DATE (mm/dd/yy)	TIME	DOSE mg	CONC µg/ml
70	5/22/97	09:31	.	3.28
71	5/21/97	04:37	300	.
71	5/21/97	13:12	.	2.11
71	5/21/97	17:23	.	0.64
71	5/22/97	05:33	.	0.025
71	5/22/97	05:34	300	.
71	5/22/97	06:39	.	8.49
71	5/22/97	07:37	.	6.31
71	5/22/97	09:53	.	3.53
72	5/21/97	04:38	300	.
72	5/21/97	12:57	.	2.72
72	5/21/97	17:17	.	1.33
72	5/22/97	05:53	.	0.32
72	5/22/97	05:54	300	.
72	5/22/97	07:04	.	8.64
72	5/22/97	07:58	.	7.78
72	5/22/97	09:45	.	4.79
73	5/21/97	04:39	300	.
73	5/21/97	13:06	.	0.09
73	5/21/97	17:46	.	0.025
73	5/22/97	06:04	.	0.025
73	5/22/97	06:05	300	.
73	5/22/97	06:50	.	7.6
73	5/22/97	07:47	.	8.89
73	5/22/97	10:10	.	4.24
74	5/21/97	04:15	300	.
74	5/21/97	13:56	.	0.34
74	5/21/97	18:06	.	0.13
74	5/22/97	06:17	300	.
74	5/22/97	07:03	.	5.41
74	5/22/97	08:01	.	4.42
74	5/22/97	10:26	.	1.26
75	5/21/97	04:25	400	.
75	5/21/97	14:03	.	1.03
75	5/21/97	18:02	.	0.63
75	5/22/97	06:25	400	.
75	5/22/97	07:18	.	8.31
75	5/22/97	08:12	.	6.6
75	5/22/97	10:19	.	3.25
76	5/21/97	04:20	300	.
76	5/21/97	14:12	.	1.46
76	5/21/97	17:59	.	0.78
76	5/22/97	06:10	.	0.28
76	5/22/97	06:13	300	.
76	5/22/97	07:08	.	9.4
76	5/22/97	08:10	.	8.09
76	5/22/97	10:31	.	4.06

Appendix F

ISONIAZID DOSE AND CONCENTRATION DATA

ID	DATE (mm/dd/yy)	TIME	DOSE mg	CONC μg/ml
77	5/21/97	04:15	300	.
77	5/21/97	13:53	.	0.78
77	5/21/97	17:55	.	0.24
77	5/22/97	06:10	300	.
77	5/22/97	07:01	.	3.28
77	5/22/97	08:06	.	6.5
77	5/22/97	10:23	.	2.87
78	5/21/97	04:42	400	.
78	5/21/97	13:41	.	0.32
78	5/21/97	17:14	.	0.25
78	5/22/97	05:22	.	0.54
78	5/22/97	05:25	400	.
78	5/22/97	06:26	.	5.65
78	5/22/97	07:26	.	4.58
78	5/22/97	09:25	.	1.98
79	5/28/97	04:56	400	.
79	5/28/97	12:35	.	0.26
79	5/28/97	16:05	.	0.025
79	5/29/97	05:37	400	.
79	5/29/97	05:37	.	0.025
79	5/29/97	06:41	.	9.2
79	5/29/97	07:42	.	4.79
79	5/29/97	09:55	.	1.99
80	5/28/97	04:58	400	.
80	5/28/97	08:00	133	.
80	5/28/97	12:20	.	8.32
80	5/28/97	14:00	133	.
80	5/28/97	15:52	.	5.21
80	5/28/97	18:00	133	.
80	5/29/97	05:42	400	.
80	5/29/97	05:42	.	2.46
80	5/29/97	06:45	.	10.55
80	5/29/97	07:49	.	11.62
81	5/28/97	04:51	400	.
81	5/28/97	12:40	.	2.88
81	5/28/97	16:22	.	1.11
81	5/29/97	05:49	.	0.025
81	5/29/97	05:50	400	.
81	5/29/97	06:51	.	9.59
81	5/29/97	07:55	.	6.88
81	5/29/97	10:04	.	4.72
82	5/28/97	04:55	400	.
82	5/28/97	08:00	133	.
82	5/28/97	12:30	.	0.69
82	5/28/97	14:00	133	.
82	5/28/97	18:00	133	.
82	5/29/97	05:44	400	.

ID	DATE (mm/dd/yy)	TIME	DOSE mg	CONC μg/ml
82	5/29/97	07:04	.	4.67
82	5/29/97	08:01	.	4.2
82	5/29/97	08:42	133	.
82	5/29/97	10:00	.	4.47
83	5/28/97	05:00	400	.
83	5/28/97	12:25	.	0.58
83	5/28/97	15:56	.	0.28
83	5/29/97	05:32	400	.
83	5/29/97	05:32	.	0.025
83	5/29/97	06:37	.	7.62
83	5/29/97	07:37	.	5.93
84	5/28/97	04:50	400	.
84	5/28/97	12:58	.	2.29
84	5/28/97	15:43	.	0.94
84	5/29/97	05:55	400	.
84	5/29/97	08:45	.	3.37
85	5/28/97	04:50	400	.
85	5/28/97	08:15	133	.
85	5/28/97	12:44	.	2.19
85	5/28/97	14:00	133	.
85	5/28/97	17:00	133	.
85	5/28/97	17:52	.	0.46
85	5/29/97	05:22	400	.
85	5/29/97	05:22	.	0.025
85	5/29/97	06:28	.	5.29
85	5/29/97	07:29	.	4.88
85	5/29/97	08:25	133	.
85	5/29/97	09:32	.	3.28
86	5/28/97	05:15	400	.
86	5/28/97	12:36	.	4.15
86	5/28/97	17:48	.	1.5
86	5/29/97	05:11	400	.
86	5/29/97	05:11	.	0.97
86	5/29/97	06:16	.	8.23
86	5/29/97	07:16	.	6.69
86	5/29/97	09:16	.	4.33
87	5/28/97	05:15	400	.
87	5/28/97	12:26	.	0.74
87	5/28/97	17:43	.	0.29
87	5/29/97	05:15	400	.
87	5/29/97	05:15	.	0.025
87	5/29/97	06:22	.	3.16
87	5/29/97	07:23	.	3.04
87	5/29/97	09:23	.	1.55
88	6/11/97	05:22	300	.
88	6/11/97	12:48	.	1.76
88	6/11/97	17:15	.	0.2

Appendix F

ISONIAZID DOSE AND CONCENTRATION DATA

ID	DATE (mm/dd/yy)	TIME	DOSE mg	CONC µg/ml
88	6/12/97	05:50	300	.
88	6/12/97	05:50	.	0.025
88	6/12/97	06:58	.	10.01
88	6/12/97	07:46	.	7.76
88	6/12/97	09:50	.	5.22
89	6/11/97	05:10	400	.
89	6/11/97	13:03	.	2.49
89	6/11/97	17:10	.	1.05
89	6/12/97	05:30	400	.
89	6/12/97	05:30	.	0.1
89	6/12/97	06:31	.	6.98
89	6/12/97	07:32	.	9.17
89	6/12/97	09:43	.	5.32
90	6/11/97	05:17	300	.
90	6/11/97	12:37	.	2.07
90	6/11/97	17:25	.	0.57
90	6/12/97	05:51	300	.
90	6/12/97	06:35	.	9.06
90	6/12/97	07:48	.	8.41
90	6/12/97	09:53	.	6.02
91	6/11/97	05:15	300	.
91	6/11/97	13:09	.	3.04
91	6/11/97	17:38	.	1.01
91	6/12/97	05:27	300	.
91	6/12/97	05:27	.	0.18
91	6/12/97	06:27	.	3.69
91	6/12/97	07:29	.	5.45
91	6/12/97	09:25	.	6.74
92	6/11/97	05:20	400	.
92	6/11/97	12:29	.	1.63
92	6/11/97	17:07	.	0.85
92	6/12/97	05:18	400	.
92	6/12/97	05:18	.	0.025
92	6/12/97	06:18	.	5.15
92	6/12/97	07:20	.	4.51
92	6/12/97	09:14	.	2.46
93	6/11/97	05:21	300	.
93	6/11/97	12:55	.	0.98
93	6/11/97	17:30	.	0.36
93	6/12/97	05:36	300	.
93	6/12/97	05:36	.	0.025
93	6/12/97	06:44	.	7.78
93	6/12/97	07:37	.	6.16
93	6/12/97	09:38	.	3.5
94	6/11/97	05:16	600	.
94	6/11/97	13:02	.	4.97
94	6/11/97	17:32	.	2.43

ID	DATE (mm/dd/yy)	TIME	DOSE mg	CONC µg/ml
94	6/12/97	05:44	600	.
94	6/12/97	05:44	.	6.4
94	6/12/97	06:46	.	13.73
94	6/12/97	07:41	.	11.6
94	6/12/97	09:45	.	9.3
95	6/11/97	05:20	400	.
95	6/11/97	08:55	133	.
95	6/11/97	13:12	.	2.56
95	6/11/97	14:10	133	.
95	6/11/97	17:20	.	3.01
95	6/11/97	18:02	133	.
95	6/12/97	05:22	400	.
95	6/12/97	05:22	.	0.22
95	6/12/97	06:21	.	4.97
95	6/12/97	07:25	.	5.96
95	6/12/97	08:25	133	.
95	6/12/97	09:19	.	4.72
96	6/11/97	05:00	400	.
96	6/11/97	13:24	.	0.44
96	6/11/97	17:52	.	0.22
96	6/12/97	06:03	400	.
96	6/12/97	06:03	.	0.025
96	6/12/97	07:08	.	4.4
96	6/12/97	08:05	.	4.36
96	6/12/97	10:05	.	2.06
97	7/9/97	05:15	300	.
97	7/9/97	13:56	.	1.59
97	7/9/97	17:21	.	0.82
97	7/10/97	05:42	.	0.025
97	7/10/97	05:43	300	.
97	7/10/97	06:48	.	8.23
97	7/10/97	07:42	.	8.28
97	7/10/97	09:39	.	4.76
98	7/9/97	05:19	400	.
98	7/9/97	13:37	.	0.66
98	7/9/97	17:12	.	0.31
98	7/10/97	05:40	400	.
98	7/10/97	05:40	.	0.025
98	7/10/97	06:45	.	4.7
98	7/10/97	07:45	.	3.72
98	7/10/97	09:43	.	1.71
99	7/9/97	05:10	300	.
99	7/9/97	14:33	.	0.96
99	7/9/97	17:31	.	0.38
99	7/10/97	05:37	300	.
99	7/10/97	05:37	.	0.025
99	7/10/97	06:44	.	6.32

Appendix F

ISONIAZID DOSE AND CONCENTRATION DATA

ID	DATE (mm/dd/yy)	TIME	DOSE mg	CONC µg/ml
99	7/10/97	07:39	.	4.51
99	7/10/97	09:35	.	1.78
100	7/9/97	05:11	400	.
100	7/9/97	14:04	.	1.58
100	7/9/97	17:28	.	0.91
100	7/10/97	05:52	400	.
100	7/10/97	05:52	.	0.025
100	7/10/97	06:55	.	9.81
100	7/10/97	07:49	.	8.69
101	7/9/97	05:14	300	.
101	7/9/97	14:30	.	0.46
101	7/9/97	17:25	.	0.21
101	7/10/97	05:47	300	.
101	7/10/97	08:31	.	3.16
102	7/9/97	05:10	300	.
102	7/9/97	13:25	.	0.36
102	7/10/97	05:25	300	.
102	7/10/97	08:34	.	1.3
103	7/9/97	05:18	300	.
103	7/9/97	13:45	.	0.5
103	7/9/97	17:37	.	0.24
103	7/10/97	05:28	300	.
103	7/10/97	05:28	.	0.025
103	7/10/97	06:33	.	4.88
103	7/10/97	08:25	.	3.9
104	7/9/97	05:22	300	.
104	7/9/97	13:50	.	0.31
104	7/9/97	17:19	.	0.1
104	7/10/97	05:34	300	.
104	7/10/97	05:34	.	0.025
104	7/10/97	06:38	.	6.01
105	7/9/97	11:22	400	.
105	7/9/97	13:30	.	1.21
105	7/9/97	17:08	.	1.91
105	7/10/97	05:23	.	0.32
105	7/10/97	05:24	400	.
105	7/10/97	06:26	.	7.96
105	7/10/97	08:23	.	3.72
106	7/9/97	05:15	300	.
106	7/9/97	14:15	.	0.92
106	7/10/97	05:30	300	.
106	7/10/97	08:38	.	3.31
107	7/23/97	05:40	300	.
107	7/23/97	13:50	.	0.91
107	7/23/97	17:52	.	0.34
107	7/24/97	05:15	300	.
107	7/24/97	05:15	.	0.08

ID	DATE (mm/dd/yy)	TIME	DOSE mg	CONC µg/ml
107	7/24/97	06:30	.	6
107	7/24/97	07:20	.	4.9
107	7/24/97	09:25	.	2.4
108	7/23/97	05:40	400	.
108	7/23/97	13:03	.	0.82
108	7/23/97	17:28	.	0.24
108	7/24/97	05:10	.	0.025
108	7/24/97	05:11	400	.
108	7/24/97	07:19	.	4.58
108	7/24/97	09:55	.	1.9
109	7/23/97	05:40	400	.
109	7/23/97	12:43	.	1.56
109	7/23/97	17:25	.	0.47
109	7/24/97	05:27	400	.
109	7/24/97	05:27	.	0.025
109	7/24/97	06:42	.	7.9
109	7/24/97	07:31	.	5.64
109	7/24/97	09:42	.	4.01
110	7/23/97	05:38	300	.
110	7/23/97	12:53	.	0.99
110	7/23/97	17:34	.	0.24
110	7/24/97	05:19	300	.
110	7/24/97	05:19	.	0.025
110	7/24/97	06:23	.	5.74
110	7/24/97	07:15	.	4.55
110	7/24/97	09:16	.	2.11
111	7/23/97	05:38	300	.
111	7/23/97	14:04	.	0.9
111	7/23/97	17:47	.	0.32
111	7/24/97	05:34	300	.
111	7/24/97	05:34	.	0.025
111	7/24/97	06:49	.	7.91
111	7/24/97	07:35	.	5.64
111	7/24/97	09:47	.	2.42
112	7/23/97	05:36	400	.
112	7/23/97	08:45	133	.
112	7/23/97	13:38	.	3.12
112	7/23/97	14:00	133	.
112	7/23/97	17:37	.	3.19
112	7/23/97	18:00	133	.
112	7/24/97	05:22	400	.
112	7/24/97	05:22	.	0.62
112	7/24/97	06:35	.	14.06
112	7/24/97	07:26	.	12.93
112	7/24/97	08:45	133	.
112	7/24/97	09:30	.	9.4
113	7/23/97	05:37	400	.

Appendix F

ISONIAZID DOSE AND CONCENTRATION DATA

ID	DATE (mm/dd/yy)	TIME	DOSE mg	CONC μg/ml
113	7/23/97	13:23	.	1.3
113	7/23/97	17:41	.	0.4
113	7/24/97	05:25	400	.
113	7/24/97	05:25	.	0.025
113	7/24/97	06:38	.	10
113	7/24/97	07:28	.	6.78
113	7/24/97	09:37	.	3.44
114	8/5/97	05:15	400	.
114	8/5/97	09:05	.	2.97
114	8/6/97	05:15	400	.
114	8/6/97	13:04	.	0.81
114	8/6/97	17:57	.	0.17
114	8/7/97	05:26	.	0.025
114	8/7/97	05:27	400	.
114	8/7/97	06:27	.	7.97
114	8/7/97	07:27	.	5.38
114	8/7/97	09:25	.	3.58
115	8/6/97	05:11	300	.
115	8/6/97	08:32	133	.
115	8/6/97	12:45	.	2.31
115	8/6/97	14:14	133	.
115	8/6/97	17:05	133	.
115	8/7/97	05:41	.	0.38
115	8/7/97	05:42	300	.
115	8/7/97	06:41	.	6.09
115	8/7/97	07:48	.	4.96
115	8/7/97	09:00	133	.
115	8/7/97	09:48	.	2.84
116	8/6/97	05:05	300	.
116	8/6/97	13:17	.	0.69
116	8/7/97	05:44	.	0.19
116	8/7/97	05:45	300	.
116	8/7/97	06:47	.	5.14
116	8/7/97	07:55	.	4.74
116	8/7/97	09:55	.	1.75
117	8/6/97	05:06	400	.
117	8/6/97	13:10	.	0.63
117	8/6/97	17:52	.	0.22
117	8/7/97	05:35	.	0.025
117	8/7/97	05:36	400	.
117	8/7/97	06:35	.	6.79
117	8/7/97	07:41	.	4.03
117	8/7/97	09:41	.	1.91
118	8/6/97	05:12	300	.
118	8/6/97	12:49	.	0.47
118	8/6/97	17:50	.	0.16
118	8/7/97	05:36	.	0.025

ID	DATE (mm/dd/yy)	TIME	DOSE mg	CONC μg/ml
118	8/7/97	05:37	300	.
118	8/7/97	06:31	.	6.19
118	8/7/97	07:32	.	2.87
118	8/7/97	09:39	.	1.26
119	8/6/97	05:10	300	.
119	8/6/97	13:01	.	1.99
119	8/6/97	17:39	.	0.88
119	8/7/97	05:23	.	0.025
119	8/7/97	05:24	300	.
119	8/7/97	06:24	.	7.72
119	8/7/97	07:23	.	8.13
119	8/7/97	09:22	.	4.87
120	8/6/97	05:10	300	.
120	8/6/97	12:53	.	0.59
120	8/7/97	05:50	300	.
120	8/7/97	05:50	.	0.025
120	8/7/97	06:51	.	2.71
120	8/7/97	08:00	.	3.16
120	8/7/97	10:02	.	1.17
121	8/6/97	05:08	400	.
121	8/6/97	13:08	.	2.84
121	8/6/97	17:30	.	0.68
121	8/7/97	05:38	400	.
121	8/7/97	05:38	.	0.025
121	8/7/97	06:39	.	9.63
121	8/7/97	07:47	.	9.11
121	8/7/97	09:45	.	4.47
122	8/6/97	05:20	400	.
122	8/6/97	13:36	.	0.85
122	8/6/97	18:02	.	0.43
122	8/7/97	06:02	400	.
122	8/7/97	07:04	.	0.71
122	8/7/97	08:09	.	6.38
122	8/7/97	10:09	.	4.85
123	8/6/97	04:55	400	.
123	8/6/97	13:31	.	1.02
123	8/6/97	18:18	.	0.26
123	8/7/97	06:05	.	0.025
123	8/7/97	06:06	400	.
123	8/7/97	07:08	.	6.12
123	8/7/97	10:13	.	2.26
124	8/6/97	05:05	400	.
124	8/6/97	10:13	.	1.02
124	8/6/97	13:41	.	0.31
124	8/6/97	18:08	.	0.025
125	8/13/97	05:00	400	.
125	8/13/97	08:55	133	.

Appendix F

ISONIAZID DOSE AND CONCENTRATION DATA

ID	DATE (mm/dd/yy)	TIME	DOSE mg	CONC µg/ml
125	8/13/97	11:55	.	6.04
125	8/13/97	14:05	133	.
125	8/13/97	16:40	133	.
125	8/13/97	17:30	.	7.58
125	8/14/97	06:09	.	3.96
125	8/14/97	06:10	400	.
125	8/14/97	07:30	.	10.42
125	8/14/97	08:21	.	9.15
125	8/14/97	08:50	133	.
125	8/14/97	10:03	.	8.92
126	8/13/97	05:00	300	.
126	8/13/97	12:15	.	1.01
126	8/13/97	17:42	.	0.7
126	8/14/97	06:25	.	0.025
126	8/14/97	06:26	300	.
126	8/14/97	07:42	.	4.73
126	8/14/97	08:27	.	3.28
126	8/14/97	10:13	.	1.68
127	8/13/97	05:00	400	.
127	8/13/97	13:10	.	2.51
127	8/13/97	17:10	.	1.21
127	8/14/97	06:18	.	0.2
127	8/14/97	06:19	400	.
127	8/14/97	07:36	.	8.42
127	8/14/97	08:31	.	6.64
127	8/14/97	10:20	.	4.71
128	8/13/97	05:00	300	.
128	8/13/97	13:05	.	0.62
128	8/13/97	17:57	.	0.36
128	8/14/97	05:53	.	0.025
128	8/14/97	05:57	300	.
128	8/14/97	07:10	.	6.72
128	8/14/97	08:11	.	4.41
128	8/14/97	09:52	.	1.88
129	8/13/97	04:30	400	.
129	8/13/97	12:34	.	0.35
129	8/13/97	18:08	.	0.025
129	8/14/97	04:45	400	.
129	8/14/97	05:20	400	.
129	8/14/97	05:20	.	2.38
129	8/14/97	06:41	.	11.1
129	8/14/97	07:33	.	8.63
129	8/14/97	09:18	.	2.53
130	8/13/97	04:30	400	.
130	8/13/97	12:49	.	0.84
130	8/13/97	18:32	.	0.3
130	8/14/97	05:37	.	0.025

ID	DATE (mm/dd/yy)	TIME	DOSE mg	CONC µg/ml
130	8/14/97	05:38	400	.
130	8/14/97	06:58	.	0.84
130	8/14/97	07:57	.	4.9
130	8/14/97	09:38	.	1.38
131	8/13/97	04:30	400	.
131	8/13/97	12:40	.	2.21
131	8/13/97	18:28	.	0.78
131	8/14/97	04:45	400	.
131	8/14/97	05:28	400	.
131	8/14/97	05:28	.	11.76
131	8/14/97	06:47	.	23.58
131	8/14/97	07:50	.	11.21
131	8/14/97	09:33	.	11.81
132	8/13/97	04:30	300	.
132	8/13/97	12:45	.	1.74
132	8/13/97	18:18	.	0.34
132	8/14/97	04:45	300	.
132	8/14/97	05:41	300	.
132	8/14/97	05:41	.	10.28
132	8/14/97	09:15	.	11.17
133	8/13/97	04:30	300	.
133	8/13/97	12:43	.	2.56
133	8/13/97	18:35	.	1.07
133	8/14/97	04:45	300	.
133	8/14/97	05:45	.	10.26
133	8/14/97	05:46	300	.
133	8/14/97	07:02	.	21.96
133	8/14/97	08:05	.	14.03
133	8/14/97	09:43	.	13.28
134	8/13/97	04:35	300	.
134	8/13/97	12:56	.	0.025
134	8/13/97	18:24	.	0.025
134	8/14/97	04:45	300	.
134	8/14/97	05:15	300	.
134	8/14/97	05:15	.	11.03
134	8/14/97	06:42	.	18.39
134	8/14/97	08:00	.	9.18
134	8/14/97	09:15	.	7.3
135	8/13/97	04:35	300	.
135	8/13/97	12:46	.	1.34
135	8/13/97	18:14	.	0.64
135	8/14/97	04:45	300	.
135	8/14/97	05:31	.	7.9
135	8/14/97	05:32	300	.
135	8/14/97	06:44	.	10.97
135	8/14/97	07:38	.	8.19
135	8/14/97	09:26	.	6.01

Appendix F

ISONIAZID DOSE AND CONCENTRATION DATA

ID	DATE (mm/dd/yy)	TIME	DOSE mg	CONC µg/ml
136	8/13/97	04:35	300	.
136	8/13/97	12:50	.	0.025
136	8/13/97	18:21	.	0.025
136	8/14/97	04:45	300	.
136	8/14/97	05:25	300	.
136	8/14/97	05:25	.	8.37
136	8/14/97	06:53	.	13.12
136	8/14/97	07:58	.	8.95
136	8/14/97	09:40	.	6.45
137	8/13/97	04:35	400	.
137	8/13/97	12:38	.	0.1
137	8/13/97	18:39	.	0.025
137	8/14/97	04:45	400	.
137	8/14/97	05:23	400	.
137	8/14/97	05:23	.	0.025
137	8/14/97	06:46	.	7.01
137	8/14/97	07:48	.	6.44
137	8/14/97	09:22	.	5.01
138	8/13/97	04:10	300	.
138	8/13/97	13:20	.	0.025
138	8/13/97	18:44	.	0.025
138	8/14/97	05:42	300	.
138	8/14/97	05:42	.	0.53
138	8/14/97	07:16	.	4.75
138	8/14/97	09:30	.	2.69

Appendix G   NONMEM RUN SUMMARY - RIFAMPICIN

Appendix G

NONMEM RUN SUMMARY - RIFAMPICIN

RUN	ADVAN	ERROR	KA	CL/F	V/F	TLAG	η_{KA} %	$\eta_{CL/F}$ %	$\eta_{V/F}$ %	η_{TLAG} %	σ_{CCV} %	σ_{ADD} µg/ml	DATA	OBF	COMMENTS
1	2	ADD + CCV	$\theta 1\exp(\eta 1)$	$\theta 2\exp(\eta 2)$	$\theta 3\exp(\eta 3)$	-	60	44	223	-	4540	10^{-4}	RFA1.CSV	1803.550	Prepared sdtab for data checkout in Xpose. Model is under-predicting but appears concentrated at PRED=11. $ERROR code appears incorrect.
2	2	ADD + CCV	$\theta 1\exp(\eta 1)$	$\theta 2\exp(\eta 2)$	$\theta 3\exp(\eta 3)$	-	60	45	223	-	4540	10^{-4}	RFA1.CSV	1803.548	Removed upper bound on V/F. No difference w.r.t. Concentration of points at PRED=11. $ERROR code appears incorrect.
3	2	ADD+CCV	$\theta 1\exp(\eta 1)$	$\theta 2\exp(\eta 2)$	$\theta 3\exp(\eta 3)$	-	60	51	<1	-	45.6	0.53	RFA1.CSV	1474.664	Removed constraint on additive portion of error model. Concentration of points now at PRED=7.5. Additive error = $\sigma^*\theta 4$
4	2	ADD+CCV	$\theta 1\exp(\eta 1)$	$\theta 2\exp(\eta 2)$	$\theta 3\exp(\eta 3)$	-	15	49	62	-	39.9	0.57	RFA1.CSV	1486.416	Removed upper bound on CL/F as well. No difference w.r.t. concentration of points now at PRED=7.5
5	2	ADD+CCV	$\theta 1\exp(\eta 1)$	$\theta 2\exp(\eta 2)$	$\theta 3\exp(\eta 3)$	-	60	52	3	-	45.6	0.53	RFA1.CSV	1474.665	Removed upper bound on ALL θ's. No difference w.r.t. concentration of points now at PRED=7.5
6	2	ADD+CCV	$\theta 1\exp(\eta 1)$	$\theta 2\exp(\eta 2)$	$\theta 3\exp(\eta 3)$	-	10^4	65	34	-	61.3	0.54	RFA1.CSV	1612.403	Fixed $SIGMA to 1. Included 2 θ's in the $ERROR block. Added scatters. Clustering of points at 7.5 now OK but KA and its CV are unrealistic.
7	2	ADD+CCV	$\theta 1\exp(\eta 1)$	$\theta 2\exp(\eta 2)$	$\theta 3\exp(\eta 3)$	-	53	52	19	-	48.9	0.05	RFA2.CSV	855.716	Removed Patient 122 (outlier) completely. No difference w.r.t. concentration of points at PRED=7.5
8	2	ADD+CCV	$\theta 1\exp(\eta 1)$	$\theta 2\exp(\eta 2)$	$\theta 3\exp(\eta 3)$	-	60	51	<1	-	45.6	0.54	RFA1.CSV	1474.664	Used the conventional method of coding $ERROR to check that the Xpose2 way of coding is correct. Results = Run3
9	2	ADD+CCV	$\theta 1\exp(\eta 1)$	$\theta 2\exp(\eta 2)$	$\theta 3\exp(\eta 3)$	-	54	53	19	-	49.2	0.05	RFA3.CSV	863.349	Now using $ERROR as per Run3. Removed Cp for patient 122 and 123 that was swapped during sample prep. No difference w.r.t. concentration of points at PRED=7.5
10	2	ADD+CCV	$\theta 1\exp(\eta 1)$ (600mg) or $\theta 6\exp(\eta 1)$ 450mg	$\theta 2\exp(\eta 2)$	$\theta 3\exp(\eta 3)$	-	55	52	20	-	49	0.05	RFA5.CSV	862.357	Data file updated to include dose amount and days since starting RFA therapy. No significant effect of dose amount on KA.
11	2	ADD+CCV	$\theta 1\exp(\eta 1)$	$\theta 2\exp(\eta 2)$	$\theta 3\exp(\eta 3)$	$\theta 6$	67	53	22	-	47	0.05	RFA5.CSV	844.418	A basic run but now with a lag time. No difference w.r.t. Concentration of points at PRED=7.5. OBF is better than previous basic run (RUN 9).

η - inter-individual variability; KA – absorption rate constant, CL/F – apparent clearance; V/F – apparent volume of distribution; TLAG – absorption lag time; σ - intra-individual variability; CCV – constant coefficient of variation; ADD – additive; OBF – minimum value of the Objective function.

NONMEM RUN SUMMARY - RIFAMPICIN

RUN	ADVAN	ERROR	KA	CL/F	V/F	TLAG	ηKA %	ηCL/F %	ηV/F %	ηTLAG %	σCCV %	σADD µg/ml	DATA	OBF	COMMENTS
12	1	ADD+CCV	-	θ2exp(η2)	θ3exp(η3)	-	-	59	37	-	52	0.05	RFA7.CSV	865.335	Implemented zero order bolus input. Data file has RATE=-2. D1 parameter estimated. Concentration of points at PRED=7.5 a little better.
13	1	ADD+CCV	-	θ2exp(η2)	θ3exp(η3)	θ6	-	59	37	-	52	0.05	RFA7.CSV	865.314	As per RUN 12 but with a lag time. Graphs are no different TLAG very small.
14	1	ADD+CCV	-	θ2exp(η2)	θ3exp(η3)	-	-	55	29	-	49	0.05	RFA7.CSV	860.823	As per RUN 12 but added in an ETA on D1 (32%).
15	2	ADD+CCV	θ1exp(η1)	θ2exp(η2)	θ3exp(η3)	θ6exp(η4)	57	51	24	59	41	0.05	RFA5.CSV	804.652	As per RUN 11 but added in an ETA on TLAG1. Concentration of points at PRED=7.5 gone.
16	2	ADD+CCV	θ1exp(η1)	θ2exp(η2)	θ3exp(η3)	-	53	52	16	-	49	0.05	RFA5.CSV	864.274	Sensitivity Analysis: KA fixed to 0.7
17	2	ADD+CCV	θ1exp(η1)	θ2exp(η2)	θ3exp(η3)	-	54	53	21	-	49	0.05	RFA5.CSV	863.833	Sensitivity Analysis: KA fixed to 0.8
18	2	ADD+CCV	θ1exp(η1)	θ2exp(η2)	θ3exp(η3)	-	58	54	23	-	49	0.05	RFA5.CSV	867.862	Sensitivity Analysis: KA fixed to 0.9
19	2	ADD+CCV	θ1exp(η1)	θ2exp(η2)	θ3exp(η3)	-	63	54	25	-	49	0.05	RFA5.CSV	874.465	Sensitivity Analysis: KA fixed to 1.0
20	2	ADD+CCV	θ1exp(η1)	θ2exp(η2)	θ3exp(η3)	-	70	54	<1	-	49	0.05	RFA5.CSV	882.273	Sensitivity Analysis: KA fixed to 1.1
21	2	ADD+CCV	θ1exp(η1)	θ2exp(η2)	θ3exp(η3)	-	52	51	<1	-	49	0.05	RFA5.CSV	871.405	Sensitivity Analysis: KA fixed to 0.6
22	2	ADD+CCV	θ1exp(η1)	θ2exp(η2)	θ3exp(η3)	-	44	51	<1	-	50	0.05	RFA5.CSV	888.473	Sensitivity Analysis: KA fixed to 0.5
23	2	ADD+CCV	θ1exp(η1)	θ2exp(η2)	θ3exp(η3)	-	<1	51	42	-	50	0.05	RFA5.CSV	902.203	Sensitivity Analysis: KA fixed to 0.4
24	2	ADD+CCV	θ1exp(η1)	θ2exp(η2)	θ3exp(η3)	-	53	52	19	-	49	0.05	RFA5.CSV	863.360	Sensitivity Analysis: KA fixed to 0.75 – lowest value of OBF
25	2	ADD+CCV	θ1exp(η1)	θ2exp(η2)	θ3exp(η3)	-	54	53	20	-	49	0.05	RFA5.CSV	863.440	Sensitivity Analysis: KA fixed to 0.775
26	2	ADD (fixed)+CCV	θ1exp(η1)	θ2exp(η2)	θ3exp(η3)	-	39	19	10	-	70	fixed	RFA5.CSV	1122.353	Fixed additive part of error to 0.0006 i.e. 0.25*LQ**2.
27	4	ADD+CCV	θ1exp(η1)	θ2exp(η2) Q	V2=θ3exp(η3) V3	-	51	53	18	-	50	0.05	RFA5.CSV	858.735	Used ADVAN4. No diff w.r.t. points at PRED=7.5
28	2	ADD+CCV	θ1exp(η1)	θ2exp(η2)	θ3exp(η3)	-	57	51	24	-	41	0.05	RFA8.CSV	875.889	Data file has some of the below LQ levels removed i.e. those with an above LQ concentration nearby.
29	2	ADD+CCV	θ1exp(η1)	θ2exp(η2)	θ3exp(η3)	θ6exp(η4)	57	51	24	-	41	0.05	RFA9.CSV	804.652	Identical to RUN 15 but data file contains ALL the covariates. Preparing the tables for GAM analysis in Xpose2.
30	2	ADD+CCV	θ1exp(η1)	Non-inducer: θ2exp(η2) Inducer: θ7exp(η2)	θ3exp(η3)	θ6exp(η4)	56	51	25	59	41	0.05	RFA9.CSV	802.498	Subsequently realised that only 3 individuals were on inducers.

η - inter-individual variability; KA – absorption rate constant, CL/F – apparent clearance; V/F – apparent volume of distribution; TLAG – absorption lag time; σ - intra-individual variability; CCV – constant coefficient of variation; ADD – additive; OBF – minimum value of the Objective function.

Appendix G

NONMEM RUN SUMMARY - RIFAMPICIN

RUN	ADVAN	ERROR	KA	CL/F	V/F	TLAG	η_{KA} %	$\eta_{CL/F}$ %	$\eta_{V/F}$ %	η_{TLAG} %	σ_{CCV} %	σ_{ADD} µg/ml	DATA	OBF	COMMENTS
31	2	ADD+CCV	$\theta1\exp(\eta1)$	Non-inhibitor: $\theta2\exp(\eta2)$ Inhibitor: $\theta7\exp(\eta2)$	$\theta3\exp(\eta3)$	$\theta6\exp(\eta4)$	54	47	26	62	40	0.05	RFA9.CSV	766.899	Enzyme inhibitor reduces CL/F by 84%. Significant effect.
32	2	ADD+CCV	$\theta1\exp(\eta1)$	$\theta2\exp(\eta2)$	Non-inhibitor: $\theta3\exp(\eta3)$ Inhibitor: $\theta7\exp(\eta3)$	$\theta6\exp(\eta4)$	57	52	25	59	41	0.05	RFA9.CSV	797.184	Effect of enzyme inhibitor on V/F. Significant effect. May disappear in the full model.
33	2	ADD+CCV	$\theta1\exp(\eta1)$ + IOV	$\theta2\exp(\eta2)$ + IOV	$\theta3\exp(\eta3)$ + IOV	-	57; 116	89; 43	51; 30	-	40	0.05	RFA10.CSV	833.171	Inter-occasion variability i.e. day1 vs day2 implemented on KA, CL/F and V/F. Removed TLAG from model because of restriction on the no. of ηs. Data file has new variable OCC. Day2 fit has less variability for CL/F & V/F but more for KA.
34	2	ADD+CCV	$\theta1\exp(\eta1)$	$\theta2\exp(\eta2)$	$\theta3\exp(\eta3)$	$\theta6\exp(\eta4)$	60	53	19	67	41	0.05	RFA11.CSV	646.148	Removed all Day2 data from the patients in Ward L who had been given the extra dose.
35	2	ADD+CCV	$\theta1\exp(\eta1)$	Females: $\theta2\exp(\eta2)$ Males: $\theta7\exp(\eta2)$	$\theta3\exp(\eta3)$	$\theta6\exp(\eta4)$	57	51	24	59	41	0.05	RFA9.CSV	804.606	SEX on CL/F. Not significant
36	2	ADD+CCV	$\theta1\exp(\eta1)$	$\theta2\exp(\eta2)$	Females: $\theta3\exp(\eta3)$ Males: $\theta7\exp(\eta3)$	$\theta6\exp(\eta4)$	58	50	22	61	40	0.05	RFA9.CSV	792.485	SEX on V/F. Significant
37	2	ADD+CCV	$\theta1\exp(\eta1)$	SENS: $\theta2\exp(\eta2)$ MDR: $\theta7\exp(\eta2)$	$\theta3\exp(\eta3)$	$\theta6\exp(\eta4)$	57	46	24	62	40	0.05	RFA9.CSV	778.309	MDR on CL/F. Significant
38	2	ADD+CCV	$\theta1\exp(\eta1)$	$\theta2\exp(\eta2)$	SENS: $\theta3\exp(\eta3)$ MDR: $\theta7\exp(\eta3)$	$\theta6\exp(\eta4)$	57	51	24	59	41	0.05	RFA9.CSV	802.326	MDR on V/F. Not significant.
39	2	ADD+CCV	$\theta1\exp(\eta1)$	Non-HIV: $\theta2\exp(\eta2)$ HIV: $\theta7\exp(\eta2)$	$\theta3\exp(\eta3)$	$\theta6\exp(\eta4)$	56	52	26	58	40	0.05	RFA9.CSV	797.720	HIV on CL/F. Significant
40	2	ADD+CCV	$\theta1\exp(\eta1)$	$\theta2\exp(\eta2)$	Non-HIV: $\theta3\exp(\eta3)$ HIV: $\theta7\exp(\eta3)$	$\theta6\exp(\eta4)$	56	51	26	59	40	0.05	RFA9.CSV	788.925	HIV on V/F. Significant
41	2	ADD+CCV	$\theta1\exp(\eta1)$ (450mg) or $\theta7\exp(\eta1)$ 600mg	$\theta2\exp(\eta2)$	$\theta3\exp(\eta3)$	$\theta6\exp(\eta4)$	59	51	25	58	40	0.05	RFA9.CSV	803.824	KA for the 2 different dose amounts. Not significant.

η - inter-individual variability; KA – absorption rate constant, CL/F – apparent clearance; V/F – apparent volume of distribution; TLAG – absorption lag time; σ - intra-individual variability; CCV – constant coefficient of variation; ADD – additive; OBF – minimum value of the Objective function.

Appendix G

NONMEM RUN SUMMARY - RIFAMPICIN

RUN	ADVAN	ERROR	KA	CL/F	V/F	TLAG	η_{KA} %	$\eta_{CL/F}$ %	$\eta_{V/F}$ %	η_{TLAG} %	σ_{CCV} %	σ_{ADD} µg/ml	DATA	OBF	COMMENTS
42	2	ADD+CCV	$\theta1\exp(\eta1)$	$\theta2\exp(\eta2)$	$\theta3\exp(\eta3)$	$\theta6\exp(\eta4)$	47	42	<1	69	37	0.05	RFA9.CSV	773.334	Estimated F1 – relative bioavailability for the 2 dose amounts i.e. 450mg and 600mg dose. Data can no longer estimate the variability in V/F once it is partitioned into F1.
43	2	ADD+CCV	$\theta1\exp(\eta1)$	$\theta2\exp(\eta2)$	$\theta3\exp(\eta3)$	$\theta6\exp(\eta4)$	56	50	24	61	40	0.05	RFA9.CSV	789.010	As for RUN 42 but no ETA on F1.
44	2	ADD+CCV	$\theta1\exp(\eta1)$	$\theta2\exp(\eta2)$	$\theta3\exp(\eta3)$ (450) $\theta7\exp(\eta3)$ (600)	$\theta6\exp(\eta4)$	57	51	24	59	41	0.05	RFA9.CSV	804.657	Effect of dose amount on V/F. Not significant.
45	2	ADD+CCV	$\theta1\exp(\eta1)$ (no DI) $\theta7\exp(\eta1)$ DI	$\theta2\exp(\eta2)$	$\theta3\exp(\eta3)$	$\theta6\exp(\eta4)$	57	51	25	59	41	0.05	RFA9.CSV	804.647	Effect of absorption drug interaction on KA. Not significant.
46	2	ADD+CCV	$\theta1\exp(\eta1)$	$\theta2\exp(\eta2)$ (no DI) or $\theta7\exp(\eta2)$ DI	$\theta3\exp(\eta3)$	$\theta6\exp(\eta4)$	58	51	24	59	41	0.05	RFA9.CSV	804.650	Effect of absorption drug interaction on CL/F. Not significant
47	2	ADD+CCV	$\theta1\exp(\eta1)$	$\theta2\exp(\eta2)$	$\theta3\exp(\eta3)$ (no DI) or $\theta7\exp(\eta3)$ DI	$\theta6\exp(\eta4)$	57	51	24	69	41	0.05	RFA9.CSV	805.115	Effect of absorption drug interaction on V/F. Not significant
48	2	ADD+CCV	$\theta1\exp(\eta1)$	$\theta2\exp(\eta2)$ (no DI) or $\theta7\exp(\eta2)$ DI	$\theta3\exp(\eta3)$	$\theta6\exp(\eta4)$	55	50	26	61	41	0.05	RFA9.CSV	799.782	Effect of NSAID use on CL/F. Significant.
49	2	ADD+CCV	$\theta1\exp(\eta1)$	$\theta2\exp(\eta2)$	$\theta3\exp(\eta3)$ (no DI) or $\theta7\exp(\eta3)$ DI	$\theta6\exp(\eta4)$	56	51	25	60	41	0.05	RFA9.CSV	802.575	Effect of NSAID use on V/F. Not significant
50	2	ADD+CCV	$\theta1\exp(\eta1)$ (no DI) or $\theta7\exp(\eta1)$ DI	$\theta2\exp(\eta2)$	$\theta3\exp(\eta3)$	$\theta6\exp(\eta4)$	62	51	24	55	41	0.05	RFA9.CSV	802.404	Effect of Iron preps on KA. Not significant
51	2	ADD+CCV	$\theta1\exp(\eta1)$	$\theta2\exp(\eta2)$ (no DI) or $\theta7\exp(\eta2)$ DI	$\theta3\exp(\eta3)$	$\theta6\exp(\eta4)$	56	50	26	65	40	0.05	RFA9.CSV	793.535	Effect of Iron preps/anaemia on CL/F. Significant
52	2	ADD+CCV	$\theta1\exp(\eta1)$	$\theta2\exp(\eta2)$	$\theta3\exp(\eta3)$ (no DI) or $\theta7\exp(\eta3)$ DI	$\theta6\exp(\eta4)$	56	52	25	61	41	0.05	RFA9.CSV	797.490	Effect of Iron preps/anaemia on V/F. Significant
53	2	ADD+CCV	$\theta1\exp(\eta1)$	$\theta2\exp(\eta2)$ (no DI) or $\theta7\exp(\eta2)$ DI	$\theta3\exp(\eta3)$	$\theta6\exp(\eta4)$	57	51	24	64	41	0.05	RFA9.CSV	804.411	Effect of antihistamines on CL/F. Not significant
54	2	ADD+CCV	$\theta1\exp(\eta1)$	$\theta2\exp(\eta2)$	$\theta3\exp(\eta3)$ (no DI) or $\theta7\exp(\eta3)$ DI	$\theta6\exp(\eta4)$	58	51	24	59	41	0.05	RFA9.CSV	804.579	Effect of antihistamines on V/F. Not significant
55	2	ADD+CCV	$\theta1\exp(\eta1)$	$\theta2\exp(\eta2)$ (no DI) or $\theta7\exp(\eta2)$ DI	$\theta3\exp(\eta3)$	$\theta6\exp(\eta4)$	58	51	24	59	41	0.05	RFA9.CSV	804.068	Effect of hypoglycaemic agents/diabetes on CL/F. Not significant
56	2	ADD+CCV	$\theta1\exp(\eta1)$	$\theta2\exp(\eta2)$	$\theta3\exp(\eta3)$ (no DI) or $\theta7\exp(\eta3)$ DI	$\theta6\exp(\eta4)$	58	51	24	59	41	0.05	RFA9.CSV	804.081	Effect of hypoglycaemic agents/diabetes on V/F. Not significant

η - inter-individual variability; KA – absorption rate constant, CL/F – apparent clearance; V/F – apparent volume of distribution; TLAG – absorption lag time; σ - intra-individual variability; CCV – constant coefficient of variation; ADD – additive; OBF – minimum value of the Objective function.

NONMEM RUN SUMMARY - RIFAMPICIN

RUN	ADVAN	ERROR	KA	CL/F	V/F	TLAG	η_{KA} %	$\eta_{CL/F}$ %	$\eta_{V/F}$ %	η_{TLAG} %	σ_{CCV} %	σ_{ADD} µg/ml	DATA	OBF	COMMENTS
57	2	ADD+CCV	$\theta1exp(\eta1)$	$\theta2exp(\eta2)$ (no DI) or $\theta7exp(\eta2)$ DI	$\theta3exp(\eta3)$	$\theta6exp(\eta4)$	57	51	24	59	41	0.05	RFA9.CSV	804.643	Effect of diuretics/hypertension on CL/F. Not significant
58	2	ADD+CCV	$\theta1exp(\eta1)$	$\theta2exp(\eta2)$	$\theta3exp(\eta3)$ (no DI) or $\theta7exp(\eta3)$ DI	$\theta6exp(\eta4)$	57	51	23	64	41	0.05	RFA9.CSV	804.004	Effect of diuretics/hypertension on V/F. Not significant
59	2	ADD+CCV	$\theta1exp(\eta1)$	$\theta2exp(\eta2)$ (no DI) or $\theta7exp(\eta2)$ DI	$\theta3exp(\eta3)$	$\theta6exp(\eta4)$	57	49	25	61	40	0.05	RFA9.CSV	790.085	Effect of INAT on CL/F. Significant
60	2	ADD+CCV	$\theta1exp(\eta1)$	$\theta2exp(\eta2)$	$\theta3exp(\eta3)$ (no DI) or $\theta7exp(\eta3)$ DI	$\theta6exp(\eta4)$	57	51	77	59	41	0.05	RFA9.CSV	804.594	Effect of INAT on V/F. Not significant
61	2	ADD+CCV	$\theta1exp(\eta1)$	$\theta2exp(\eta2)$ (no DI) or $\theta7exp(\eta2)$ DI	$\theta3exp(\eta3)$	$\theta6exp(\eta4)$	52	45	26	64	40	0.05	RFA9.CSV	766.502	Effect of quinolones on CL/F. Significant
62	2	ADD+CCV	$\theta1exp(\eta1)$	$\theta2exp(\eta2)$	$\theta3exp(\eta3)$ (no DI) or $\theta7exp(\eta3)$ DI	$\theta6exp(\eta4)$	57	51	25	58	41	0.05	RFA9.CSV	802.379	Effect of quinolones on V/F. Not significant
63	2	ADD+CCV	$\theta1exp(\eta1)$	$\theta2exp(\eta2)$ (no DI) or $\theta7exp(\eta2)$ DI	$\theta3exp(\eta3)$	$\theta6exp(\eta4)$	55	49	27	62	40	0.05	RFA9.CSV	784.252	Effect of ethambutol on CL/F. Significant
64	2	ADD+CCV	$\theta1exp(\eta1)$	$\theta2exp(\eta2)$	$\theta3exp(\eta3)$ (no DI) or $\theta7exp(\eta3)$ DI	$\theta6exp(\eta4)$	57	51	25	60	41	0.05	RFA9.CSV	802.233	Effect of ethambutol on V/F. Not significant
65	2	ADD+CCV	$\theta1exp(\eta1)$	$\theta2exp(\eta2)$ (no DI) or $\theta7exp(\eta2)$ DI	$\theta3exp(\eta3)$	$\theta6exp(\eta4)$	59	51	23	58	41	0.05	RFA9.CSV	801.707	Effect of candidiasis on CL/F. Not significant.
66	2	ADD+CCV	$\theta1exp(\eta1)$	$\theta2exp(\eta2)$	$\theta3exp(\eta3)$ (no DI) or $\theta7exp(\eta3)$ DI	$\theta6exp(\eta4)$	59	51	23	58	41	0.05	RFA9.CSV	801.630	Effect of candidiasis on V/F. Not significant.
67	2	ADD+CCV	$\theta1exp(\eta1)$	$\theta2exp(\eta2)$ (no Tx) or $\theta7exp(\eta2)$ Tx	$\theta3exp(\eta3)$	$\theta6exp(\eta4)$	58	50	23	60	41	0.05	RFA9.CSV	793.945	Effect of prior TB treatment on CL/F. Significant
68	2	ADD+CCV	$\theta1exp(\eta1)$	$\theta2exp(\eta2)$	$\theta3exp(\eta3)$ (no Tx) or $\theta7exp(\eta3)$ Tx	$\theta6exp(\eta4)$	57	51	23	60	41	0.05	RFA9.CSV	800.280	Effect of prior TB treatment on V/F. Significant.
69	2	ADD+CCV	$\theta1exp(\eta1)$	$(\theta2+\theta7*(WT-54))exp(\eta3)$	$\theta3exp(\eta3)$	$\theta6exp(\eta4)$	59	49	22	58	41	0.05	RFA9.CSV	789.177	Effect of WT on CL/F. Significant.
70	2	ADD+CCV	$\theta1exp(\eta1)$	$\theta2exp(\eta2)$	$(\theta3+\theta7*(WT-54))exp(\eta3)$	$\theta6exp(\eta4)$	58	51	24	58	41	0.05	RFA9.CSV	802.493	Effect of WT on V/F. Not significant.
71	2	ADD+CCV	$\theta1exp(\eta1)$	$(\theta2+\theta7*(AGE-33))exp(\eta3)$	$\theta3exp(\eta3)$	$\theta6exp(\eta4)$	58	51	23	60	41	0.05	RFA9.CSV	796.967	Effect of AGE on CL/F. Significant.
72	2	ADD+CCV	$\theta1exp(\eta1)$	$\theta2exp(\eta2)$	$(\theta3+\theta7*(AGE-33))exp(\eta3)$	$\theta6exp(\eta4)$	57	51	24	59	41	0.05	RFA9.CSV	802.282	Effect of AGE on V/F. Not significant.

η - inter-individual variability; KA – absorption rate constant, CL/F – apparent clearance; V/F – apparent volume of distribution; TLAG – absorption lag time; σ - intra-individual variability; CCV – constant coefficient of variation; ADD – additive; OBF – minimum value of the Objective function.

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NONMEM RUN SUMMARY - RIFAMPICIN

RUN	ADVAN	ERROR	KA	CL/F		V/F	TLAG	η_{KA} %	$\eta_{CL/F}$ %	$\eta_{V/F}$ %	η_{TLAG} %	σ_{CCV} %	σ_{ADD} µg/ml	DATA	OBF	COMMENTS
73	2	ADD+CCV	$\theta 1 exp(\eta 1)$	$(\theta 2+\theta 7*(DAYS-41)) exp(\eta 3)$	$\theta 3 exp(\eta 3)$		$\theta 6 exp(\eta 4)$	55	47	24	65	40	0.05	RFA9.CSV	747.816	Effect of no. of days since starting RFA therapy on CL/F. Significant
74	2	ADD+CCV	$\theta 1 exp(\eta 1)$	$\theta 2 exp(\eta 2)$		$(\theta 3+\theta 7*(DAYS-41)) exp(\eta 3)$	$\theta 6 exp(\eta 4)$	57	52	26	60	42	0.05	RFA9.CSV	795.039	Effect of no. of days since starting RFA therapy on V/F. Significant
75	2	ADD+CCV	$\theta 1 exp(\eta 1)$	$(\theta 2+\theta 7*(ALKP-84)) exp(\eta 3)$	$\theta 3 exp(\eta 3)$		$\theta 6 exp(\eta 4)$	57	51	25	59	41	0.05	RFA9.CSV	804.643	Liver Function tests – ALK PHOS on CL/F. Not significant
76	2	ADD+CCV	$\theta 1 exp(\eta 1)$	$\theta 2 exp(\eta 2)$		$(\theta 3+\theta 7*(ALKP-84)) exp(\eta 3)$	$\theta 6 exp(\eta 4)$	58	51	23	59	41	0.05	RFA9.CSV	803.910	Liver Function tests – ALK PHOS on V/F. Not significant
77	2	ADD+CCV	$\theta 1 exp(\eta 1)$	$(\theta 2+\theta 7*(BILI-10)) exp(\eta 3)$	$\theta 3 exp(\eta 3)$		$\theta 6 exp(\eta 4)$	57	51	25	59	40	0.05	RFA9.CSV	803.856	Liver Function tests – Total Bilirubin on CL/F. Not significant.
78	2	ADD+CCV	$\theta 1 exp(\eta 1)$	$\theta 2 exp(\eta 2)$		$(\theta 3+\theta 7*(BILI-10)) exp(\eta 3)$	$\theta 6 exp(\eta 4)$	57	51	23	61	41	0.05	RFA9.CSV	803.307	Liver Function tests – Total Bilirubin on V/F. Not significant.
79	2	ADD+CCV	$\theta 1 exp(\eta 1)$	$(\theta 2+\theta 7*(ALB-25)) exp(\eta 3)$	$\theta 3 exp(\eta 3)$		$\theta 6 exp(\eta 4)$	57	51	24	59	41	0.05	RFA9.CSV	804.269	Liver Function tests – Albumin on CL/F. Not significant.
80	2	ADD+CCV	$\theta 1 exp(\eta 1)$	$\theta 2 exp(\eta 2)$		$(\theta 3+\theta 7*(ALB-25)) exp(\eta 3)$	$\theta 6 exp(\eta 4)$	58	51	24	59	41	0.05	RFA9.CSV	801.302	Liver Function tests – Albumin on V/F. Not significant.
81	2	ADD+CCV	$\theta 1 exp(\eta 1)$	$(\theta 2+\theta 7*(GLOB-52)) exp(\eta 3)$	$\theta 3 exp(\eta 3)$		$\theta 6 exp(\eta 4)$	59	50	22	59	41	0.05	RFA9.CSV	789.276	Liver Function tests – Globulin on CL/F. Significant.
82	2	ADD+CCV	$\theta 1 exp(\eta 1)$	$\theta 2 exp(\eta 2)$		$(\theta 3+\theta 7*(GLOB-52)) exp(\eta 3)$	$\theta 6 exp(\eta 4)$	60	51	22	58	41	0.05	RFA9.CSV	790.857	Liver Function tests – Globulin on V/F. Significant.
83	2	ADD+CCV	$\theta 1 exp(\eta 1)$	$(\theta 2+\theta 7*(ALT-16)) exp(\eta 3)$	$\theta 3 exp(\eta 3)$		$\theta 6 exp(\eta 4)$	57	51	24	59	41	0.05	RFA9.CSV	804.666	Liver Function tests – ALT on CL/F. Not significant.
84	2	ADD+CCV	$\theta 1 exp(\eta 1)$	$\theta 2 exp(\eta 2)$		$(\theta 3+\theta 7*(ALT-16)) exp(\eta 3)$	$\theta 6 exp(\eta 4)$	57	51	24	59	41	0.05	RFA9.CSV	804.501	Liver Function tests – ALT on V/F. Not significant.
85	2	ADD+CCV	$\theta 1 exp(\eta 1)$	$(\theta 2+\theta 7*(AST-31)) exp(\eta 3)$	$\theta 3 exp(\eta 3)$		$\theta 6 exp(\eta 4)$	54	51	26	61	41	0.05	RFA9.CSV	798.580	Liver Function tests – AST on CL/F. Significant.
86	2	ADD+CCV	$\theta 1 exp(\eta 1)$	$\theta 2 exp(\eta 2)$		$(\theta 3+\theta 7*(AST-31)) exp(\eta 3)$	$\theta 6 exp(\eta 4)$	57	51	25	59	41	0.05	RFA9.CSV	803.666	Liver Function tests – AST on V/F. Not significant.
87	2	ADD+CCV	$\theta 1 exp(\eta 1)$	$(\theta 2+\theta 7*(GGT-37)) exp(\eta 3)$	$\theta 3 exp(\eta 3)$		$\theta 6 exp(\eta 4)$	58	50	24	59	41	0.05	RFA9.CSV	803.624	Liver Function tests – GGT on CL/F. Significant.
88	2	ADD+CCV	$\theta 1 exp(\eta 1)$	$\theta 2 exp(\eta 2)$		$(\theta 3 + \theta 7*(GGT-37)) exp(\eta 3)$	$\theta 6 exp(\eta 4)$	57	51	24	61	41	0.05	RFA9.CSV	804.253	Liver Function tests – GGT on V/F. Not significant.
89	2	ADD+CCV	$\theta 1 exp(\eta 1)$	$(\theta 2+\theta 7*(VIRU-65183.49)) exp(\eta 3)$	$\theta 3 exp(\eta 3)$		$\theta 6 exp(\eta 4)$	56	52	27	60	42	0.05	RFA9.CSV	803.561	Effect of HIV viral load on CL/F. Not significant.
90	2	ADD+CCV	$\theta 1 exp(\eta 1)$	$(\theta 2+\theta 7*(VIR-5)) exp(\eta 3)$	$\theta 3 exp(\eta 3)$		$\theta 6 exp(\eta 4)$	57	52	29	59	440	0.05	RFA9.CSV	800.541	Effect of log HIV viral load on CL/F. Not significant.

η - inter-individual variability; KA – absorption rate constant, CL/F – apparent clearance; V/F – apparent volume of distribution; TLAG – absorption lag time; σ - intra-individual variability; CCV – constant coefficient of variation; ADD – additive; OBF – minimum value of the Objective function.

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NONMEM RUN SUMMARY - RIFAMPICIN

RUN	ADVAN	ERROR	KA	CL/F	V/F	TLAG	η_{KA} %	$\eta_{CL/F}$ %	$\eta_{V/F}$ %	η_{TLAG} %	σ_{CCV} %	σ_{ADD} µg/ml	DATA	OBF	COMMENTS
91	2	ADD+CCV	$\theta1\exp(\eta1)$	$\theta2\exp(\eta2)$	$(\theta3 + \theta7*(VIRU-65183.49))\exp(\eta3)$	$\theta6\exp(\eta4)$	56	50	24	57	42	0.04	RFA9.CSV	783.170	Effect of HIV viral load on V/F. Significant.
92	2	ADD+CCV	$\theta1\exp(\eta1)$	$\theta2\exp(\eta2)$	$(\theta3+\theta7*(VIR-5))\exp(\eta3)$	$\theta6\exp(\eta4)$	55	51	27	61	40	0.05	RFA9.CSV	788.569	Effect of log HIV viral load on V/F. Significant.
93	2	ADD+CCV	$\theta1\exp(\eta1)$	$(\theta2+\theta7*(NMDR-7))\exp(\eta3)$	$\theta3\exp(\eta3)$	$\theta6\exp(\eta4)$	58	50	24	61	41	0.05	RFA9.CSV	794.096	Effect of severity of MDR on CL/F i.e. no. of drugs patient was resistant to concurrently. Significant.
94	2	ADD+CCV	$\theta1\exp(\eta1)$	$(\theta2+\theta7*MDR+\theta8*(NMDR-7))\exp(\eta3)$	$\theta3\exp(\eta3)$	$\theta6\exp(\eta4)$	57	46	24	63	40	0.05	RFA9.CSV	774.075	Effect of PRESENCE and SEVERITY of MDR on CL/F i.e. RUN 37+RUN 93. Significant.
95	2	ADD+CCV	$\theta1\exp(\eta1)$	$\theta2\exp(\eta2)$	$(\theta3 +\theta7*(NMDR-7))\exp(\eta3)$	$\theta6\exp(\eta4)$	57	51	25	59	41	0.05.	RFA9.CSV	803.322	Effect of severity of MDR on V/F i.e. no. of drugs patient was resistant to concurrently. Significant.
96	2	ADD+CCV	$\theta1\exp(\eta1)$	$(\theta2+\theta7*(EXT-5))\exp(\eta3)$	$\theta3\exp(\eta3)$	$\theta6\exp(\eta4)$	57	51	24	59	41	0.05	RFA9.CSV	804.594	Effect of Extent of X-ray on CL/F. Not significant.
97	2	ADD+CCV	$\theta1\exp(\eta1)$	$\theta2\exp(\eta2)$	$(\theta3+\theta7*(EXT-5))\exp(\eta3)$	$\theta6\exp(\eta4)$	56	51	24	59	41	0.05	RFA9.CSV	803.806	Effect of Extent of X-ray on V/F. Not significant.
98	2	ADD+CCV	$\theta1\exp(\eta1)$	$(\theta2+\theta7*(CAV-4))\exp(\eta3)$	$\theta3\exp(\eta3)$	$\theta6\exp(\eta4)$	60	50	21	60	41	0.05	RFA9.CSV	786.806	Effect of Extent of cavitation on X-ray on CL/F. significant.
99	2	ADD+CCV	$\theta1\exp(\eta1)$	$\theta2\exp(\eta2)$	$(\theta3+\theta7*(CAV-4))\exp(\eta3)$	$\theta6\exp(\eta4)$	58	51	24	60	.41	0.05	RFA9.CSV	801.763	Effect of Extent of cavitation on X-ray on V/F. Not significant.
100	2	ADD+CCV	$\theta1\exp(\eta1)$	$(\theta2+\theta7*(DAYS-10))\exp(\eta2)$	$\theta3\exp(\eta3)$	$\theta6\exp(\eta4)$	50	43	25	75	40	0.05	RFA9.CSV	730.138	Model Build-up starts. Days – max=10. Compare with RUN 15 (OBF=804.652). OBF ↓ by 74.5
101	2	ADD+CCV	$\theta1\exp(\eta1)$	$(\theta2+\theta7*(DAYS-10)-\theta8*QUI)\exp(\eta2)$	$\theta3\exp(\eta3)$	$\theta6\exp(\eta4)$	50	42	25	76	40	0.05	RFA9.CSV	729.478	Add in quinolones. Change in OBF (0.7) not significant.
102	2	ADD+CCV	$\theta1\exp(\eta1)$	$(\theta2+\theta7*(DAYS-10)-\theta8*INHI)\exp(\eta2)$	$\theta3\exp(\eta3)$	$\theta6\exp(\eta4)$	51	43	26	67	40	0.05	RFA9.CSV	730.389	Removed quinolones, add in inhibitors. Change in OBF (↑) not significant.
103	2	ADD+CCV	$\theta1\exp(\eta1)$	$(\theta2+\theta7*(DAYS-10)-\theta8*MDR)\exp(\eta2)$	$\theta3\exp(\eta3)$	$\theta6\exp(\eta4)$	27	41	42	83	145	0.05	RFA9.CSV	726.885	Removed inhibitors, added MDR. Change in OBF (↓ 3.3) not significant.
104	2	ADD+CCV	$\theta1\exp(\eta1)$	$(\theta2+\theta7*(DAYS-10)-\theta8*EMB)\exp(\eta2)$	$\theta3\exp(\eta3)$	$\theta6\exp(\eta4)$	50	43	25	75	40	0.05	RFA9.CSV	729.995	Removed MDR, added in EMB. Change in OBF (↓ 0.2) not significant.
105	2	ADD+CCV	$\theta1\exp(\eta1)$	$(\theta2+\theta7*(DAYS-10)-\theta8*(CAV-4))\exp(\eta2)$	$\theta3\exp(\eta3)$	$\theta6\exp(\eta4)$	53	43	24	67	40	0.05	RFA9.CSV	728.051	Removed EMB, added in severity score for cavitation. Change in OBF (↓ 2.1) not significant.

η - inter-individual variability; KA – absorption rate constant, CL/F – apparent clearance; V/F – apparent volume of distribution; TLAG – absorption lag time; σ - intra-individual variability; CCV – constant coefficient of variation; ADD – additive; OBF – minimum value of the Objective function.

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NONMEM RUN SUMMARY - RIFAMPICIN

RUN	ADVAN	ERROR	KA	CL/F	V/F	TLAG	η_{KA} %	$\eta_{CL/F}$ %	$\eta_{V/F}$ %	η_{TLAG} %	σ_{CCV} %	σ_{ADD} µg/ml	DATA	OBF	COMMENTS
106	2	ADD+CCV	$\theta 1exp(\eta 1)$	$(\theta 2+\theta 7*(DAYS-10)-\theta 8*(CAV-4))$ $exp(\eta 2)$	$(\theta 3+\theta 9*(VIR-5))$ $exp(\eta 3)$	$\theta 6exp(\eta 4)$	46	44	30	73	36	0.05	RFA9.CSV	707.986	Did not remove severity score for cavitation (mistake). Added in Log viral load on V/F. OBF ↓ 22.2
107	2	ADD+CCV	$\theta 1exp(\eta 1)$	$(\theta 2+\theta 7*(DAYS-10)-\theta 8*(CAV-4))$ $exp(\eta 2)$	$(\theta 3+\theta 9*(VIR-5))$ $exp(\eta 3)$	$\theta 6exp(\eta 4)$	48	44	28	89	40	0.05	RFA9.CSV	710.681	As for previous run but coded in all patients – not just the HIV +ve patients. HIV-ve patients recorded as log viral load=0. OBF ↓ 19.5. *NB Inclusion of CAV on CL/F in the model is an error.*
108	2	ADD+CCV	$\theta 1exp(\eta 1)$	$(\theta 2+\theta 7*(DAYS-10)-\theta 8*(CAV-4))$ $exp(\eta 2)$	$(\theta 3+\theta 9*(VIR-5)+\theta 10*HIV)$ $exp(\eta 3)$	$\theta 6exp(\eta 4)$	47	44	29	74	39	0.05	RFA9.CSV	710.024	Added in HIV status on V/F as well. No additional effect over log viral load (OBJ ↓ 0.7). *NB Inclusion of CAV on CL/F in the model is an error.*
109	2	ADD+CCV	$\theta 1exp(\eta 1)$	$(\theta 2+\theta 7*(DAYS-10)-\theta 8*(CAV-4))$ $exp(\eta 2)$	$(\theta 3+\theta 9*(VIR-5)+\theta 10*HIV)$ $exp(\eta 3)$	$\theta 6exp(\eta 4)$	45	44	31	71	39	0.05	RFA9.CSV	711.045	Fixed CAV to zero. OBJ ↑ 0.4. Therefore confirmed its exclusion and ability to continue with further additions to the model with it included. To be removed at the end.
110	2	ADD+CCV	$\theta 1exp(\eta 1)$	$(\theta 2+\theta 7*(DAYS-10)-\theta 8*(CAV-4)+\theta 10*(WT-54))$ $exp(\eta 2)$	$(\theta 3+\theta 9*(VIR-5))$ $exp(\eta 3)$	$\theta 6exp(\eta 4)$	47	37	26	82	39	0.05	RFA9.CSV	652.622	Removed HIV on V/F. Added in WT on CL/F. OBF ↓ 58.1. *NB Inclusion of CAV on CL/F in the model is an error.*
111	2	ADD+CCV	$\theta 1exp(\eta 1)$	$(\theta 2+\theta 7*(DAYS-10)-\theta 8*(CAV-4)+\theta 10*(WT-54)+\theta 11*(GLOB-52))$ $exp(\eta 2)$	$(\theta 3+\theta 9*(VIR-5))$ $exp(\eta 3)$	$\theta 6exp(\eta 4)$	48	38	24	85	39	0.05	RFA9.CSV	648.267	Added in GLOB. OBF ↓ 4.4. *NB Inclusion of CAV on CL/F in the model is an error.*
112	2	ADD+CCV	$\theta 1exp(\eta 1)$	$(\theta 2+\theta 7*(DAYS-10)-\theta 8*(CAV-4)+\theta 10*(WT-54)+\theta 11*(GLOB-52)+\theta 12*INAT)exp(\eta 2)$	$(\theta 3+\theta 9*(VIR-5))$ $exp(\eta 3)$	$\theta 6exp(\eta 4)$	48	38	24	86	39	0.05	RFA9.CSV	648.115	Added in effect of INAT on CL/F. No significant change in OBF (↓ 0.2). *NB Inclusion of CAV on CL/F in the model is an error.*
113	2	ADD+CCV	$\theta 1exp(\eta 1)$	$(\theta 2+\theta 7*(DAYS-10)-\theta 8*(CAV-4)+\theta 10*(WT-54)+\theta 11*(GLOB-52))$ $exp(\eta 2)$	$(\theta 3+\theta 9*(VIR-5)+\theta 12*(GLOB-52))$ $exp(\eta 3)$	$\theta 6exp(\eta 4)$	48	38	26	80	38	0.05	RFA9.CSV	646.158	Removed INAT, added in GLOB on V/F. No significant change in OBF (↓ 2.0). *NB Inclusion of CAV on CL/F in the model is an error.*

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NONMEM RUN SUMMARY - RIFAMPICIN

RUN	ADVAN	ERROR	KA	CL/F	V/F	TLAG	η_{KA} %	$\eta_{CL/F}$ %	$\eta_{V/F}$ %	η_{TLAG} %	σ_{CCV} %	σ_{ADD} μg/ml	DATA	OBF	COMMENTS
114	2	ADD+CCV	$\theta 1\exp(\eta 1)$	$(\theta 2+\theta 7*(DAYS-10)-\theta 8*(CAV-4)+\theta 10*(WT-54)+\theta 11*(GLOB-52))\exp(\eta 2)$	$(\theta 3+\theta 9*(VIR-5)+\theta 12*SEX)\exp(\eta 3)$	$\theta 6\exp(\eta 4)$	48	37	72	70	38	0.05	RFA9.CSV	641.745	Removed GLOB on V/F, added in SEX on V/F. OBF ↓ 4.4. *NB Inclusion of CAV on CL/F in the model is an error.*
115	2	ADD+CCV	$\theta 1\exp(\eta 1)$	$(\theta 2+\theta 7*(DAYS-10)-\theta 8*(CAV-4)+\theta 10*(WT-54)+\theta 11*(GLOB-52)+\theta 13*FEF)\exp(\eta 2)$	$(\theta 3+\theta 9*(VIR-5)+\theta 12*SEX)\exp(\eta 3)$	$\theta 6\exp(\eta 4)$	49	37	19	94	38	0.05	RFA9.CSV	639.772	Added in effect of iron preps on CL/F. No significant effect on OBF (↓ 2.0). *NB Inclusion of CAV on CL/F in the model is an error.*
116	2	ADD+CCV	$\theta 1\exp(\eta 1)$	$(\theta 2+\theta 7*(DAYS-10)-\theta 8*(CAV-4)+\theta 10*(WT-54)+\theta 11*(GLOB-52)+\theta 13*(NMDR-7))\exp(\eta 2)$	$(\theta 3+\theta 9*(VIR-5)+\theta 12*SEX)\exp(\eta 3)$	$\theta 6\exp(\eta 4)$	51	36	14	95	38	0.05	RFA9.CSV	639.400	Removed iron preps. Added in MDR severity score. No significant change in OBF (↓ 2.4). *NB Inclusion of CAV on CL/F in the model is an error.*
117	2	ADD+CCV	$\theta 1\exp(\eta 1)$	$(\theta 2+\theta 7*(DAYS-10)-\theta 8*(CAV-4)+\theta 10*(WT-54)+\theta 11*(GLOB-52))\exp(\eta 2)$	$(\theta 3+\theta 9*(VIR-5)+\theta 12*SEX+\theta 13*(DAYS-10))\exp(\eta 3)$	$\theta 6\exp(\eta 4)$	51	36	18	81	38	0.05	RFA9.CSV	628.151	Removed MDR severity score, Added in DAYS since starting RFA on V/F. OBF ↓ 13.6. *NB Inclusion of CAV on CL/F in the model is an error.*
118	2	ADD+CCV	$\theta 1\exp(\eta 1)$	$(\theta 2+\theta 7*(DAYS-10)-\theta 8*(CAV-4)+\theta 10*(WT-54)+\theta 11*(GLOB-52)+\theta 14*(AGE-33))\exp(\eta 2)$	$(\theta 3+\theta 9*(VIR-5)+\theta 12*SEX +\theta 13*(DAYS-10))\exp(\eta 3)$	$\theta 6\exp(\eta 4)$	52	36	18	82	38	0.05	RFA9.CSV	625.875	Added in Age on CL/F. No significant change in OBF (↓ 2.3). *NB Inclusion of CAV on CL/F in the model is an error.*
119	2	ADD+CCV	$\theta 1\exp(\eta 1)$	$(\theta 2+\theta 7*(DAYS-10)-\theta 8*(CAV-4)+\theta 10*(WT-54)+\theta 11*(GLOB-52))\exp(\eta 2)$	$(\theta 3+\theta 9*(VIR-5)+\theta 12*SEX+\theta 13*(DAYS-10)+\theta 14*INHI)\exp(\eta 3)$	$\theta 6\exp(\eta 4)$	58	35	2	76	38	0.05	RFA9.CSV	628.584	Added in inhibitors on V/F. OBF ↑ 0.4. *NB Inclusion of CAV on CL/F in the model is an error.*
120	2	ADD+CCV	$\theta 1\exp(\eta 1)$	$(\theta 2+\theta 7*(DAYS-10)-\theta 8*(CAV-4)+\theta 10*(WT-54)+\theta 11*(GLOB-52))\exp(\eta 2)$	$(\theta 3+\theta 9*(VIR-5)+\theta 12*SEX+\theta 13*(DAYS-10)+\theta 14*INHI -\theta 15*FEF)\exp(\eta 3)$	$\theta 6\exp(\eta 4)$	52	36	18	83	38	0.05	RFA9.CSV	626.919	Added in Iron preps/anaemia on V/F. OBF ↓1.2. *NB Inclusion of CAV on CL/F and INHI on V/F in the model is an error.*

η - inter-individual variability; KA – absorption rate constant, CL/F – apparent clearance; V/F – apparent volume of distribution; TLAG – absorption lag time; σ - intra-individual variability; CCV – constant coefficient of variation; ADD – additive; OBF – minimum value of the Objective function.

Appendix G

NONMEM RUN SUMMARY - RIFAMPICIN

RUN	ADVAN	ERROR	KA	CL/F	V/F	TLAG	η_{KA} %	$\eta_{CL/F}$ %	$\eta_{V/F}$ %	η_{TLAG} %	σ_{CCV} %	σ_{ADD} µg/ml	DATA	OBF	COMMENTS
121	2	ADD+CCV	$\theta 1 \exp(\eta 1)$	$(\theta 2+\theta 7^*(DAYS-10)-\theta 8^*(CAV-4)+\theta 10^*(WT-54)+\theta 11^*(GLOB-52)-\theta 15^*HIV)\exp(\eta 2)$	$(\theta 3+\theta 9^*(VIR-5)+\theta 12^*SEX+\theta 13^*(DAYS-10)+\theta 14^*INHI)\exp(\eta 3)$	$\theta 6 \exp(\eta 4)$	50	36	21	70	37	0.05	RFA9.CSV	626.014	Added in HIV on CL/F. OBF ↓ 2.1. *NB Inclusion of CAV on CL/F and INHI on V/F in the model is an error.*
122	2	ADD+CCV	$\theta 1 \exp(\eta 1)$	$(\theta 2+\theta 7^*(DAYS-10)-\theta 8^*(CAV-4)+\theta 10^*(WT-54)+\theta 11^*(GLOB-52)-\theta 15^*(AST-31))\exp(\eta 2)$	$(\theta 3+\theta 9^*(VIR-5)+\theta 12^*SEX+\theta 13^*(DAYS-10)+\theta 14^*INHI)\exp(\eta 3)$	$\theta 6 \exp(\eta 4)$	52	36	17	84	38	0.05	RFA9.CSV	627.048	Added in AST on CL/F. OBF ↓ 0.5. *NB Inclusion of CAV on CL/F and INHI on V/F in the model is an error.*
123	2	ADD+CCV	$\theta 1 \exp(\eta 1)$	$(\theta 2+\theta 7^*(DAYS-10)-\theta 8^*(CAV-4)+\theta 10^*(WT-54)+\theta 11^*(GLOB-52)+\theta 15^*NSID)\exp(\eta 2)$	$(\theta 3+\theta 9^*(VIR-5)+\theta 12^*SEX+\theta 13^*(DAYS-10)+\theta 14^*INHI)\exp(\eta 3)$	$\theta 6 \exp(\eta 4)$	52	36	17	83	38	0.05	RFA9.CSV	626.859	Added in NSAIDS on CL/F. OBF ↓ 1.3. *NB Inclusion of CAV on CL/F and INHI on V/F in the model is an error.*
124	2	ADD+CCV	$\theta 1 \exp(\eta 1)$	$(\theta 2+\theta 7^*(DAYS-10)-\theta 8^*(CAV-4)+\theta 10^*(WT-54)+\theta 11^*(GLOB-52)-\theta 15^*(VIR-5))\exp(\eta 2)$	$(\theta 3+\theta 9^*(VIR-5)+\theta 12^*SEX+\theta 13^*(DAYS-10)+\theta 14^*INHI)\exp(\eta 3)$	$\theta 6 \exp(\eta 4)$	52	35	17	84	38	0.05	RFA9.CSV	627.026	Added in Log Viral load on CL/F. OBF ↓1.1. *NB Inclusion of CAV on CL/F and INHI on V/F in the model is an error.*
125	2	ADD+CCV	$\theta 1 \exp(\eta 1)$	$(\theta 2+\theta 7^*(DAYS-10)-\theta 8^*(CAV-4)+\theta 10^*(WT-54)+\theta 11^*(GLOB-52))\exp(\eta 2)$	$(\theta 3+\theta 9^*(VIR-5)+\theta 12^*SEX+\theta 13^*(DAYS-10)+\theta 14^*INHI)\exp(\eta 3)$	$\theta 6 \exp(\eta 4)$	51	39	27	77	35	0.05	RFA9.CSV	606.918	Implemented BLOCK OMEGA on CL/F & V/F. Found a high correlation (0.967). COV step does not work with the BLOCK OMEGA. *NB Inclusion of CAV on CL/F and INHI on V/F in the model is an error.*
126	2	ADD+CCV	$\theta 1 \exp(\eta 1)$	$(\theta 2+\theta 7^*(DAYS-10)-\theta 8^*(CAV-4)+\theta 10^*(WT-54)+\theta 11^*(GLOB-52))\exp(\eta 2)$	$(\theta 3+\theta 9^*(VIR-5)+\theta 12^*SEX+\theta 13^*(DAYS-10)+\theta 14^*INHI)\exp(\eta 3)$	$\theta 6 \exp(\eta 4)$	46	39	33	73	35	0.05	RFA9.CSV	630.799	Fixed CAV, GLOB on CL/F. Also SEX, DAYS & INHI on V/F. All except DAYS on V/F were not significant at p=0.005 on model buildup. *IGNORE THIS RUN.*
127	2	ADD+CCV	$\theta 1 \exp(\eta 1)$	$(\theta 2+\theta 7^*(DAYS-10)-\theta 8^*(CAV-4)+\theta 10^*(WT-54)+\theta 11^*(GLOB-52))\exp(\eta 2)$	$(\theta 3+\theta 9^*(VIR-5)+\theta 12^*SEX+\theta 13^*(DAYS-10)+\theta 14^*INHI)\exp(\eta 3)$	$\theta 6 \exp(\eta 4)$	58	35	0.2	76	38	0.05	RFA9.CSV	628.584	Reverted to DIAG OMEGA. Implemented COV on RUN119.KA vs V/F (0.868); KA vs TLAG (0.843). *NB Inclusion of CAV on CL/F and INHI on V/F in the model is an error.*

η - inter-individual variability; KA – absorption rate constant, CL/F – apparent clearance; V/F – apparent volume of distribution; TLAG – absorption lag time; σ - intra-individual variability; CCV – constant coefficient of variation; ADD – additive; OBF – minimum value of the Objective function.

NONMEM RUN SUMMARY - RIFAMPICIN

RUN	ADVAN	ERROR	KA	CL/F	V/F	TLAG	η_{KA} %	$\eta_{CL/F}$ %	$\eta_{V/F}$ %	η_{TLAG} %	σ_{CCV} %	σ_{ADD} μg/ml	DATA	OBF	COMMENTS
128	2	ADD+CCV	θ1exp(η1)	(θ2+θ7*(DAYS-10)-θ8*(CAV-4)+θ10*(WT-54)+θ11*(GLOB-52))exp(η2)	(θ3+θ9*(VIR-5)+θ12*SEX+θ13*(DAYS-10)+θ14*INHI)exp(η3)	θ6exp(η4)	52	39	26	79	35	0.05	RFA9.CSV	607.751	CAV fixed to 0 i.e. reconfirmed RUN 106. Establishing rank order for backward deletion. BLOCK OMEGA hence compare with RUN 125. (CORR=0.82). OBF no signficant change. *NB Inclusion of INHI on V/F in the model is an error.*
129	2	ADD+CCV	θ1exp(η1)	(θ2+θ7*(DAYS-10)-θ8*(CAV-4)+θ10*(WT-54)+θ11*(GLOB-52))exp(η2)	(θ3+θ9*(VIR-5)+θ12*SEX+θ13*(DAYS-10)+θ14*INHI)exp(η3)	θ6exp(η4)	50	39	28	76	35	0.05	RFA9.CSV	611.296	GLOB fixed to 0. Establishing rank order for backward deletion. BLOCK OMEGA hence compare with RUN 125. (CORR=0.78). OBF ↑ 4.38. *NB Inclusion of CAV on CL/F and INHI on V/F in the model is an error.*
130	2	ADD+CCV	θ1exp(η1)	(θ2+θ7*(DAYS-10)-θ8*(CAV-4)+θ10*(WT-54)+θ11*(GLOB-52))exp(η2)	(θ3+θ9*(VIR-5)+θ12*SEX+θ13*(DAYS-10)+θ14*INHI)exp(η3)	θ6exp(η4)	51	38	26	77	35	0.05	RFA9.CSV	616.410	LOGHIV fixed to 0. Establishing rank order for backward deletion. BLOCK OMEGA hence compare with RUN 125. (CORR=0.80). OBF ↑ by 9.5. *NB Inclusion of CAV on CL/F and INHI on V/F in the model is an error.*
131	2	ADD+CCV	θ1exp(η1)	(θ2+θ7*(DAYS-10)-θ8*(CAV-4)+θ10*(WT-54)+θ11*(GLOB-52))exp(η2)	(θ3+θ9*(VIR-5)+θ12*SEX+θ13*(DAYS-10)+θ14*INHI)exp(η3)	θ6exp(η4)	50	39	40	74	35	0.05	RFA9.CSV	613.148	SEX fixed to 0. Establishing rank order for backward deletion. BLOCK OMEGA hence compare with RUN 125. (CORR=0.71). OBF ↑ by 6.2. *NB Inclusion of CAV on CL/F and INHI on V/F in the model is an error.*
132	2	ADD+CCV	θ1exp(η1)	(θ2+θ7*(DAYS-10)-θ8*(CAV-4)+θ10*(WT-54)+θ11*(GLOB-52))exp(η2)	(θ3+θ9*(VIR-5)+θ12*SEX+θ13*(DAYS-10)+θ14*INHI)exp(η3)	θ6exp(η4)	50	39	27	76	35	0.05	RFA9.CSV	609.105	INHI on V/F fixed to 0. Establishing rank order for backward deletion. BLOCK OMEGA hence compare with run 125. (CORR=0.69). OBF ↑ by 2.2. *NB Inclusion of CAV on CL/F and INHI on V/F in the model is an error.*
133	2	ADD+CCV	θ1exp(η1)	(θ2+θ7*(DAYS-10)-θ8*(CAV-4)+θ10*(WT-54)+θ11*(GLOB-52))exp(η2)	(θ3+θ9*(VIR-5)+θ12*SEX+θ13*(DAYS-10)+θ14*INHI)exp(η3)	θ6exp(η4)	50	40	29	77	35	0.05	RFA9.CSV	617.531	DAYS on V/F fixed to 0. Establishing rank order for backward deletion. BLOCK OMEGA hence compare with RUN 125. (CORR=0.97). OBF ↑ by 10.6. *NB Inclusion of CAV on CL/F and INHI on V/F in the model is an error.*

η - inter-individual variability; KA – absorption rate constant, CL/F – apparent clearance; V/F – apparent volume of distribution; TLAG – absorption lag time; σ - intra-individual variability; CCV – constant coefficient of variation; ADD – additive; OBF – minimum value of the Objective function.

NONMEM RUN SUMMARY - RIFAMPICIN

RUN	ADVAN	ERROR	KA	CL/F	V/F	TLAG	η_{KA} %	$\eta_{CL/F}$ %	$\eta_{V/F}$ %	η_{TLAG} %	σ_{CCV} %	σ_{ADD} µg/ml	DATA	OBF	COMMENTS
134	2	ADD+CCV	$\theta 1 exp(\eta 1)$	$(\theta 2+\theta 7*(DAYS-10)-\theta 8*(CAV-4)+\theta 10*(WT-54)+\theta 11*(GLOB-52)) exp(\eta 2)$	$(\theta 3+\theta 9*(VIR-5)+\theta 12*SEX+\theta 13*(DAYS-10)+\theta 14*INHI) exp(\eta 3)$	$\theta 6 exp(\eta 4)$	49	51	31	93	38	0.05	RFA9.CSV	698.802	DAYS on CL fixed to 0. Establishing rank order for backward deletion. BLOCK OMEGA hence compare with RUN 125. (CORR=0.81). OBF ↑ by 91.9. *NB Inclusion of CAV on CL/F and INHI on V/F in the model is an error.*
135	2	ADD+CCV	$\theta 1 exp(\eta 1)$	$(\theta 2+\theta 7*(DAYS-10)-\theta 8*(CAV-4)+\theta 10*(WT-54)+\theta 11*(GLOB-52)) exp(\eta 2)$	$(\theta 3+\theta 9*(VIR-5)+\theta 12*SEX+\theta 13*(DAYS-10)+\theta 14*INHI) exp(\eta 3)$	$\theta 6 exp(\eta 4)$	64	40	20	92	34	0.05	RFA9.CSV	658.203	WT fixed to 0. Establishing rank order for backward deletion. BLOCK OMEGA hence compare with RUN 125. (CORR=1). OBF ↑ by 51.3. *NB Inclusion of CAV on CL/F and INHI on V/F in the model is an error.*
136	2	ADD+CCV	$\theta 1 exp(\eta 1)$	$(\theta 2+\theta 7*(DAYS-10)-\theta 8*(CAV-4)+\theta 10*(WT-54)+\theta 11*(GLOB-52)) exp(\eta 2)$	$(\theta 3+\theta 9*(VIR-5)+\theta 12*SEX+\theta 13*(DAYS-10)+\theta 14*INHI) exp(\eta 3)$	-	54	38	12	-	45	0.05	RFA9.CSV	684.098	Removed lag time. COV does not work. *NB Inclusion of CAV on CL/F and INHI on V/F in the model is an error.*
137	2	ADD+CCV	$\theta 1 exp(\eta 1)$	$(\theta 2+\theta 7*(DAYS-10)+\theta 9*(WT-54)+\theta 10*(GLOB-52)) exp(\eta 2)$	$(\theta 3+\theta 8*(VIR-5)+\theta 11*SEX+\theta 12*(DAYS-10)) exp(\eta 3)$	$\theta 6 exp(\eta 4)$	51	38	26	77	35	0.05	RFA9.CSV	610.291	Full Model
138	2	ADD+CCV	$\theta 1 exp(\eta 1)$	$(\theta 2+\theta 7*(DAYS-10)+\theta 9*(WT-54)+\theta 10*(GLOB-52)) exp(\eta 2)$	$(\theta 3+\theta 8*(VIR-5)+\theta 11*SEX+\theta 12*(DAYS-10)) exp(\eta 3)$	$\theta 6 exp(\eta 4)$	51	38	27	77	35	0.05	RFA9.CSV	617.187	Stepwise Rank Order Deletion to Final Model. GLOB fixed to 0. OBF ↑ 6.9 Hence exclude.
139	2	ADD+CCV	$\theta 1 exp(\eta 1)$	$(\theta 2+\theta 7*(DAYS-10)+\theta 9*(WT-54)+\theta 10*(GLOB-52)) exp(\eta 2)$	$(\theta 3+\theta 8*(VIR-5)+\theta 11*SEX+\theta 12*(DAYS-10)) exp(\eta 3)$	$\theta 6 exp(\eta 4)$	49	38	30	75	35	0.05	RFA9.CSV	623.253	Stepwise Rank Order Deletion to Final Model. GLOB and SEX fixed to 0. OBF ↑ 6.1. Hence exclude.
140	2	ADD+CCV	$\theta 1 exp(\eta 1)$	$(\theta 2+\theta 7*(DAYS-10)+\theta 9*(WT-54)+\theta 10*(GLOB-52)) exp(\eta 2)$	$(\theta 3+\theta 8*(VIR-5)+\theta 11*SEX+\theta 12*(DAYS-10)) exp(\eta 3)$	$\theta 6 exp(\eta 4)$	48	38	30	73	36	0.05	RFA9.CSV	630.091	Stepwise Rank Order Deletion to Final Model. GLOB, SEX, LOGHIV fixed to 0. OBF ↑ 6.8. Hence exclude.
141	2	ADD+CCV	$\theta 1 exp(\eta 1)$	$(\theta 2+\theta 7*(DAYS-10)+\theta 9*(WT-54)+\theta 10*(GLOB-52)) exp(\eta 2)$	$(\theta 3+\theta 8*(VIR-5)+\theta 11*SEX+\theta 12*(DAYS-10)) exp(\eta 3)$	$\theta 6 exp(\eta 4)$	33	38	20	158	38	0.05	RFA9.CSV	675.854	Stepwise Rank Order Deletion to Final Model. GLOB, SEX, LOGHIV and DAYS on V/F fixed to 0. OBF ↑ 45.8. Hence can't exclude DAYS on V/F.

η - inter-individual variability; KA – absorption rate constant, CL/F – apparent clearance; V/F – apparent volume of distribution; TLAG – absorption lag time; σ - intra-individual variability; CCV – constant coefficient of variation; ADD – additive; OBF – minimum value of the Objective function.

Appendix G

NONMEM RUN SUMMARY - RIFAMPICIN

RUN	ADVAN	ERROR	KA	CL/F	V/F	TLAG	η_{KA} %	$\eta_{CL/F}$ %	$\eta_{V/F}$ %	η_{TLAG} %	σ_{CCV} %	σ_{ADD} µg/ml	DATA	OBF	COMMENTS
142	2	ADD+CCV	θ1exp(η1)	(θ2+θ7*(DAYS-10)+θ9*(WT-54)+θ10*(GLOB-52))exp(η2)	(θ3+θ8*(VIR-5)+θ11*SEX+θ12*(DAYS-10)) exp(η3)	θ6exp(η4)	51	41	31	75	36	0.05	RFA9.CSV	684.214	Stepwise Rank Order Deletion to Final Model. GLOB, SEX, LOGHIV and WT fixed to 0. OBF ↑ 8.4. Hence exclude.
143	2	ADD+CCV	θ1exp(η1)	(θ2+θ7*(DAYS-10)+θ9*(WT-54)+θ10*(GLOB-52))exp(η2)	(θ3+θ8*(VIR-5)+θ11*SEX+θ12*(DAYS-10)) exp(η3)	θ6exp(η4)	47	53	34	70	38	0.05	RFA9.CSV	760.214	Stepwise Rank Order Deletion to Final Model. GLOB, SEX, LOGHIV, WT and DAYS on CL/F fixed to 0. OBF ↑ 76. Hence can't exclude DAYS on CL/F.
144	2	ADD+CCV	θ1exp(η1)	(θ2+θ7*(DAYS-10)+θ9*(WT-54)+θ10*(GLOB-52))exp(η2)	(θ3+θ8*(VIR-5)+θ11*SEX+θ12*(DAYS-10)) exp(η3)	θ6exp(η4)	52	39	26	69	39	0.05	RFA9.CSV	701.922	FINAL RUN. Removed all covariates that caused a ≤11 DOBF when fixed to NULL during backward deletion i.e. GLOB, SEX, LOGHIV, WT. Implemented COV. No BLOCK OMEGA
145	2	ADD+CCV	θ1exp(η1)	(θ2+θ7*(DAYS-30)-θ8*(CAV-4)+θ10*(WT-54)+θ11*(GLOB-52))exp(η2)	(θ3+θ9*(VIR-5)+θ12*SEX+θ13*(DAYS-30))exp(η3)	θ6exp(η4)	56	37	19	69	39	0.05	RFA9.CSV	673.780	Runs 144 to 149 are a record of the attempt to find the best fit for maximal enzyme induction. MAX=30 Arbitrary choice.
146	2	ADD+CCV	θ1exp(η1)	(θ2+θ7*(DAYS-4.5)-θ8*(CAV-4)+θ10*(WT-54)+θ11*(GLOB-52))exp(η2)	(θ3+θ9*(VIR-5)+θ12*SEX+θ13*(DAYS-4.5)+θ14*INHI)exp(η3)	θ6exp(η4)	48	38	20	127	41	0.05	RFA9.CSV	668.526	MAX=4.5 as suggested by TREE modelling.
147	2	ADD+CCV	θ1exp(η1)	(θ2+θ7*(DAYS-7)-θ8*(CAV-4)+θ10*(WT-54)+θ11*(GLOB-52))exp(η2)	(θ3+θ9*(VIR-5)+θ12*SEX+θ13*(DAYS-7)+θ14*INHI)exp(η3)	θ6exp(η4)	49	34	18	130	41	0.05	RFA9.CSV	660.323	MAX=7 (literature says between 7 and 14 days)
148	2	ADD+CCV	θ1exp(η1)	(θ2+θ7*(DAYS-14)-θ8*(CAV-4)+θ10*(WT-54)+θ11*(GLOB-52))exp(η2)	(θ3+θ9*(VIR-5)+θ12*SEX+θ13*(DAYS-14)+θ14*INHI)exp(η3)	θ6exp(η4)	51	35	15	134	40	0.05	RFA9.CSV	675.287	MAX=14 (literature says between 7 and 14 days)
149	2	ADD+CCV	θ1exp(η1)	(θ2+θ7*(DAYS-10)-θ8*(CAV-4)+θ10*(WT-54)+θ11*(GLOB-52))exp(η2)	(θ3+θ9* (VIR-5)+θ12*SEX+θ13*(DAYS-10)+θ14*INHI)exp(η3)	θ6exp(η4)	54	34	19	74	39	0.05	RFA9.CSV	654.089	MAX=10 as suggested by GAM natural cubic spline at 9.7. No correlations of significance in the matrix. CORR of etas CL/F vs V/F = 0.75.

η - inter-individual variability; KA – absorption rate constant, CL/F – apparent clearance; V/F – apparent volume of distribution; TLAG – absorption lag time; σ - intra-individual variability; CCV – constant coefficient of variation; ADD – additive; OBF – minimum value of the Objective function.

Appendix H   NONMEM CONTROL STREAM - RIFAMPICIN

Appendix H

NONMEM CONTROL STREAM - RIFAMPICIN

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$PROB RIFAMPICIN KINETICS IN TUBERCULOSIS PATIENTS
$INPU ID DATE=DROP TIME DOSE=AMT RATE=DROP DV=CONC SS II
 SEX TBHX=DROP MDR HIV WT AGE=DROP DAMT=DROP DAYS
 ALKP=DROP BILI=DROP PROT=DROP ALB=DROP GLOB GGT=DROP ALT=DROP
AST=DROP ALP=DROP PLAT=DROP LYMC=DROP LYMP=DROP VIRU  INDU=DROP INHI
ABS1=DROP ABS2=DROP MIS1=DROP MIS2=DROP NSID=DROP FEFL H1B=DROP
PEN=DROP BS=DROP DIUR=DROP INAT=DROP QUIN=DROP  AG=DROP EMB PZA=DROP
ANAE=DROP HPT=DROP DM=DROP EPIL=DROP CAND=DROP NMDR EXT=DROP CAV
WALL=DROP
$DATA        RFA9.CSV IGNORE=#
$SUBROUTINES        ADVAN2 TRANS2
$PK
     DAY=DAYS
     IF (DAYS.GE.10) DAY = 10
TVKA = THETA(1)  ; KA
     TVCL = THETA(2)+THETA(7)*(DAY-10)+THETA(8)*(WT-54)
     IF (TVCL.LE.0) EXIT 1 100
     TVV  = THETA(3)+THETA(9)*(DAY-10)
IF (TVV.LE.0) EXIT 1 200
     KA   = TVKA*EXP(ETA(1))
     CL   = TVCL*EXP(ETA(2))
     V    = TVV*EXP(ETA(3))
     S2   = V
   TVALG1 = THETA(6)
   ALAG1  = TVALG1*EXP(ETA(4))
  CALLFL  = -2
$ERROR
     IPRED=F
     W=(F*F*THETA(4)*THETA(4)+THETA(5)**2)**0.5
     IRES=DV-F
     IWRES=IRES/W
     Y=IPRED + W*EPS(1)
$SIGMA    1 FIXED
$THETA   (0,1,50)  ; 1: KA
                (10)             ; 2: CL intercept
                (0,36)           ; 3: V intercept
                (0,0.2)          ; 4: CCV part of residual error
                (0.09)           ; 5: additive part of residual error
                (0,0.1)          ; 6: lag time
                (0.06)           ; 7: Slope for DAYS since starting RFA
therapy on CL
                (0.06)           ; 8: Effect of WT on CL
                (0.05)           ; 9: Slope for DAYS since starting RFA
therapy on V
$OMEGA   0.25 0.25 0.25 0.25
$EST NOABORT MAXEVAL=9999 POSTHOC MSF=rfa
$TABLES ID TIME IPRED IWRES KA CL V ALAG1 DAY ETA1 ETA2 ETA3 ETA4
        ONEHEADER NOPRINT FILE=sdtab148
$COV
```

Appendix I   COVARIATE MODEL BUILDING - RIFAMPICIN

Appendix I

COVARIATE MODEL BUILDING - RIFAMPICIN

Covariate Effect	DOBF[1]	Retained[2] in Forward Model Build-up	Retained[3] in Final Model
RFA auto-induction on CL/F	74.5	Yes	Yes
Quinolones on CL/F	38.15	No	No
Enzyme inhibitor on CL/F	37.753	No	No
MDR-TB on CL/F	26.343	No	No
Ethambutol on CL/F	20.4	No	No
Severity score for extent of lung cavitation on CL/F	17.846	No	No
Log HIV viral load on V/F	16.083	Yes	No
HIV on V/F	15.727	No	No
WT on CL/F	15.475	Yes	No
Globulin on CL/F	15.376	Yes	No
Thiacetazone on CL/F	14.567	No	No
Globulin on V/F	13.795	No	No
Sex on V/F	12.167	Yes	No
Iron preparations/anaemia on CL/F	11.117	No	No
Drug resistance severity score on CL/F	10.556	No	No
RFA auto-induction on V/F	9.613	Yes	Yes
AGE on CL/F	7.685	No	No
Enzyme inhibitor on V/F	7.468	No	No
Iron preparations/anaemia on V/F	7.162	No	No
HIV on CL/F	6.932	No	No
AST on CL/F	6.072	No	No
Non-steroidal anti-inflammatory drug use on CL/F	4.87	No	No
Log HIV viral load on CL/F	4.081	No	No
Albumin on V/F	3.35	n/a	n/a
Candidiasis on V/F	3.022	n/a	n/a
Candidiasis on CL/F	2.945	n/a	n/a
Severity score for extent of lung cavitation on V/F	2.889	n/a	n/a
Ethambutol on V/F	2.419	n/a	n/a
AGE on V/F	2.37	n/a	n/a
MDR-TB on V/F	2.326	n/a	n/a
Quinolones on V/F	2.273	n/a	n/a
Iron preparations on KA	2.248	n/a	n/a
WT on V/F	2.159	n/a	n/a
Non-steroidal anti-inflammatory drugs on V/F	2.077	n/a	n/a
Total Bilirubin on V/F	1.345	n/a	n/a
Drug resistance severity score on V/F	1.33	n/a	n/a
GGT on CL/F	1.028	n/a	n/a
AST on V/F	0.986	n/a	n/a
Severity score for extent of lung involvement on V/F	0.846	n/a	n/a
Dose amount on KA	0.828	n/a	n/a
Total Bilirubin on CL/F	0.796	n/a	n/a
ALK PHOS on V/F	0.742	n/a	n/a
Diuretics/hypertension on V/F	0.648	n/a	n/a
Hypoglycaemic agents/diabetes mellitus on CL/F	0.584	n/a	n/a
Hypoglycaemic agents/diabetes mellitus on V/F	0.571	n/a	n/a

[1]DOBF – difference in the minimum value of the objective function between 2 NONMEM runs. Chi square distributed – [2]DOBF ≥ 3.84 , $p \leq 0.05$ df=1; [3]DOBF ≥ 11, $p \leq 0.001$ df=1

Appendix I

COVARIATE MODEL BUILDING - RIFAMPICIN

Covariate Effect	DOBF[1]	Retained[2] in Forward Model Build-up	Retained[3] in Final Model
GGT on V/F	0.399	n/a	n/a
Albumin on CL/F	0.383	n/a	n/a
Antihistamines on CL/F	0.241	n/a	n/a
ALT on V/F	0.151	n/a	n/a
Antihistamines on V/F	0.073	n/a	n/a
Severity score for extent of lung involvement on CL/F	0.058	n/a	n/a
Thiacetazone on V/F	0.058	n/a	n/a
Sex on CL/F	0.046	n/a	n/a
ALK PHOS on CL/F	0.009	n/a	n/a
Diuretics/hypertension on CL/F	0.009	n/a	n/a
Absorption drug interaction on KA	0.005	n/a	n/a
Absorption drug interaction on CL/F	0.002	n/a	n/a
Dose amount on V/F	-0.005	n/a	n/a
ALT on CL/F	-0.014	n/a	n/a
Absorption drug interaction on V/F	-0.463	n/a	n/a

[1]DOBF – difference in the minimum value of the objective function between 2 NONMEM runs. Chi square distributed – [2]DOBF $\geq$ 3.84 , p $\leq$ 0.05 df=1; [3]DOBF $\geq$ 11, p $\leq$ 0.001 df=1

Appendix J   NONMEM RUN SUMMARY - ISONIAZID

Appendix J

NONMEM RUN SUMMARY - ISONIAZID

RUN	ADVAN	ERROR	KA	CL/F	VD	TLAG	η_{KA} %	$\eta_{CL/F}$ %	η_V %	σ_{CCV} %	σ_{ADD} µg/ml	DATA	OBF	COMMENTS
1	2	ADD+CCV	$\theta1\exp(\eta1)$	$\theta2\exp(\eta2)$	$\theta3\exp(\eta3)$	-	68	45	439	40	0.05	INH2.CSV	1128.050	Prepared tables for data checkout in Xpose2. Model is under-predicting. Some parameter estimates are unrealistic. Run times are long.
2	2	ADD+CCV	$\theta1\exp(\eta1)$	$\theta2\exp(\eta2)$	$\theta3\exp(\eta3)$	-	2102	27	15	23	0.5	INH5.CSV	851.055	Implemented new $ERROR code as per Mats suggestion. IWRES has unit variance. KA estimate and η is very large. Data file has ALL the covariates.
3	2	ADD+CCV	$\theta1\exp(\eta1)$	$\theta2\exp(\eta2)$	$\theta3\exp(\eta3)$	-	80	39	16	17	0.7	INH5.CSV	1196.276	Sensitivity Analysis: KA fixed to 0.5. V estimate unrealistic.
4	2	ADD+CCV	$\theta1\exp(\eta1)$	$\theta2\exp(\eta2)$	$\theta3\exp(\eta3)$	-	40	42	<1	29	0.7	INH5.CSV	1160.628	Sensitivity Analysis: KA fixed to 0.6. V estimate still small.
5	2	ADD+CCV	$\theta1\exp(\eta1)$	$\theta2\exp(\eta2)$	$\theta3\exp(\eta3)$	-	61	44	<1	29	0.6	INH5.CSV	1131.429	Sensitivity Analysis: KA fixed to 0.7. V estimate still small
6	2	ADD+CCV	$\theta1\exp(\eta1)$	$\theta2\exp(\eta2)$	$\theta3\exp(\eta3)$	-	97	50	22	23	0.6	INH5.CSV	1017.531	Sensitivity Analysis: KA fixed to 1.0. V estimate now realistic.
7	2	ADD+CCV	$\theta1\exp(\eta1)$	$\theta2\exp(\eta2)$	$\theta3\exp(\eta3)$	-	78	47	34	18	0.5	INH5.CSV	930.859	Sensitivity Analysis: KA fixed to 1.5.
8	2	ADD+CCV	$\theta1\exp(\eta1)$	$\theta2\exp(\eta2)$	$\theta3\exp(\eta3)$	-	76	46	35	18	0.5	INH5.CSV	897.293	Sensitivity Analysis: KA fixed to 2.0 OBF ↓
9	2	ADD+CCV	$\theta1\exp(\eta1)$	$\theta2\exp(\eta2)$	$\theta3\exp(\eta3)$	-	118	45	36	18	0.5	INH5.CSV	882.867	Sensitivity Analysis: KA fixed to 3.0 OBF ↓
10	2	ADD+CCV	$\theta1\exp(\eta1)$	$\theta2\exp(\eta2)$	$\theta3\exp(\eta3)$	-	225	44	36	18	0.5	INH5.CSV	881.798	Sensitivity Analysis: KA fixed to 4.0 η_{KA} is large. OBF ↓.
11	2	ADD+CCV	$\theta1\exp(\eta1)$	$\theta2\exp(\eta2)$	$\theta3\exp(\eta3)$	-	315	42	35	19	0.5	INH5.CSV	881.112	Sensitivity Analysis: KA fixed to 4.5. η_{KA} is larger. OBF ↓.
12	2	ADD+CCV	$\theta1\exp(\eta1)$	$\theta2\exp(\eta2)$	$\theta3\exp(\eta3)$	-	442	40	34	19	0.5	INH5.CSV	879.296	Sensitivity Analysis: KA fixed to 5.0 η_{KA} is larger. OBF ↓.
13	2	ADD+CCV	$\theta1\exp(\eta1)$	$\theta2\exp(\eta2)$	$\theta3\exp(\eta3)$	-	773	33	31	21	0.5	INH5.CSV	870.946	Sensitivity Analysis: KA fixed to 6.0. η_{KA} is larger. OBF ↓.
14	2	ADD+CCV	$\theta1\exp(\eta1)$	$\theta2\exp(\eta2)$	$\theta3\exp(\eta3)$	-	1204	27	26	22	0.5	INH5.CSV	860.979	Sensitivity Analysis: KA fixed to 8.0. η_{KA} is larger. OBF ↓.
15	2	ADD+CCV	$\theta1\exp(\eta1)$	$\theta2\exp(\eta2)$	$\theta3\exp(\eta3)$	$\theta4$	116	46	36	18	0.5	INH5.CSV	882.085	Added in a lag time and KA is not fixed. Goodness of fits plots are better and estimates are more realistic.

η - inter-individual variability – where 2 values are recorded they are the estimates for the first and second populations; KA – absorption rate constant; CL/F – apparent clearance; CL(1)/F– apparent clearance (fast); CL(2)/F – apparent clearance for slow acetylators; V/F – apparent volume of distribution; TLAG – absorption lag time; σ - intra-individual variability; CCV – constant coefficient of variation; ADD – additive; OBF – minimum value of the Objective function.

NONMEM RUN SUMMARY - ISONIAZID

RUN	ADVAN	ERROR	KA	CL/F	VD	TLAG	η_{KA} %	$\eta_{CL/F}$ %	η_V %	σ_{CCV} %	σ_{ADD} µg/ml	DATA	OBF	COMMENTS
16	2	ADD+CCV	$\theta1\exp(\eta1)$	$\theta2\exp(\eta2)$	$\theta3\exp(\eta3)$	$\theta4\exp(\eta4)$	444	22	22	19	0.52	INH5.CSV	838.242	As per RUN 15 but with an η on TLAG OCCURS DURING SEARCH FOR ETA AT A NONZERO VALUE OF ETA. PK PARAMETER FOR ABSORPTION LAG IS GREATER THAN OR EQUAL TO STEADY STATE DOSE INTERVAL. PROGRAM TERMINATED BY FNLETA. MESSAGE ISSUED FROM TABLE STEP TLAG very small (10^{-4}) and variability very large (10^8).
17	1	ADD+CCV	-	$\theta1\exp(\eta1)$	$\theta2\exp(\eta2)$	-	-	45	34	23	0.53	INH5.CSV	901.258	ADVAN 1 and estimated D1 parameter. Duration parameter very small.
18	1	ADD+CCV	-	$\theta1\exp(\eta1)$	$\theta2\exp(\eta2)$	$\theta3$	-	47	34	23	0.5	INH5.CSV	901.117	ADVAN 1, D1 and TLAG parameter.
19	4	ADD+CCV	$\theta1\exp(\eta1)$	$\theta2\exp(\eta2)$	$\theta3\exp(\eta3)$	-	68	52	37	20	0.46	INH5.CSV	800.274	2 compartment model. Although the OBF is smaller, goodness of fits plots are not any better than using RUN 15. Hence the simpler model was chosen as the basic model.
20	2	ADD+CCV	$\theta1$(INAT) $\theta1+\theta6$(INH)) $\exp\eta1$	$\theta2\exp(\eta2)$	$\theta3\exp(\eta3)$	$\theta4$	37	46	34	22	0.49	INH5.CSV	846.845	Effect of PREP on KA but illogical to try and determine KA for INAT as there were no samples drawn at descriptive times. Therefore ignore this run.
21	2	ADD+CCV	$\theta1\exp(\eta1)$	$\theta2\exp(\eta2)$	$\theta3\exp(\eta3)$	$\theta4$	-	-	-	-	-	INH5.CSV	801.676	F1 for PREP. Rounding errors and see comments for RUN 20.
22	2	ADD+CCV	$\theta2\exp(\eta1)$	CL(1)/F=$\theta6\exp(\eta2)$ CL(2)/F=$\theta6*\theta7\exp(\eta4)$	V1=$\theta8\exp(\eta3)$ V2=$\theta8*\theta9\exp(\eta5)$	$\theta3$	127	42/42	41/41	29	0.02	INH5.CSV	296.172	Mixture Modelling on both CL/F and V. Dramatic ↓ in OBF.
23	2	ADD+CCV	$\theta2\exp(\eta1)$	CL(1)/F=$\theta6\exp(\eta2)$ CL(2)/F=$\theta6*\theta7\exp(\eta4)$	$\theta8\exp(\eta3)$	$\theta3$	127	43/43	43	29	0.02	INH5.CSV	289.890	Full BLOCK matrix on $OMEGA. Mixture modelling on CL/F only. Significant ↓ in OBF.
24	2	ADD+CCV	$\theta2\exp(\eta1)$	CL(1)/F=$\theta6\exp(\eta2)$ CL(2)/F=$\theta6*\theta7\exp(\eta4)$	$\theta8\exp(\eta3)$	$\theta3$	4393	14	22	32	0.03	INH5.CSV	301.755	Diagonal matrix on $OMEGA. Mixture modelling on CL/F only. Very large estimate and η for KA. OBF ↑.
25	2	ADD+CCV	$\theta2\exp(\eta1)$	CL(1)/F=$\theta6\exp(\eta2)$ CL(2)/F=$\theta6*\theta7\exp(\eta4)$	$\theta8\exp(\eta3)$	$\theta3$	100	44/38	44	28	0.02	INH5.CSV	303.829	Mixture Modelling. Fixed KA to2.0. Estimate of TLAG very small. KA variability is more reasonable.
26	2	ADD+CCV	$\theta2\exp(\eta1)$	CL(1)/F=$\theta6\exp(\eta2)$ CL(2)/F=$\theta6*\theta7\exp(\eta4)$	$\theta3\exp(\eta3)$	-	100	44/38	44	28	0.02	INH5.CSV	303.829	Mixture Modelling. Removed TLAG. OBF no change.

η - inter-individual variability – where 2 values are recorded they are the estimates for the first and second populations; KA – absorption rate constant; CL/F – apparent clearance; CL(1)/F– apparent clearance (fast); CL(2)/F – apparent clearance for slow acetylators; V/F – apparent volume of distribution; TLAG – absorption lag time; σ - intra-individual variability; CCV – constant coefficient of variation; ADD – additive; OBF – minimum value of the Objective function.

Appendix J

NONMEM RUN SUMMARY - ISONIAZID

RUN	ADVAN	ERROR	KA	CL/F	VD	TLAG	η_{KA} %	$\eta_{CL/F}$ %	η_V %	σ_{CCV} %	σ_{ADD} μg/ml	DATA	OBF	COMMENTS
27	2	ADD+CCV	$\theta 2\exp(\eta 1)$	$CL(1)/F=\theta 6\exp(\eta 2)$ $CL(2)/F=\theta 6^*\theta 7\exp(\eta 4)$	$\theta 3\exp(\eta 3)$	-	117	43/38	43	29	0.02	INH5.CSV	290.405	Mixture Modelling. Sensitivity Analysis: KA fixed to 3.0
28	2	ADD+CCV	$\theta 2\exp(\eta 1)$	$CL(1)/F=\theta 6\exp(\eta 2)$ $CL(2)/F=\theta 6^*\theta 7\exp(\eta 4)$	$\theta 3\exp(\eta 3)$	-	1479	43/35	49	30	<1	INH5.CSV	365.167	Mixture Modelling. Sensitivity Analysis: KA fixed to 1.0. OBF ↑ η_{KA} ↑
29	2	ADD+CCV	$\theta 2\exp(\eta 1)$	$CL(1)/F=\theta 6\exp(\eta 2)$ $CL(2)/F=\theta 6^*\theta 7\exp(\eta 4)$	$\theta 3\exp(\eta 3)$	-	185	43/38	43	29	0.02	INH5.CSV	289.910	Mixture Modelling. Sensitivity Analysis: KA fixed to 4.0. OBF ↓
30	2	ADD+CCV	$\theta 2\exp(\eta 1)$	$CL(1)/F=\theta 6\exp(\eta 2)$ $CL(2)/F=\theta 6^*\theta 7\exp(\eta 4)$	$\theta 3\exp(\eta 3)$	-	293	42/37	42	30	0.02	INH5.CSV	291.432	Mixture Modelling. Sensitivity Analysis: KA fixed to 5.0. OBF ↓
31	2	ADD+CCV	$\theta 2\exp(\eta 1)$	$CL(1)/F=\theta 6\exp(\eta 2)$ $CL(2)/F=\theta 6^*\theta 7\exp(\eta 4)$	$\theta 3\exp(\eta 3)$	-	423	41/37	41	30	0.02	INH5.CSV	293.259	Mixture Modelling. Sensitivity Analysis: KA fixed to 6.0. OBF ↓.
32	2	ADD+CCV	$\theta 2\exp(\eta 1)$	$CL(1)/F=\theta 6\exp(\eta 2)$ $CL(2)/F=\theta 6^*\theta 7\exp(\eta 4)$	$\theta 3\exp(\eta 3)$	-	146	43/38	43	29	0.02	INH5.CSV	289.640	Mixture Modelling. Sensitivity Analysis: KA fixed to 3.5. Lowest value. This value used as the fixed value for KA in all subsequent runs.
33	2	ADD+CCV	$\theta 2\exp(\eta 1)$	$CL(1)/F=(\theta 6+\theta 8^*(AGE-33))\exp(\eta 2)$ $CL(2)/F=(\theta 6+\theta 8^*(AGE-33))$ $^*\theta 7\exp(\eta 4)$	$\theta 3\exp(\eta 3)$	-	145	43/38	43	29	0.02	INH5.CSV	289.232	Mixture Modelling. Age on CL/F. Not significant.
34	2	ADD+CCV	$\theta 2\exp(\eta 1)$	$CL(1)/F=\theta 6\exp(\eta 2)$ $CL(2)/F=\theta 6^*\theta 7\exp(\eta 4)$	$(\theta 3+\theta 8^*(AGE-33))\exp(\eta 3)$	-	145	43/38	43	29	0.02	INH5.CSV	288.714	Age on V. Not significant.
35	2	ADD+CCV	$\theta 2\exp(\eta 1)$	$CL(1)/F=(\theta 6+\theta 8^*(ALK-84))\exp(\eta 2)$ $CL(2)/F=(\theta 6+\theta 8^*(ALK-84)^*\theta 7\exp(\eta 4)$	$\theta 3\exp(\eta 3)$	-	146	43/38	43	29	0.02	INH5.CSV	289.529	Liver Function Tests. Alk Phos on CL/F. Not significant.
36	2	ADD+CCV	$\theta 2\exp(\eta 1)$	$CL(1)/F=\theta 6\exp(\eta 2)$ $CL(2)/F=\theta 6^*\theta 7\exp(\eta 4)$	$(\theta 3+\theta 8^*(ALK-84))\exp(\eta 3)$	-	145	43/38	43	29	0.02	INH5.CSV	289.262	Liver Function Tests. Alk Phos on V. Not significant.
37	2	ADD+CCV	$\theta 2\exp(\eta 1)$	$CL(1)/F=(\theta 6+\theta 8^*(BIL-10))\exp(\eta 2)$ $CL(2)/F=(\theta 6+\theta 8^*(BIL-10)^*\theta 7\exp(\eta 4)$	$\theta 3\exp(\eta 3)$	-	148	43/38	43	29	0.02	INH5.CSV	289.122	Liver Function Tests. Total Bilirubin on CL/F. Not significant.
38	2	ADD+CCV	$\theta 2\exp(\eta 1)$	$CL(1)/F=\theta 6\exp(\eta 2)$ $CL(2)/F=\theta 6^*\theta 7\exp(\eta 4)$	$(\theta 3+\theta 8^*(BIL-10))\exp(\eta 3)$	-	149	43/37	42	29	0.02	INH5.CSV	284.743	Liver Function Tests. Total Bilirubin on V. Significant
39	2	ADD+CCV	$\theta 2\exp(\eta 1)$	$CL(1)/F=(\theta 6+\theta 8^*(ALBU-25))\exp(\eta 2)$ $CL(2)/F=(\theta 6+\theta 8^*(ALBU-25))^*\theta 7\exp(\eta 4)$	$\theta 3\exp(\eta 3)$	-	143	43/38	43	29	0.02	INH5.CSV	288.621	Liver Function Tests. Albumin on CL/F. Not significant
40	2	ADD+CCV	$\theta 2\exp(\eta 1)$	$CL(1)/F=\theta 6\exp(\eta 2)$ $CL(2)/F=\theta 6^*\theta 7\exp(\eta 4)$	$(\theta 3+\theta 8^*(ALBU-25))\exp(\eta 3)$	-	140	43/38	43	29	0.02	INH5.CSV	285.967	Liver Function Tests. Albumin on V. Not significant

η - inter-individual variability – where 2 values are recorded they are the estimates for the first and second populations; KA – absorption rate constant; CL/F – apparent clearance; CL(1)/F– apparent clearance (fast); CL(2)/F – apparent clearance for slow acetylators; V/F – apparent volume of distribution; TLAG – absorption lag time; σ - intra-individual variability; CCV – constant coefficient of variation; ADD – additive; OBF – minimum value of the Objective function.

Appendix J

NONMEM RUN SUMMARY - ISONIAZID

RUN	ADVAN	ERROR	KA	CL/F	VD	TLAG	η_{KA} %	$\eta_{CL/F}$ %	η_V %	σ_{CCV} %	σ_{ADD} µg/ml	DATA	OBF	COMMENTS
41	2	ADD+CCV	$\theta2exp(\eta1)$	CL(1)/F=$(\theta6+\theta8*(GLOB-52))$ $exp(\eta2)$ CL(2)/F=$(\theta6+\theta8*(GLOB-52))*\theta7$ $exp(\eta4)$	$\theta3exp(\eta3)$	-	153	41/39	43	29	0.02	INH5.CSV	265.483	Liver Function Tests. Globulin on CL/F. Significant
42	2	ADD+CCV	$\theta2exp(\eta1)$	CL(1)/F=$\theta6exp(\eta2)$ CL(2)/F=$\theta6*\theta7exp(\eta4)$	$(\theta3+\theta8*(GLOB-52))exp(\eta3)$	-	148	44/40	44	29	0.02	INH5.CSV	284.418	Liver Function Tests. Globulin on V. Significant.
43	2	ADD+CCV	$\theta2exp(\eta1)$	CL(1)/F=$(\theta6+\theta8*(GGT-37))exp(\eta2)$ CL(2)/F=$(\theta6+\theta8*(GGT-37))*\theta7$ $exp(\eta4)$	$\theta3exp(\eta3)$	-	147	43/38	43	29	0.02	INH5.CSV	288.910	Liver Function Tests. GGT on CL/F. Not significant.
44	2	ADD+CCV	$\theta2exp(\eta1)$	CL(1)/F=$\theta6exp(\eta2)$ CL(2)/F=$\theta6*\theta7exp(\eta4)$	$(\theta3+\theta8*(GGT-37))exp(\eta3)$	-	145	43/38	43	29	0.02	INH5.CSV	289.338	Liver Function Tests. GGT on V. Not significant.
45	2	ADD+CCV	$\theta2exp(\eta1)$	CL(1)/F=$(\theta6+\theta8*(ALT-16))exp(\eta2)$ CL(2)/F=$(\theta6+\theta8*(ALT-16))*\theta7$ $exp(\eta4)$	$\theta3exp(\eta3)$	-	145	43/38	43	29	0.02	INH5.CSV	287.662	Liver Function Tests. ALT on CL/F. Not significant.
46	2	ADD+CCV	$\theta2exp(\eta1)$	CL(1)/F=$\theta6exp(\eta2)$ CL(2)/F=$\theta6*\theta7exp(\eta4)$	$(\theta3+\theta8*(ALT-16))exp(\eta3)$	-	145	43/38	43	29	0.02	INH5.CSV	289.592	Liver Function Tests. ALT on V. Not significant.
47	2	ADD+CCV	$\theta2exp(\eta1)$	CL(1)/F=$(\theta6+\theta8*(AST-31))exp(\eta2)$ CL(2)/F=$(\theta6+\theta8*(AST-31))*\theta7$ $exp(\eta4)$	$\theta3exp(\eta3)$	-	145	43/38	43	29	0.02	INH5.CSV	289.596	Liver Function Tests. AST on CL/F. Not significant.
48	2	ADD+CCV	$\theta2exp(\eta1)$	CL(1)/F=$\theta6exp(\eta2)$ CL(2)/F=$\theta6*\theta7exp(\eta4)$	$(\theta3+\theta8*(AST-31))exp(\eta3)$	-	146	43/38	43	29	0.02	INH5.CSV	288.350	Liver Function Tests. AST on V. Not significant.
49	2	ADD+CCV	$\theta2exp(\eta1)$	CL(1)/F=$(\theta6+\theta8*(VIRU-65183.49))$ $exp(\eta2)$ CL(2)/F=$(\theta6+\theta8*(VIRU-65183.49))$ $*\theta7exp(\eta4)$	$\theta3exp(\eta3)$	-	146	43/38	43	29	0.02	INH5.CSV	288.765	Viral Load on CL/F. Not significant.
50	2	ADD+CCV	$\theta2exp(\eta1)$	CL(1)/F=$\theta6*(exp(\eta2)$ CL(2)/F=$\theta6*\theta7exp(\eta4)$	$(\theta3+\theta8*(VIRU-65183.49))exp(\eta3)$	-	146	43/38	43	29	0.02	INH5.CSV	289.616	Viral load on V. Not significant.
51	2	ADD+CCV	$\theta2exp(\eta1)$	CL(1)/F=$(\theta6+\theta8*(VIR-5))exp(\eta2)$ CL(2)/F=$(\theta6+\theta8*(VIR-5))$ $*\theta7exp(\eta4)$	$\theta3exp(\eta3)$	-	147	43/38	43	29	0.02	INH5.CSV	287.667	Log Viral Load on CL/F. Not significant.
52	2	ADD+CCV	$\theta2exp(\eta1)$	CL(1)/F=$\theta6*(exp(\eta2)$ CL(2)/F=$\theta6*\theta7exp(\eta4)$	$(\theta3+\theta8*(VIR-5))exp(\eta3)$	-	148	43/38	43	29	0.02	INH5.CSV	281.546	Log Viral load on V. Significant.
53	2	ADD+CCV	$\theta2exp(\eta1)$	CL(1)/F=$(\theta6*(1-ABS)$ $+\theta8*ABS)*exp(\eta2)$ CL(2)/F=$(\theta6*(1-ABS)+\theta8*ABS)*\theta7exp(\eta4)$	$\theta3exp(\eta3)$	-	145	45/33	42	30	0.02	INH5.CSV	273.929	Drug interactions. Drugs affecting absorption on CL/F. Significant but illogical grouping of drugs together. See next run.

η - inter-individual variability – where 2 values are recorded they are the estimates for the first and second populations; KA – absorption rate constant; CL/F – apparent clearance; CL(1)/F– apparent clearance (fast); CL(2)/F – apparent clearance for slow acetylators; V/F – apparent volume of distribution; TLAG – absorption lag time; σ - intra-individual variability; CCV – constant coefficient of variation; ADD – additive; OBF – minimum value of the Objective function.

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NONMEM RUN SUMMARY - ISONIAZID

RUN	ADVAN	ERROR	KA	CL/F	VD	TLAG	η_{KA} %	$\eta_{CL/F}$ %	η_V %	σ_{CCV} %	σ_{ADD} $\mu g/ml$	DATA	OBF	COMMENTS
54	2	ADD+CCV	$\theta2\exp(\eta1)$	CL(1)/F=$\theta6^*\exp(\eta2)$ CL(2)/F=$\theta6^*\theta7\exp(\eta4)$	$\theta3\exp(\eta3)$	-	145	43/38	43	29	0.02	INH5.CSV	289.639	Drug interactions. Drugs affecting absorption. Teased out the effect of antacids only on F1. Not significant.
55	2	ADD+CCV	$\theta2\exp(\eta1)$	CL(1)/F=$\theta6\exp(\eta2)$ CL(2)/F=$\theta6^*\theta7\exp(\eta4)$	$(\theta3^*(1-ABS)$ $+\theta8^*ABS)\exp(\eta3)$	-	146	43/38	43	29	0.02	INH5.CSV	289.322	Drug interactions. Drugs affecting absorption on V. Not significant.
56	2	ADD+CCV	$\theta2\exp(\eta1)$	CL(1)/F=$(\theta6^*(1-NSID)$ $+\theta8^*NSID)\exp(\eta2)$ CL(2)/F=$(\theta6^*(1-NSID)$ $+\theta8^*NSID)^*\theta7\exp(\eta4)$	$\theta3\exp(\eta3)$	-	145	43/37	43	29	0.02	INH5.CSV	287.404	Drug interactions. NSAID's on CL/F. Not significant.
57	2	ADD+CCV	$\theta2\exp(\eta1)$	CL(1)/F=$\theta6\exp(\eta2)$ CL(2)/F=$\theta6^*\theta7\exp(\eta4)$	$(\theta3^*(1-NSID)$ $+\theta8^*NSID)$ $\exp(\eta3)$	-	145	43/38	43	29	0.02	INH5.CSV	286.970	Drug interactions. NSAID's on V. Not significant.
58	2	ADD+CCV	$\theta2\exp(\eta1)$	CL(1)/F=$(\theta6^*(1-FEFL)$ $+\theta8^*FEFL)\exp(\eta2)$ CL(2)/F=$(\theta6^*(1-FEFL) +$ $\theta8^*FEFL)^*\theta7)\exp(\eta4)$	$\theta3\exp(\eta3)$	-	145	43/38	43	29	0.02	INH5.CSV	288.818	Drug interactions. Iron Preps/Anaemia on CL/F. Not significant
59	2	ADD+CCV	$\theta2\exp(\eta1)$	CL(1)/F=$\theta6\exp(\eta2)$ CL(2)/F=$\theta6^*\theta7\exp(\eta4)$	$(\theta3^*(1-FEFL)+$ $\theta8^*FEFL)\exp(\eta3)$	-	146	43/38	43	29	0.02	INH5.CSV	287.807	Drug interactions. Iron Preps/Anaemia on V. Not significant.
60	2	ADD+CCV	$\theta2\exp(\eta1)$	CL(1)/F=$(\theta6^*(1-H1B)$ $+\theta8^*H1B)\exp(\eta2)$ CL(2)/F=$\theta6^*(1-H1B)$ $+\theta8^*H1B)^*\theta7\exp(\eta4)$	$\theta3\exp(\eta3)$	-	148	43/39	43	29	0.02	INH5.CSV	281.260	Drug interactions. Antihistamines on CL/F. Not significant.
61	2	ADD+CCV	$\theta2\exp(\eta1)$	CL(1)/F=$(\theta6\exp(\eta2)$ CL(2)/F=$\theta6^*\theta7\exp(\eta4)$	$(\theta3^*(1-H1B)$ $+\theta8^*H1B)\exp(\eta3)$	-	144	43/38	43	29	0.02	INH5.CSV	289.132	Drug interactions. Antihistamines on V. Not significant.
62	2	ADD+CCV	$\theta2\exp(\eta1)$	CL(1)/F=$(\theta6^*(1-PEN)$ $+\theta8^*PEN)\exp(\eta2)$ CL(2)/F=$(\theta6^*(1-PEN)$ $+\theta8^*PEN)^*\theta7\exp(\eta4)$	$\theta3\exp(\eta3)$	-	145	43/38	43	29	0.02	INH5.CSV	283.709	Drug interactions. Penicillin on CL/F Significant.
63	2	ADD+CCV	$\theta2\exp(\eta1)$	CL(1)/F=$\theta6\exp(\eta2)$ CL(2)/F=$\theta6^*\theta7\exp(\eta4)$	$\theta3^*(1-$ $PEN)+\theta8^*PEN)\exp(\eta3)$	-	138	43/38	43	29	0.02	INH5.CSV	282.263	Drug interactions. Penicillin on V. Significant.
64	2	ADD+CCV	$\theta2\exp(\eta1)$	CL(1)/F=$(\theta6^*(1-BS)+\theta8^*BS)\exp(\eta2)$ CL(2)/F=$(\theta6^*(1-BS)$ $+\theta8^*BS)^*\theta7\exp(\eta4)$	$\theta3\exp(\eta3)$	-	139	43/38	43	29	0.02	INH5.CSV	282.644	Drug interactions. Hypoglycaemic agents/diabetes on CL/F. Significant but ? reason.

η - Inter-individual variability – where 2 values are recorded they are the estimates for the first and second populations; KA – absorption rate constant; CL/F – apparent clearance; CL(1)/F– apparent clearance (fast); CL(2)/F – apparent clearance for slow acetylators; V/F – apparent volume of distribution; TLAG – absorption lag time; σ - intra-individual variability; CCV – constant coefficient of variation; ADD – additive; OBF – minimum value of the Objective function.

Appendix J

NONMEM RUN SUMMARY - ISONIAZID

RUN	ADVAN	ERROR	KA	CL/F	VD	TLAG	η_{KA} %	$\eta_{CL/F}$ %	η_V %	σ_{CCV} %	σ_{ADD} µg/ml	DATA	OBF	COMMENTS
65	2	ADD+CCV	$\theta2\exp(\eta1)$	$CL(1)/F=\theta6\exp(\eta2)$ $CL(2)/F=\theta6^*\theta7\exp(\eta4)$	$(\theta3^*(1-BS)$ $+\theta8^*BS) \exp(\eta3)$	-	145	43/38	43	29	0.02	INH5.CSV	289.284	Drug interactions. Hypoglycaemic agents/diabetes on V. Not significant.
66	2	ADD+CCV	$\theta2\exp(\eta1)$	$CL(1)/F=(\theta6^*(1-DIUR)$ $+\theta8^*DIUR)\exp(\eta2)$ $CL(2)/F=(\theta6^*(1-DIUR)$ $+\theta8^*DIUR)^*\theta7 \exp(\eta4)$	$\theta3\exp(\eta3)$	-	149	42/38	43	29	0.02	INH5.CSV	284.603	Drug interactions. Diuretics/hypertension on CL/F. Significant but ? reason.
67	2	ADD+CCV	$\theta2\exp(\eta1)$	$CL(1)/F=\theta6\exp(\eta2)$ $CL(2)/F=\theta6^*\theta7\exp(\eta4)$	$(\theta3^*(1-DIUR)+$ $\theta8^*DIUR)\exp(\eta3)$	-	145	43/38	43	29	0.02	INH5.CSV	286.636	Drug interactions. Diuretics/hypertension on V. Not significant.
68	2	ADD+CCV	$\theta2\exp(\eta1)$	$CL(1)/F=(\theta6^*(1-INAT)$ $+\theta8^*INAT)\exp(\eta2)$ $CL(2)/F=(\theta6^*(1-INAT)+$ $\theta8^*INAT)^*\theta7\exp(\eta4)$	$\theta3\exp(\eta3)$	-	148	43/38	43	29	0.02	INH5.CSV	289.037	Drug interactions. INAT on CL/F. Not significant.
69	2	ADD+CCV	$\theta2\exp(\eta1)$	$CL(1)/F=\theta6\exp(\eta2)$ $CL(2)/F=\theta6^*\theta7\exp(\eta4)$	$(\theta3^*(1-INAT)+$ $\theta8^*INAT)\exp(\eta3)$	-	145	43/38	43	29	0.02	INH5.CSV	289.607	Drug interactions. INAT on V. Not significant.
70	2	ADD+CCV	$\theta2\exp(\eta1)$	$CL(1)/F=(\theta6^*(1-QUIN)$ $+\theta8^*QUIN)\exp(\eta2)$ $CL(2)/F=(\theta6^*(1-QUIN)+$ $\theta8^*QUIN)^*\theta7 \exp(\eta4)$	$\theta3\exp(\eta3)$	-	145	43/39	43	29	0.02	INH5.CSV	287.926	Drug interactions. Quinolones on CL/F. Not significant.
71	2	ADD+CCV	$\theta2\exp(\eta1)$	$CL(1)/F=\theta6\exp(\eta2)$ $CL(2)/F=\theta6^*\theta7\exp(\eta4)$	$(\theta3^*(1-QUIN)$ $+\theta8^*QUIN)$ $\exp(\eta3)$	-	145	43/38	43	29	0.02	INH5.CSV	289.334	Drug interactions. Quinolones on V. Not significant.
72	2	ADD+CCV	$\theta2\exp(\eta1)$	$CL(1)/F=(\theta6^*(1-INHI)$ $+\theta8^*INHI)\exp(\eta2)$ $CL(2)/F=(\theta6^*(1-INHI)$ $+\theta8^*INHI)^*\theta7\exp(\eta4)$	$\theta3\exp(\eta3)$	-	143	43/37	43	29	0.02	INH5.CSV	279.555	Drug interactions. Enzyme inhibitors on CL/F. Significant.
73	2	ADD+CCV	$\theta2\exp(\eta1)$	$CL(1)/F=\theta6\exp(\eta2)$ $CL(2)/F=\theta6^*\theta7\exp(\eta4)$	$(\theta3^*(1-INHI)$ $+\theta8^*INHI)\exp(\eta3)$	-	146	43/38	43	29	0.02	INH5.CSV	289.640	Drug interactions. Enzyme inhibitors on V. Not significant.
74	2	ADD+CCV	$\theta2\exp(\eta1)$	$CL(1)/F=(\theta6^*(1-INHIQ)$ $+\theta8^*INHIQ)\exp(\eta2)$ $CL(2)/F=(\theta6(\theta6^*(1-INHIQ)$ $+\theta8^*INHIQ)^*\theta7\exp(\eta4)$	$\theta3\exp(\eta3)$	-	146	43/38	43	29	0.02	INH5.CSV	288.739	Drug interactions. Enzyme inhibitors + quinolones on CL/F. Not significant.
75	2	ADD+CCV	$\theta2\exp(\eta1)$	$CL(1)/F=\theta6\exp(\eta2)$ $CL(2)/F=\theta6^*\theta7\exp(\eta4)$	$(\theta3(\theta6^*(1-INHIQ)$ $+\theta8^*INHIQ)$ $\exp(\eta3)$	-	145	43/38	43	29	0.02	INH5.CSV	289.639	Drug interactions. Enzyme inhibitors + quinolones on V. Not significant.

η - inter-individual variability – where 2 values are recorded they are the estimates for the first and second populations; KA – absorption rate constant; CL/F – apparent clearance; CL(1)/F– apparent clearance (fast); CL(2)/F – apparent clearance for slow acetylators; V/F – apparent volume of distribution; TLAG – absorption lag time; σ - intra-individual variability; CCV – constant coefficient of variation; ADD – additive; OBF – minimum value of the Objective function.

Appendix J

NONMEM RUN SUMMARY - ISONIAZID

RUN	ADVAN	ERROR	KA	CL/F	VD	TLAG	η_{KA} %	$\eta_{CL/F}$ %	η_V %	σ_{CCV} %	σ_{ADD} µg/ml	DATA	OBF	COMMENTS
76	2	ADD+CCV	$\theta2\exp(\eta1)$	CL(1)/F=($\theta6^*$(1-EMB) +$\theta8^*$EMB)exp($\eta2$) CL(2)/F=($\theta6$(1-EMB)+ $\theta8^*$EMB)$^*\theta7\exp(\eta4)$	$\theta3\exp(\eta3)$	-	146	43/38	43	29	0.02	INH5.CSV	289.515	Drug interactions. Ethambutol on CL/F. Not significant.
77	2	ADD+CCV	$\theta2\exp(\eta1)$	CL(1)/F=$\theta6\exp(\eta2)$ CL(2)/F=$\theta6^*\theta7\exp(\eta4)$	($\theta3$(1-EMB) +$\theta8^*$EMB)exp($\eta3$)	-	146	43/38	43	29	0.02	INH5.CSV	289.478	Drug interactions. Ethambutol on V. Not significant.
78	2	ADD+CCV	$\theta2\exp(\eta1)$	CL(1)/F=($\theta6^*$(1-CAND) +$\theta8^*$CAND)exp($\eta2$) CL(2)/F=($\theta6^*$(1-CAND)+ $\theta8^*$CAND)$^*\theta7\exp(\eta4)$	$\theta3\exp(\eta3)$	-	146	43/38	43	29	0.02	INH5.CSV	289.351	Disease interactions. Candidiasis on CL/F. Not significant.
79	2	ADD+CCV	$\theta2\exp(\eta1)$	CL(1)/F=$\theta6\exp(\eta2)$ CL(2)/F=$\theta6^*\theta7\exp(\eta4)$	$\theta3^*$(1-CAND)+ $\theta8^*$CAND)exp($\eta3$)	-	145	43/38	43	29	0.02	INH5.CSV	289.551	Disease interactions. Candidiasis on V. Not significant.
80	2	ADD+CCV	$\theta2\exp(\eta1)$	CL(1)/F=($\theta6+\theta8^*$(NMDR-7)) exp($\eta2$) CL(2)/F=($\theta6+\theta8^*$(NMDR-7)) $^*\theta7\exp(\eta4)$	$\theta3\exp(\eta3)$	-	155	43/38	43	29	0.02	INH5.CSV	283.657	Effect of No. of drugs resistant to on CL/F. Significant.
81	2	ADD+CCV	$\theta2\exp(\eta1)$	CL(1)/F=$\theta6\exp(\eta2)$ CL(2)/F=$\theta6^*\theta7\exp(\eta4)$	($\theta3+ \theta8^*$(NMDR-7)) exp($\eta3$)	-	147	43/38	43	29	0.02	INH5.CSV	288.953	Effect of no. of drugs resistant to on V. Not significant.
82	2	ADD+CCV	$\theta2\exp(\eta1)$	CL(1)/F=($\theta6+\theta8^*$(EXT-5)) exp($\eta2$) CL(2)/F=($\theta6+\theta8^*$(EXT-5))$^*\theta7$ exp($\eta4$)	$\theta3\exp(\eta3)$	-	145	43/37	43	29	0.02	INH5.CSV	282.737	Extent of X-ray severity on CL/F. Significant but ? reason.
83	2	ADD+CCV	$\theta2\exp(\eta1)$	CL(1)/F=$\theta6\exp(\eta2)$ CL(2)/F=$\theta6^*\theta7\exp(\eta4)$	($\theta3+\theta8^*$(EXT-5)) exp($\eta3$)	-	145	43/37	42	29	0.02	INH5.CSV	282.778	Extent of X-ray severity on V. Significant but ? reason.
84	2	ADD+CCV	$\theta2\exp(\eta1)$	CL(1)/F=($\theta6+\theta8^*$(CAV-4))exp($\eta2$) CL(2)/F=($\theta6+\theta8^*$(CAV-4)) $^*\theta7\exp(\eta4)$	$\theta3\exp(\eta3)$	-	146	43/38	43	29	0.02	INH5.CSV	288.020	Severity of cavitation on CL/F. Not significant.
85	2	ADD+CCV	$\theta2\exp(\eta1)$	CL(1)/F=$\theta6\exp(\eta2)$ CL(2)/F=$\theta6^*\theta7\exp(\eta4)$	($\theta3+\theta8^*$(CAV-4))exp($\eta3$)	-	145	43/38	43	29	0.02	INH5.CSV	285.697	Severity of cavitation on V. Marginally significant.
86	2	ADD+CCV	$\theta2\exp(\eta1)$	CL(1)/F=($\theta6+\theta8^*$(WT-54))exp($\eta2$) CL(2)/F=($\theta6+\theta8^*$(WT-54))$^*\theta7$ exp($\eta4$)	$\theta3\exp(\eta3)$	-	139	43/37	42	29	0.02	INH5.CSV	286.765	WT on CL/F. Not significant.
87	2	ADD+CCV	$\theta2\exp(\eta1)$	CL(1)/F=($\theta6^*$(1-MDR)+$\theta8^*$MDR) exp($\eta2$) CL(2)/F=($\theta6^*$(1-MDR)+$\theta8^*$MDR) $^*\theta7\exp(\eta4)$	$\theta3\exp(\eta3)$	-	145	43/38	43	29	0.02	INH5.CSV	289.172	MDR on CL/F. Not significant
88	2	ADD+CCV	$\theta2\exp(\eta1)$	CL(1)/F=$\theta6\exp(\eta2)$ CL(2)/F=$\theta6^*\theta7\exp(\eta4)$	($\theta3^*$(1-SEX) +$\theta8^*$SEX)exp($\eta3$)	-	153	41/35	43	27	0.05	INH5.CSV	288.580	Sex on V. Not significant

η - inter-individual variability – where 2 values are recorded they are the estimates for the first and second populations; KA – absorption rate constant; CL/F – apparent clearance; CL(1)/F– apparent clearance (fast); CL(2)/F – apparent clearance for slow acetylators; V/F – apparent volume of distribution; TLAG – absorption lag time; σ - intra-individual variability; CCV – constant coefficient of variation; ADD – additive; OBF – minimum value of the Objective function.

Appendix J

NONMEM RUN SUMMARY - ISONIAZID

RUN	ADVAN	ERROR	KA	CL/F	VD	TLAG	η_{KA} %	$\eta_{CL/F}$ %	η_V %	σ_{CCV} %	σ_{ADD} μg/ml	DATA	OBF	COMMENTS
89	2	ADD+CCV	$\theta2\exp(\eta1)$	CL(1)/F=$\theta6\exp(\eta2)$ CL(2)/F=$\theta6*\theta7\exp(\eta4)$	$\theta3*(1-MDR)$ $+\theta8*MDR)\exp(\eta3)$	-	147	43/38	43	29	0.02	INH5.CSV	289.348	MDR on V. Not significant
90	2	ADD+CCV	$\theta2\exp(\eta1)$	CL(1)/F=$(\theta6*(1-SEX)$ $+\theta8*SEX)\exp(\eta2)$ CL(2)/F=$(\theta6*(1-SEX)$ $+\theta8*SEX)*\theta7\exp(\eta4)$	$\theta3*\exp(\eta3)$	-	147	42/38	43	29	0.02	INH5.CSV	285.301	Sex on CL/F. Significant
91	2	ADD+CCV	$\theta2\exp(\eta1)$	CL(1)/F=$(\theta6*(1-HIV)+$ $\theta8*HIV)\exp(\eta2)$ CL(2)/F=$(\theta6*(1-HIV)+$ $\theta8*HIV)*\theta7\exp(\eta4)$	$\theta3*\exp(\eta3)$	-	148	43/37	43	29	0.02	INH5.CSV	284.489	HIV on CL/F. Not significant
92	2	ADD+CCV	$\theta2\exp(\eta1)$	CL(1)/F=$\theta6\exp(\eta2)$ CL(2)/F=$\theta6*\theta7\exp(\eta4)$	$(\theta3*(1-HIV)+$ $\theta8*HIV)\exp(\eta3)$	-	147	43/38	43	29	0.02	INH5.CSV	287.798	HIV on V. Not significant
93	2	ADD+CCV	$\theta2\exp(\eta1)$	CL(1)/F=$\theta6\exp(\eta2)$ CL(2)/F=$\theta6*\theta7\exp(\eta4)$	$\theta3\exp(\eta3)$	-	154	42/33	41	28	0.02	INH9.CSV	204.112	Data File has the 7 outlier values removed i.e. those values that were high prior to dosing.
94	2	ADD+CCV	$\theta2\exp(\eta1)$	$\theta1\exp(\eta1)$	$\theta2\exp(\eta2)$	$\theta3\exp(\eta3)$	77	46	36	18	0.5	INH9.CSV	877.033	REPEATED run with no mixture modelling with new data file.
95	2	ADD+CCV	$\theta2\exp(\eta1)$	CL(1)/F=$(\theta6-\theta8*(GLOB-52))$ $\exp(\eta2)$ CL(2)/F=$(\theta6-\theta8*(GLOB-52))*\theta7$ $\exp(\eta4)$	$\theta3\exp(\eta3)$	-	151	41/35	41	27	0.02	INH9.CSV	192.538	Globulin on CL/F. OBF ↓ 11.6
96	2	ADD+CCV	$\theta2\exp(\eta1)$	CL(1)/F=$(\theta6-\theta8*(GLOB-52)$ $+\theta9*ETH)\exp(\eta2)$ CL(2)/F=$(\theta6-\theta8*(GLOB-52)$ $+\theta9*ETH)*\theta7\exp(\eta4)$	$\theta3\exp(\eta3)$	-	148	41/35	41	28	0.02	INH9.CSV	189.342	Globulin & Ethionamide on CL/F. OBF ↓ 3.2
97	2	ADD+CCV	$\theta2\exp(\eta1)$	CL(1)/F=$(\theta6-\theta8*(GLOB-52)$ $+\theta9*H1RB)\exp(\eta2)$ CL(2)/F=$(\theta6-\theta8*(GLOB-52)$ $+\theta9*H1RB)*\theta7\exp(\eta4)$	$\theta3\exp(\eta3)$	-	157	41/34	41	28	0.02	INH9.CSV	188.108	Globulin and antihistamines on CL/F. OBF ↓ 4.43
98	2	ADD+CCV	$\theta2\exp(\eta1)$	CL(1)/F=$(\theta6-\theta8*(GLOB-52)$ $+\theta9*H1RB-\theta10*(VIR-5))\exp(\eta2)$ CL(2)/F=$(\theta6-\theta8*(GLOB-52)$ $+\theta9*H1RB-\theta10*(VIR-5))*\theta7\exp(\eta4)$	$\theta3\exp(\eta3)$	-	160	40/35	41	27	0.02	INH9.CSV	188.328	Globulin, antihistamines and log viral load on CL/F. OBF - no change.
99	2	ADD+CCV	$\theta2\exp(\eta1)$	CL(1)/F=$(\theta6-\theta8*(GLOB-52)$ $+\theta9*H1RB)\exp(\eta2)$ CL(2)/F=$(\theta6-\theta8*(GLOB-52)$ $+\theta9*H1RB)*\theta7\exp(\eta4)$	$\theta3*(1-PEN)$ $+\theta10*PEN)$ $\exp(\eta3)$	-	153	40/34	41	28	0.02	INH9.CSV	182.459	Globulin and antihistamines on CL/F. Penicillin on V. OBF ↓ 5.7

η - inter-individual variability – where 2 values are recorded they are the estimates for the first and second populations; KA – absorption rate constant; CL/F – apparent clearance; CL(1)/F– apparent clearance (fast); CL(2)/F– apparent clearance for slow acetylators; V/F – apparent volume of distribution; TLAG – absorption lag time; σ - intra-individual variability; CCV – constant coefficient of variation; ADD – additive; OBF – minimum value of the Objective function.

Appendix J

NONMEM RUN SUMMARY - ISONIAZID

RUN	ADVAN	ERROR	KA	CL/F	VD	TLAG	η_{KA} %	$\eta_{CL/F}$ %	η_V %	σ_{CCV} %	σ_{ADD} μg/ml	DATA	OBF	COMMENTS
100	2	ADD+CCV	θ2exp(η1)	CL(1)/F=(θ6-θ8*(GLOB-52) +θ9*H1RB +θ11*BS) exp(η2) CL(2)/F=(θ6-θ8*(GLOB-52) +θ9*H1RB+θ11*BS)*θ7exp(η4)	θ3*(1-PEN) +θ10*PEN) exp(η3)	-	158	40/32	41	28	0.02	INH9.CSV	181.339	Globulin, antihistamines and hypoglycaemics / diabetes on CL/F. Penicillin on V. OBF ↓ 1
101	2	ADD+CCV	θ2exp(η1)	CL(1)/F=(θ6-θ8*(GLOB-52) +θ9*H1RB+θ11*EXT-5)) exp(η2) CL(2)/F=(θ6-θ8*(GLOB-52) +θ9*H1RB+θ11*EXT-5))*θ7exp(η4)	θ3*(1-PEN) +θ10*PEN) exp(η3)	-	156	40/32	41	28	0.02	INH9.CSV	175.729	Globulin , antihistamines and extent of X-ray involvement on CL/F. Penicillin on V. OBF ↓ 5.7
102	2	ADD+CCV	θ2exp(η1)	CL(1)/F=(θ6-θ8*(GLOB-52) +θ9*H1RB+θ11*EXT-5)) exp(η2) CL(2)/F=(θ6-θ8*(GLOB-52) +θ9*H1RB+θ11*EXT-5))*θ7exp(η4)	(θ3+θ10*PEN+θ12 *(EXT-5))exp(η3)	-	157	40/32	40	28	0.02	INH9.CSV	174.837	Globulin , antihistamines and extent of X-ray involvement on CL/F. Penicillin and extent of x-ray involvement on V. OBF ↓ 1
103	2	ADD+CCV	θ2exp(η1)	CL(1)/F=(θ6-θ8*(GLOB-52) +θ9*H1RB+θ11*EXT-5) +θ12*(NMDR-7)) exp(η2) CL(2)/F=(θ6-θ8*(GLOB-52) +θ9*H1RB+θ11*EXT-5) +θ12*(NMDR-7))*θ7exp(η4)	θ3*(1-PEN) +θ10*PEN) exp(η3)	-	156	40/32	41	28	0.02	INH9.CSV	175.514	Globulin, antihistamines, extent of X-ray involvement and "No. of drugs resistant to" on CL/F. Penicillin on V. OBF no change.
104	2	ADD+CCV	θ2exp(η1)	CL(1)/F=(θ6-θ8*(GLOB-52) +θ9*H1RB+θ11*EXT-5))+θ12*PEN exp(η2) CL(2)/F=(θ6-θ8*(GLOB-52) +θ9*H1RB+θ11*EXT-5) +θ12*PEN)*θ7exp(η4)	θ3*(1-PEN) +θ10*PEN) exp(η3)	-	157	40/32	40	28	0.02	INH9.CSV	175.716	Globulin, antihistamines, extent of X-ray involvement and penicillin on CL/F. Penicillins on V. OBF no change.
105	2	ADD+CCV	θ2exp(η1)	CL(1)/F=(θ6-θ8*(GLOB-52) +θ9*H1RB+θ11*EXT-5)) exp(η2) CL(2)/F=(θ6-θ8*(GLOB-52) +θ9*H1RB+θ11*EXT-5))*θ7exp(η4)	(θ3*+θ10*PEN+θ1 2*(WT-54)) exp(η3)	-	165	39/33	39	26	0.03	INH9.CSV	176.436	Globulin, antihistamines and extent of X-ray involvement on CL/F. Penicillin and weight on V. OBF ↑
106	2	ADD+CCV	θ2exp(η1)	CL(1)/F=(θ6-θ8*(GLOB-52) +θ9*H1RB+θ11*EXT-5)) exp(η2) CL(2)/F=(θ6-θ8*(GLOB-52) +θ9*H1RB+θ11*EXT-5))*θ7exp(η4)	(θ3*+θ10*PEN+θ1 2*(GLOB- 52))exp(η3)	-	156	40/32	40	28	0.02	INH9.CSV	175.414	Globulin , antihistamines and extent of X-ray involvement on CL/F. Penicillin and globulin on V. OBF no change

η - inter-individual variability – where 2 values are recorded they are the estimates for the first and second populations; KA – absorption rate constant; CL/F – apparent clearance; CL(1)/F– apparent clearance (fast); CL(2)/F – apparent clearance for slow acetylators; V/F – apparent volume of distribution; TLAG – absorption lag time; σ - intra-individual variability; CCV – constant coefficient of variation; ADD – additive; OBF – minimum value of the Objective function.

Appendix J

NONMEM RUN SUMMARY - ISONIAZID

RUN	ADVAN	ERROR	KA	CL/F	VD	TLAG	η_{KA} %	$\eta_{CL/F}$ %	η_V %	σ_{CCV} %	σ_{ADD} µg/ml	DATA	OBF	COMMENTS
107	2	ADD+CCV	$\theta2\exp(\eta1)$	CL(1)/F=($\theta6$-$\theta8$*(GLOB-52)+$\theta9$*H1RB+$\theta11$*EXT-5)+$\theta12$*HPT) exp($\eta2$) CL(2)/F=($\theta6$-$\theta8$*(GLOB-52)+$\theta9$*H1RB+$\theta11$*EXT-5)+$\theta12$*HPT)*$\theta7$exp($\eta4$)	($\theta3$*(1-PEN)+$\theta10$*PEN) exp($\eta3$)	-	159	40/32	40	28	0.02	INH9.CSV	168.516	Globulin, antihistamines, extent of X-ray involvement and anti-hypertensives on CL/F. Penicillin on V. OBF ↓ 7.2
108	2	ADD+CCV	$\theta2\exp(\eta1)$	CL(1)/F=($\theta6$-$\theta8$*(GLOB-52)+$\theta9$*H1RB+$\theta11$*EXT-5)+$\theta12$*HPT) exp($\eta2$) CL(2)/F=($\theta6$-$\theta8$*(GLOB-52)+$\theta9$*H1RB+$\theta11$*EXT-5)+$\theta12$*HPT)*$\theta7$exp($\eta4$)	($\theta3$+$\theta10$*PEN-$\theta13$*(BILI-10)) exp($\eta3$)	-	164	39/32	40	28	0.02	INH9.CSV	163.445	Globulin, antihistamines, antihypertensivess and extent of X-ray involvement on CL/F. Penicillin and bilirubin on V. OBF ↓ 5. Decision after RUNs 109 and 110 is that this is the FULL MODEL.
109	2	ADD+CCV	$\theta2\exp(\eta1)$	CL(1)/F=($\theta6$-$\theta8$*(GLOB-52)+$\theta9$*H1RB+$\theta11$*EXT-5)+$\theta12$*HPT+$\theta14$*SEX) exp($\eta2$) CL(2)/F=($\theta6$-$\theta8$*(GLOB-52) + $\theta9$*H1RB+$\theta11$*EXT-5)+$\theta12$*HPT+$\theta14$*SEX)*$\theta7$exp($\eta4$)	($\theta3$+$\theta10$*PEN-$\theta13$*(BILI-10)) exp($\eta3$)	-	164	40/22	42	27	0.02	INH9.CSV	173.562	Globulin, antihistamines, extent of X-ray involvement, anti-hypertensives and sex on CL/F. Penicillin, bilirubin on V. OBF ↑
110	2	ADD+CCV	$\theta2\exp(\eta1)$	CL(1)/F=($\theta6$-$\theta8$*(GLOB-52)+$\theta9$*H1RB+$\theta11$*EXT-5)+$\theta12$*HPT+$\theta14$*CAV-4)) exp($\eta2$) CL(2)/F=($\theta6$-$\theta8$*(GLOB-52)+$\theta9$*H1RB+$\theta11$*EXT-5)+$\theta12$*HPT+$\theta14$*(CAV-4))*$\theta7$exp($\eta4$)	($\theta3$+$\theta10$*PEN-$\theta13$*(BILI-10)) exp($\eta3$)	-	162	40/19	43	27	0.02	INH9.CSV	174.421	Globulin, antihistamines, anti-hypertensives and extent of X-ray involvement on CL/F. Penicillin, bilirubin, and lung cavitation on V. OBF ↑
111	2	ADD+CCV	$\theta2\exp(\eta1)$	CL(1)/F=($\theta6$-$\theta8$*(GLOB-52)+$\theta9$*H1RB+$\theta11$*EXT-5)+$\theta12$*HPT) exp($\eta2$) CL(2)/F=($\theta6$-$\theta8$*(GLOB-52)+$\theta9$*H1RB+$\theta11$*EXT-5)+$\theta12$*HPT)*$\theta7$exp($\eta4$)	($\theta3$+$\theta10$*PEN-$\theta13$*(BILI-10)) exp($\eta3$)	-	162	40/32	40	28	0.02	INH9.CSV	167.613	Establishing Rank Order for Backward Deletion: Globulin (FIXED to 0), antihistamines, antihypertensives and extent of X-ray involvement on CL/F. Penicillin and bilirubin on V. OBF ↑ 4.2

η - inter-individual variability – where 2 values are recorded they are the estimates for the first and second populations; KA – absorption rate constant; CL/F – apparent clearance; CL(1)/F– apparent clearance (fast); CL(2)/F – apparent clearance for slow acetylators; V/F – apparent volume of distribution; TLAG – absorption lag time; σ - intra-individual variability; CCV – constant coefficient of variation; ADD – additive; OBF – minimum value of the Objective function.

Appendix J

NONMEM RUN SUMMARY - ISONIAZID

RUN	ADVAN	ERROR	KA	CL/F	VD	TLAG	η_{KA} %	$\eta_{CL/F}$ %	η_V %	σ_{CCV} %	σ_{ADD} µg/ml	DATA	OBF	COMMENTS
112	2	ADD+CCV	$\theta 2\exp(\eta 1)$	CL(1)/F=($\theta 6-\theta 8$*(GLOB-52)+$\theta 9$*H1RB+$\theta 11$*EXT-5)+$\theta 12$*HPT)exp($\eta 2$) CL(2)/F=($\theta 6-\theta 8$*(GLOB-52)+$\theta 9$*H1RB+$\theta 11$*EXT-5)+$\theta 12$*HPT)*$\theta 7\exp(\eta 4)$	($\theta 3+\theta 10$*PEN-$\theta 13$*(BILI-10))exp($\eta 3$)	-	160	39/32	40	28	0.02	INH9.CSV	164.945	Establishing Rank Order for Backward Deletion: Globulin , antihistamines (FIXED to 0), antihypertensives and extent of X-ray involvement on CL/F. Penicillin and bilirubin on V. OBF ↑ 1.5
113	2	ADD+CCV	$\theta 2\exp(\eta 1)$	CL(1)/F=($\theta 6-\theta 8$*(GLOB-52)+$\theta 9$*H1RB+$\theta 11$*EXT-5)+$\theta 12$*HPT)exp($\eta 2$) CL(2)/F=($\theta 6-\theta 8$*(GLOB-52)+$\theta 9$*H1RB+$\theta 11$*EXT-5)+$\theta 12$*HPT)*$\theta 7\exp(\eta 4)$	($\theta 3+\theta 10$*PEN-$\theta 13$*(BILI-10))exp($\eta 3$)	-	165	39/32	40	28	0.02	INH9.CSV	167.334	Establishing Rank Order for Backward Deletion: Globulin , antihistamines, antihypertensives and extent of X-ray involvement (FIXED to 0) on CL/F. Penicillin and bilirubin on V. OBF ↑ 3.88
114	2	ADD+CCV	$\theta 2\exp(\eta 1)$	CL(1)/F=($\theta 6-\theta 8$*(GLOB-52)+$\theta 9$*H1RB+$\theta 11$*EXT-5)+$\theta 12$*HPT)exp($\eta 2$) CL(2)/F=($\theta 6-\theta 8$*(GLOB-52)+$\theta 9$*H1RB+$\theta 11$*EXT-5)+$\theta 12$*HPT)*$\theta 7\exp(\eta 4)$	($\theta 3+\theta 10$*PEN-$\theta 13$*(BILI-10))exp($\eta 3$)	-	163	40/32	40	27	0.02	INH9.CSV	172.042	Establishing Rank Order for Backward Deletion: Globulin , antihistamines, antihypertensives (FIXED to 0) and extent of X-ray involvement on CL/F. Penicillin and bilirubin on V. OBF ↑ 8.6
115	2	ADD+CCV	$\theta 2\exp(\eta 1)$	CL(1)/F=($\theta 6-\theta 8$*(GLOB-52)+$\theta 9$*H1RB+$\theta 11$*EXT-5)+$\theta 12$*HPT)exp($\eta 2$) CL(2)/F=($\theta 6-\theta 8$*(GLOB-52)+$\theta 9$*H1RB+$\theta 11$*EXT-5)+$\theta 12$*HPT)*$\theta 7\exp(\eta 4)$	($\theta 3+\theta 10$*PEN-$\theta 13$*(BILI-10))exp($\eta 3$)	-	168	39/32	40	27	0.02	INH9.CSV	172.203	Establishing Rank Order for Backward Deletion: Globulin , antihistamines, antihypertensives and extent of X-ray involvement on CL/F. Penicillin (FIXED to 0) and bilirubin on V. OBF ↑ 8.8
116	2	ADD+CCV	$\theta 2\exp(\eta 1)$	CL(1)/F=($\theta 6-\theta 8$*(GLOB-52)+$\theta 9$*H1RB+$\theta 11$*EXT-5)+$\theta 12$*HPT)exp($\eta 2$) CL(2)/F=($\theta 6-\theta 8$*(GLOB-52)+$\theta 9$*H1RB+$\theta 11$*EXT-5)+$\theta 12$*HPT)*$\theta 7\exp(\eta 4)$	($\theta 3+\theta 10$*PEN-$\theta 13$*(BILI-10))exp($\eta 3$)	-	159	40/32	40	28	0.02	INH9.CSV	168.516	Establishing Rank Order for Backward Deletion: Globulin , antihistamines, antihypertensives and extent of X-ray involvement on CL/F. Penicillin and bilirubin (FIXED to 0) on V. OBF ↑ 5
117	2	ADD+CCV	$\theta 2\exp(\eta 1)$	CL(1)/F=($\theta 6-\theta 8$*(GLOB-52)+$\theta 9$*H1RB+$\theta 11$*EXT-5)+$\theta 12$*HPT)exp($\eta 2$) CL(2)/F=($\theta 6-\theta 8$*(GLOB-52)+$\theta 9$*H1RB+$\theta 11$*EXT-5)+$\theta 12$*HPT)*$\theta 7\exp(\eta 4)$	($\theta 3+\theta 10$*PEN-$\theta 13$*(BILI-10))exp($\eta 3$)	-	160	39/32	40	28	0.02	INH9.CSV	164.945	Backward Deletion: Antihistamines on CL/F fixed to 0. OBF ↑ 1.5. Hence exclude.

η - Inter-individual variability – where 2 values are recorded they are the estimates for the first and second populations; KA – absorption rate constant; CL/F – apparent clearance; CL(1)/F– apparent clearance (fast); CL(2)/F – apparent clearance for slow acetylators; V/F – apparent volume of distribution; TLAG – absorption lag time; σ - intra-individual variability; CCV – constant coefficient of variation; ADD – additive; OBF – minimum value of the Objective function.

Appendix J

NONMEM RUN SUMMARY - ISONIAZID

RUN	ADVAN	ERROR	KA	CL/F	VD	TLAG	η_{KA} %	$\eta_{CL/F}$ %	η_V %	σ_{CCV} %	σ_{ADD} μg/ml	DATA	OBF	COMMENTS
118	2	ADD+CCV	$\theta 2\exp(\eta 1)$	CL(1)/F=($\theta 6$-$\theta 8$*(GLOB-52) +$\theta 9$*H1RB+$\theta 11$*EXT-5)+$\theta 12$*HPT) exp($\eta 2$) CL(2)/F=($\theta 6$-$\theta 8$*(GLOB-52) +$\theta 9$*H1RB+$\theta 11$*EXT-5) +$\theta 12$*HPT)*$\theta 7$exp($\eta 4$)	($\theta 3$+$\theta 10$*PEN-$\theta 13$*(BILI-10)) exp($\eta 3$)	-	162	39/32	40	28	0.02	INH9.CSV	168.238	Backward Deletion: Antihistamines and extent of X-ray involvement on CL/F fixed to 0. OBF ↑ 3.3. Hence exclude.
119	2	ADD+CCV	$\theta 2\exp(\eta 1)$	CL(1)/F=($\theta 6$-$\theta 8$*(GLOB-52) +$\theta 9$*H1RB+$\theta 11$*EXT-5)+$\theta 12$*HPT) exp($\eta 2$) CL(2)/F=($\theta 6$-$\theta 8$*(GLOB-52) +$\theta 9$*H1RB+$\theta 11$*EXT-5) +$\theta 12$*HPT)*$\theta 7$exp($\eta 4$)	($\theta 3$+$\theta 10$*PEN-$\theta 13$*(BILI-10)) exp($\eta 3$)	-	163	39/29	41	26	0.03	INH9.CSV	179.136	Backward Deletion: Antihistamines, extent of X-ray involvement and Globulin on CL/F fixed to 0. OBF ↑ 10.9. Marginally significant, hence include globulin.
120	2	ADD+CCV	$\theta 2\exp(\eta 1)$	CL(1)/F=($\theta 6$-$\theta 8$*(GLOB-52) +$\theta 9$*H1RB+$\theta 11$*EXT-5)+$\theta 12$*HPT) exp($\eta 2$) CL(2)/F=($\theta 6$-$\theta 8$*(GLOB-52) +$\theta 9$*H1RB+$\theta 11$*EXT-5) +$\theta 12$*HPT)*$\theta 7$exp($\eta 4$)	($\theta 3$+$\theta 10$*PEN-$\theta 13$*(BILI-10)) exp($\eta 3$)	-	150	40/35	41	28	0.02	INH9.CSV	179.071	Backward Deletion: Antihistamines, extent of X-ray involvement on CL/F and bilirubin on V/F fixed to 0. OBF ↑ 0.1. Hence exclude.
121	2	ADD+CCV	$\theta 2\exp(\eta 1)$	CL(1)/F=($\theta 6$-$\theta 8$*(GLOB-52) +$\theta 9$*H1RB+$\theta 11$*EXT-5)+$\theta 12$*HPT) exp($\eta 2$) CL(2)/F=($\theta 6$-$\theta 8$*(GLOB-52) +$\theta 9$*H1RB+$\theta 11$*EXT-5) +$\theta 12$*HPT)*$\theta 7$exp($\eta 4$)	($\theta 3$+$\theta 10$*PEN-$\theta 13$*(BILI-10)) exp($\eta 3$)	-	146	41/35	41	28	0.02	INH9.CSV	186.251	Backward Deletion: Antihistamines, extent of X-ray involvement and antihypertensives on CL/F and bilirubin on V/F fixed to 0. OBF ↑ 7.2. Hence exclude.
122	2	ADD+CCV	$\theta 2\exp(\eta 1)$	CL(1)/F=($\theta 6$-$\theta 8$*(GLOB-52) +$\theta 9$*H1RB+$\theta 11$*EXT-5)+$\theta 12$*HPT) exp($\eta 2$) CL(2)/F=($\theta 6$-$\theta 8$*(GLOB-52) +$\theta 9$*H1RB+$\theta 11$*EXT-5) +$\theta 12$*HPT)*$\theta 7$exp($\eta 4$)	($\theta 3$+$\theta 10$*PEN-$\theta 13$*(BILI-10)) exp($\eta 3$)	-	151	41/35	42	28	0.02	INH9.CSV	192.538	Backward Deletion: Antihistamines, extent of X-ray involvement and antihypertensives on CL/F and bilirubin and penicillins on V/F fixed to 0. OBF ↑ 6.3. Hence exclude. FINAL MODEL

η - inter-individual variability – where 2 values are recorded they are the estimates for the first and second populations; KA – absorption rate constant; CL/F – apparent clearance; CL(1)/F– apparent clearance (fast); CL(2)/F – apparent clearance for slow acetylators; V/F – apparent volume of distribution; TLAG – absorption lag time; σ - intra-individual variability; CCV – constant coefficient of variation; ADD – additive; OBF – minimum value of the Objective function.

Appendix K NONMEM CONTROL STREAM - ISONIAZID

Appendix K

NONMEM CONTROL STREAM - ISONIAZID

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$PROB  ISONIAZID KINETICS IN TUBERCULOSIS PATIENTS MIXTURE MODEL
$INPU ID DATE=DROP TIME DOSE=AMT DAMT RATE=DROP DV=CONC SS II
SEX=DROP TBHX=DROP MDR=DROP HIV=DROP WT AGE=DROP PREP ALKP=DROP
BILI=DROP PROT=DROP ALB=DROP GLOB=DROP GGT=DROP ALT=DROP AST=DROP
ALP=DROP PLAT=DROP LYMC=DROP LYMP=DROP VIRU=DROP INDU=DROP
INHI=DROP ABS1=DROP ABS2=DROP MIS1=DROP MIS2=DROP NSID=DROP
FEFL=DROP H1B=DROP PEN=DROP BS=DROP DIUR=DROP INAT=DROP QUIN=DROP
AG=DROP EMB=DROP PZA=DROP ANAE=DROP HPT=DROP DM=DROP
EPIL=DROP CAND=DROP NMDR=DROP EXT=DROP CAV=DROP WALL=DROP
$DATA        INH9.CSV IGNORE=#
$SUBROUTINES        ADVAN2 TRANS2 MIX=mix
$PK
     EST  = MIXEST
     TVKA = THETA(2) ; KA
     KA   = TVKA*EXP(ETA(1))
      CL1 = THETA(6)*EXP(ETA(2))
      TVV = THETA(3)
        V = TVV*EXP(ETA(3))
      CL2 = THETA(7)*THETA(6)*EXP(ETA(4))
      MM  = MIXNUM
      IF (COMACT.NE.0) MM=MIXEST
        Q = 1
      IF (MM.EQ.2) Q=0
      CL  = Q*CL1+(1-Q)*CL2
      S2  = V
$ERROR
     IPRED=F
     W=(F*F*THETA(4)*THETA(4)+THETA(5)**2)**0.5
     IRES=DV-F
     IWRES=IRES/W
     Y=IPRED+W*EPS(1)
   AUC = DAMT/CL
    KE = CL/V
  TMAX = DLOG(KA/KE)/(KA-KE)
    CB = EXP(-KE*TMAX)-EXP(-KA*TMAX)
    CC = DAMT*KA
    CD = V*(KA-KE)
  CMAX = (CC/CD)*CB
$SIGMA   1 FIXED
$THETA  (0,0.5,1)    ; 1: Mixing Fraction
        (3.5 FIXED)  ; 2: KA
        (0,36)       ; 3: V
        (0,0.5)      ; 4: CCV
        (0,0.2)      ; 5: ADDITIVE
        (0,15)       ; 6: CL(1)
        (0,0.5)      ; 7: CL(2)
$OMEGA  .25                       ; Ka
$OMEGA BLOCK(3) 0.25              ; CL1
                0.01  0.25       ; V
                0.001 0.01 0.25 ; CL2
$EST NOABORT MAXEVAL=9999 POSTHOC MSF=INH
$TABLES ID TIME CL1 CL2 V CL IPRED IWRES ETA1 DAMT EST DOSE WT
 ETA2 ETA3 ETA4 AUC TMAX CMAX ONEHEADER NOPRINT FILE=sdtab93
$COV
```

Appendix L   NONMEM MIX SUBROUTINE

Appendix L

NONMEM MIX SUBROUTINE

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SUBROUTINE MIX (ICALL,NSPOP,P)
COMMON /ROCM0/ THETA(20)
DIMENSION P(*)
DOUBLE PRECISION P,THETA
P(1)=THETA(1)
P(2)=1.-THETA(1)
NSPOP=2
RETURN
END
```

Appendix M   COVARIATE MODEL BUILDING - ISONIAZID

Appendix M

COVARIATE MODEL BUILDING - ISONIAZID

Covariate Effect	DOBF[1]	Retained[2] in Forward Model Buildup	Retained[3] in Final Model
Globulin on CL/F	11.6	Yes	No
Enzyme inhibitors on CL/F	10.085	Yes	No
Antihistamines on CL/F	8.38	No	No
Log Viral Load on CL/F	8.094	No	No
Penicillins on V/F	7.377	No	No
Hypoglycaemic agents/Diabetes mellitus on CL/F	6.996	No	No
Severity score for extent of lung involvement on CL/F	6.903	No	No
Severity score for extent of lung involvement on V/F	6.862	No	No
Drug resistance severity score on CL/F	5.983	No	No
Penicillins on CL/F	5.931	No	No
Weight on V/F	5.486	Yes	No
Globulin on V/F	5.222	Yes	Yes
HIV on CL/F	5.151	No	No
Diuretics/hypertension on CL/F	5.037	No	No
Total Bilirubin on V/F	4.897	No	No
Sex on CL/F	4.339	No	No
Severity score for extent of lung cavitation on V/F	3.943	No	No
Albumin on V/F	3.673	n/a	n/a
Diuretics/hypertension on V/F	3.004	n/a	n/a
Weight on CL/F	2.875	n/a	n/a
Non-steroidal anti-inflammatory drugs on V/F	2.67	n/a	n/a
Non-steroidal anti-inflammatory drugs on CL/F	2.236	n/a	n/a
ALT on CL/F	1.978	n/a	n/a
Log Viral Load on V/F	1.973	n/a	n/a
HIV on V/F	1.842	n/a	n/a
Iron Preparations on V/F	1.833	n/a	n/a
Quinolones on CL/F	1.714	n/a	n/a
Severity score for extent of lung cavitation on CL/F	1.62	n/a	n/a
AST on V/F	1.29	n/a	n/a
Sex on V/F	1.06	n/a	n/a
Albumin on CL/F	1.019	n/a	n/a
Age on V/F	0.926	n/a	n/a
Iron Preparations on CL/F	0.822	n/a	n/a
GGT on CL/F	0.73	n/a	n/a
Drug resistance severity score on V/F	0.687	n/a	n/a
Thiacetazone on CL/F	0.603	n/a	n/a
Total Bilirubin on CL/F	0.518	n/a	n/a
Antihistamines on V/F	0.508	n/a	n/a
MDR-TB on CL/F	0.468	n/a	n/a
Age on CL/F	0.408	n/a	n/a
Alkaline Phosphatase on V/F	0.378	n/a	n/a
Hypoglycaemic agents/Diabetes mellitus on V/F	0.356	n/a	n/a

[1]DOBF – difference in the minimum value of the objective function between 2 NONMEM runs. Chi square distributed – [2]DOBF $\geq$ 3.84 , p $\leq$ 0.05 df=1; [3]DOBF $\geq$ 11, p $\leq$ 0.001 df=1

Appendix M

COVARIATE MODEL BUILDING - ISONIAZID

Covariate Effect	DOBF[1]	Retained[2] in Forward Model Buildup	Retained[3] in Final Model
Drugs affecting Absorption on V/F	0.318	n/a	n/a
Quinolones on V/F	0.306	n/a	n/a
GGT on V/F	0.302	n/a	n/a
MDR-TB on V/F	0.292	n/a	n/a
Candidiasis on CL/F	0.289	n/a	n/a
Ethambutol on V/F	0.162	n/a	n/a
Ethambutol on CL/F	0.125	n/a	n/a
Alkaline Phosphatase on CL/F	0.111	n/a	n/a
Candidiasis on V/F	0.089	n/a	n/a
AST on CL/F	0.05	n/a	n/a
ALT on V/F	0.048	n/a	n/a
Thiacetazone on V/F	0.033	n/a	n/a
Enzyme inhibitors on V/F	0	n/a	n/a

[1]DOBF – difference in the minimum value of the objective function between 2 NONMEM runs. Chi square distributed – [2]DOBF ≥ 3.84, p ≤ 0.05 df=1; [3]DOBF ≥ 11, p ≤ 0.001 df=1