DETERMINATION OF KANAMYCIN PLASMA CONCENTRATIONS USING LC/MS AND PHARMACOKINETICS OF KANAMYCIN IN PATIENTS WITH MULTIDRUG-RESISTANT TUBERCULOSIS AND IN PATIENTS WITH MULTIDRUG-RESISTANT TUBERCULOSIS CO-INFECTED WITH HIV

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A thesis submitted in fulfilment of the requirements for the degree of Magister Pharmaceuticiae in the School of Pharmacy, University of the Western Cape, Bellville, South Africa

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November 2012.

Determination of kanamycin plasma concentrations using LC/MS and pharmacokinetics of kanamycin in patients with multidrug-resistant tuberculosis and in patients with multidrug-resistant tuberculosis co-infected with HIV

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Key words

Multidrug-resistant tuberculosis

HIV

Liquid chromatography/

Mass spectrometry

Pharmacokinetics

Plasma concentrations

Kanamycin

ABSTRACT

DETERMINATION OF KANAMYCIN PLASMA CONCENTRATIONS USING LC/MS AND PHARMACOKINETICS OF KANAMYCIN IN PATIENTS WITH MULTIDRUG-RESISTANT TUBERCULOSIS AND IN PATIENTS WITH MULTIDRUG-RESISTANT TUBERCULOSIS CO-INFECTED WITH HIV.

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Pharmacokinetics (PK) of kanamycin has been studied in healthy volunteers using immuno assay methods. Little information exists on the PK of kanamycin in HIV-negative patients infected with multidrug-resistant tuberculosis (MDR-TB), and currently there is no information on its PK in South African patients with MDR-TB infection and in those with MDR-TB co-infected with HIV. Therefore, the aim of the study was to determine firstly, kanamycin plasma concentrations using liquid chromatography coupled with mass spectrometry (LC/MS); secondly, to investigate the PK parameters of kanamycin in patients infected with MDR-TB and in patients co-infected with MDR-TB and HIV; thirdly, to assess the influence of HIV infection and renal impairment on the PK of kanamycin and fourthly, to find out whether there is any interaction between antiretroviral drugs and kanamycin.

Blood samples were drawn at different times over 24 hours after kanamycin intramuscular administration. The plasma concentration-time profile for each patient was plotted manually and the non-compartmental analysis method was used to calculate PK parameters.

The LC/MS analysis method was highly sensitive and specific in the detection and quantification of kanamycin in plasma. Separation of kanamycin peak was achieved at a retention time of 1.50 minutes with a high mean recovery percentage of 92.5%.

Pharmacokinetic parameters were presented as median and range. The absorption rate (K_a), maximum plasma concentrations (C_{max}), the time to the maximum plasma concentrations (T_{max}), the area under the plasma concentration curve during 24 hours (AUC_{0-24}), the area under the plasma concentration curve from zero to infinity ($AUC_{0-\infty)}$, the elimination rate constant (K_e), the volume of distribution (V_d), the clearance (Cl_{tot}), the half-life ($t_{1/2}$) and the mean residence time (MRT) in HIV -positive patients were 0.52 (0.16-0.90) hr^{-1}, 18.19 (8.40-27.63) µg/ml, 1.00 (0.50-2.00) hrs, 146.28 (60.22-305.86) µg/ml.hr, 190.33 (71.32-453.32) µg/ml.hr, 0.18 (0.063-0.33) hr^{-1}, 30.17 (13.28-87.63) L, 4.38 (1.65-14.02) L/hr, 3.85 (2.10-11.00) hrs and 2.99 (1.11-12.96) hrs, respectively; and in HIV -negative patients, 18.39 (13.32–25.26) µg/ml, 1.00 (0.50-2.00) hours, 161.90 (54.36–192.68) µg/ml.hr, 236.60 (60.06-295.63) µg/ml.hr, 0.13 (0.081-0.35) hr^{-1}, 33.02 (14.89–45.59) L, 4.00 (3.01–15.04) L/hr, 5.37 (1.98–8.56) hours, and 4.44 (1.13–12.76) hours, respectively.

There was no statistical significant difference in the PK parameters in the HIV-positive and HIV-negative patients, with the smallest p value being 0.43. A statistical significant difference (p=0.013) was found in the renal function in HIV -positive and HIV -negative patients. However, the difference did not result into affecting the PK parameters of kanamycin (p=0.31).

We had an unequal distribution of patients in the HIV -positive and HIV -negative groups with regards to CD4 counts and viral load, imbalance in the different categories of renal dysfunction and stages of HIV disease. Therefore, we recommend further studies with a higher sample size of patients to address the unequal distribution with regards to the immunological and virological profile, categories of renal dysfunction and stages of HIV disease.

November 2012

DECLARATION

I declare that the thesis *Determination of kanamycin plasma concentrations using LC/MS and pharmacokinetics of kanamycin in patients with multidrug-resistant tuberculosis and in patients with multidrug-resistant tuberculosis co-infected with HIV* is my work, that it has not been submitted before for any degree or examination in any other university, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

Ibukunoluwa Mercy Abaniwonda November 2012

Signed: UWC, Bellville

ACKNOWLEDGEMENTS

Most importantly, I dedicate every page in this thesis to my Lord, Jesus Christ, who gave me the strength, courage and knowledge to complete this thesis.

I am indebted to my supervisor, Prof. Pierre Mugabo, for his support, guidance, and suggestions throughout the research.

I also wish to express my appreciation to my parents, Mr and Mrs Jonathan Abaniwonda, for their unwavering encouragement, excellent support, continuous love and incessant prayers during my academic studies. I would like to thank my siblings and friends for their love and support from time to time during the process of research.

Special thanks are due to Dr Ademola Oyagbemi from the Cape Peninsula University of Technology, South Africa/University of Ibadan, Nigeria for his insightful contributions and moral support during the research and writing process.

I also wish to express my thanks to Prof. George Amabeoku for his invaluable support and encouragement during the period of research.

I would like to express my gratitude to the following people and departments:

- The medical superintendent at Brewelskloof Hospital for permission to conduct the study.
- Staff members at Brewelskloof Hospital for their support.
- The Pharmaceutical Services, Provincial Administration of the Western Cape for supplying anti-tuberculosis tablets.

- The Department of Health, Province of the Western Cape for the permission to conduct the study at Brewelskloof Hospital.
- Patients who participated in the study.
- The research department, University of the Western Cape for financial support of the study.
- Prof. Richard Madsen for guidance regarding statistical analysis.

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ABBREVIATIONS AND ACRONYMS

AIDS	Acquired immune deficiency syndrome
ALT	Alanine aminotransaminase
ARV	Antiretroviral
ARVs	Antiretroviral drugs
AST	Aspartate aminotransaminase
AUC	Area under the concentration-time curve
AUC_{0-24}	Area under the concentration-time curve from zero to 24 hours
$AUC_{0-\infty}$	Area under the plasma concentration-time curve from zero to infinity
CD4	Cluster difference 4
Cl	Clearance
Cl_{tot}	Total body clearance
C_{max}	Maximum concentration
DMSO	Dimethyl sulfoxide
DOT	Directly observed treatment
ELISA	Enzyme-multiplied-immuno assay technique
GFR	Glomerular filtration rate
HIV	Human immunodeficiency virus
HPLC	High-pressure liquid chromatography
INH	Isoniazid
K_e	Elimination rate constant

LC-MS	Liquid chromatography-mass spectrometry
MIC	Minimum inhibitory concentration
MDRD	Modification of diet in renal disease
MDR-TB	Multidrug-resistant tuberculosis
MRT	Mean residence time
MTB	*Mycobacterium tuberculosis*
M/z	Mass-to-charge ratio
NRTI	Nucleoside reverse transcriptase inhibitor
NNRTI	Non-nucleoside reverse transcriptase inhibitor
PD	Pharmacodynamics
pH	Potential hydrogen
PI	Protease inhibitor
PK	Pharmacokinetic(s)
pka	Acid dissociation constant
RIF	Rifampicin
SD	Standard deviation
TDM	Therapeutic drug monitoring
TFA	Trifluroacetic acid
T_{max}	Time to reach maximum concentration
$T_{1/2}$	Half-life
UV	Ultra violet
V_d	Volume of distribution
WHO	World Health Organisation
XDR-TB	Extensively drug-resistant tuberculosis

CHAPTER 1: INTRODUCTION AND RATIONALE OF THE STUDY

1.1 Tuberculosis and human immunodeficiency viral infection: Globally and in South Africa

The scale of the tuberculosis (TB) pandemic is growing worldwide. The bacterium *Mycobacterium tuberculosis* (MTB) is responsible for this infection and is the most common cause of death among patients infected with human immunodeficiency virus (HIV). The World Health Organisation (WHO) reports that one-third of the world's population is currently infected with tuberculosis (WHO, 2008). The number of TB and HIV infections is raising concerns globally, and particularly in sub-Saharan Africa. In South Africa, 400,000 new cases of TB are reported each year (WHO, 2011), while sub-Saharan Africa has the greatest burden of TB and HIV in the world. In this region, the surge of HIV has been an important factor in the development of active TB, making it difficult for TB control programmes to be successful.

Despite the introduction of control programmes, the number of TB cases in sub-Saharan Africa has risen dramatically. In a study by WHO in 2009, it was estimated that the national directly observed treatment (DOT) success rate in South Africa was recorded to have increased from 65% in 2001 to 74% in 2006. Therefore, the increase of TB in sub-Saharan Africa is not due to the failure of control programmes, but principally due to the link between TB and HIV, because each disease accelerates the progress of the other. It has been estimated that 70%

of new adult patients with TB annually are co-infected with HIV in South Africa (WHO, 2009).

Furthermore, improper treatment of TB can lead to the development of multidrug-resistant tuberculosis (MDR-TB), which is TB that is resistant to at least isoniazid (INH) and rifampicin (RIF), the most potent anti-TB first-line drugs. The rise of MDR-TB in sub-Saharan Africa is causing concern and is complicated by HIV infection, thus making elimination of the drug-resistant bacteria difficult. It has been reported that approximately 5% of new cases of TB are MDR-TB globally (Mitnick et al., 2008; Farley et al., 2011). In addition, compared with drug-resistant tuberculosis, MDR-TB therapy is complicated and involves the use of second-line anti-TB drugs that are more expensive and less potent, but with more side effects, and treatment is for a minimum of 18–24 months.

Another factor threatening the control of TB is the presence of extensively drug-resistant tuberculosis (XDR-TB). Studies done in KwaZulu-Natal Province in South Africa reported that the prevalence of XDR-TB was found to be 6% among patients with TB and that mortality rates were extremely high (98%) in HIV-infected individuals with XDR-TB (Gandhi et al., 2006). This suggests that the development of resistance to at least INH and RIF, and to at least one of the three injectable second-line anti-TB drugs is not likely to be controlled, due to HIV infection.

1.2 Rationale of the study

HIV infection has been noted to change the pharmacokinetics (PK) of first-line anti-TB drugs in HIV-positive patients co-infected with TB. It has been suggested that approximately 5% of HIV-positive patients on anti-TB treatment do not respond to the standard regimen of TB treatment, as compared to 0.4% of HIV-negative patients (Aaron et al., 2004).

In addition, many studies have confirmed that HIV -positive patients do not sufficiently absorb anti-TB drugs, resulting in sub-therapeutic outcomes of anti-TB therapy that may result in the development of resistance to anti-TB drugs (Choudri et al. 1997; Peloquin, 2002; Gurumurthy et al., 2004). This evidence has not yet been confirmed for many of the second-line anti-TB drugs.

Furthermore, the cure rate of MDR-TB infection in HIV-infected individuals is found to be lower due to high mortality rates than in HIV-negative patients. Studies have shown that the median sputum culture conversion rate from positive to negative was similar in both HIV-positive and HIV-negative groups of patients (Brust et al., 2011; Issakidis et al., 2011). However, individual competing factors (treatment default or death) suffered by HIV-positive patients altered the sputum conversion rate (Issakidis et al., 2011). Sputum culture conversion has been reported to indicate treatment outcomes, and MDR-TB patients with no sputum conversion within two to three months had high rates of relapse and failure (Brust et al., 2011). However, the inclusion of anti-retroviral therapy (ARV) with MDR-

TB therapy might improve survival and treatment outcomes for MDR-TB patients co-infected with HIV.

Many of the patients infected with MDR-TB and co-infected with HIV are on ARV therapy, therefore the interaction between ARVs and anti-TB drugs needs to be looked into. Interactions between ARV drugs and anti-TB drugs alter the plasma drug concentrations of anti-TB drugs by changing the absorption, distribution, hepatic metabolism and renal elimination of anti-TB drugs. For first-line anti-TB drugs, major drug-to-drug interaction occurs between RIF and highly active antiretroviral therapy, protease inhibitors and non-nucleoside reverse transcriptase inhibitors (Burman et al., 1999; Aaron et al., 2004; CDC, 2007). For second-line anti-TB drugs, interaction between tenofovir and kanamycin as a result of reduced elimination by the kidneys could result in an increase in the plasma concentration of kanamycin.

Furthermore, it is known that currently used first- and second-line anti-TB drugs are different in their mechanism of action and these variations are mainly determined by their pharmacodynamic (PD) properties, that is their intrinsic activity profile, as well as their PK properties, such as absorption, disposition and tissue distribution profile (Budha et al., 2008). Consequently, a better understanding of the PK and PD of anti-TB drugs should improve treatment of mycobacterial infections and a more rational use of anti-TB therapy both in patients with MDR-TB infection and in patients co-infected with MDR-TB and HIV. In this study, only the PK of kanamycin in patients with MDR-TB and in

patients with MDR-TB co-infected with HIV will be determined due to budget and time constraints. Kanamycin is one of the drugs used in the intensive phase treatment of MDR-TB. Consideration of its PK in developing a dosage regimen could lead to better treatment outcomes in patients with MDR-TB and in patients with MDR-TB co-infected with HIV, as a poor dosage regimen could lead to treatment failure.

The PK of kanamycin has been studied in healthy volunteers using microbiological assay methods and to a limited extent in patients with MDR-TB (Kirby et al., 1976; Pechere and Dugal., 1979; Holdiness, 1984; Douglas and Mcleod, 1999; Nuermberger and Grosset, 2004; Budha, 2008; Coyne et al., 2009). To the best of my knowledge, there is no recent published study on the analysis of kanamycin plasma concentrations using liquid chromatography (LC) coupled with mass spectrometry (MS). In addition, little information exists on the PK of kanamycin in HIV -negative patients infected with MDR-TB (Yew et al., 1999), and currently there is no information on its PK in South African patients with MDR-TB infection and in patients with MDR-TB co-infected with HIV (Mugabo, 2009). Furthermore, information obtained from this study might help the future development of therapeutic drug monitoring in order to improve patients' outcome and optimise drug therapy.

Hence, the aim of the study was to determine kanamycin plasma concentrations using LC/MS and to describe the PK of kanamycin in patients with MDR-TB and in patients with MDR-TB co-infected with HIV during their course of treatment.

This was done by collecting blood samples from patients at different time intervals in a heparinised tube after patients attained the steady state concentration and then running various tests. The influence of HIV, kidney dysfunction and drug interactions on the PK of kanamycin were also determined.

For the determination and quantification of kanamycin plasma concentration, LC/MS was utilised because of its high sensitivity and specificity as compared to other methods used in previous studies (Stead, 2000; La and Feng, 2007).

1.3 Structure of the thesis

Chapter one presents an overview of TB and HIV globally and in South Africa. It also provides the rationale of the study and the method to be used in determining kanamycin plasma concentration.

The rest of the thesis is structured as follows: the literature is extensively reviewed in the following chapter. In the literature review, the pathogenesis of TB, the causes of anti-TB drug resistance, the development of MDR-TB and the influence of both TB and HIV on each other are presented. The effects of drug interaction and diseases on the PK of kanamycin are explained. The chapter also gives an overview of the risk factors for kidney and liver impairment. Furthermore, a review of the PK of kanamycin in healthy volunteers and in patients with MDR-TB is presented. Previous methods used in the analysis of kanamycin are also reviewed.

In the third chapter, a comprehensive description of methods used in the study is provided. In addition, the method used for the calculation of kanamycin PK parameters and statistical methods are explained.

The fourth chapter presents the results obtained in the study. Here, the demographic data, immunological and virological profile, and biological profile of the patients are provided. In addition, the plasma concentrations and the PK parameters of kanamycin are presented.

The fifth chapter discusses the results obtained. The first section explains the statistical analysis of the PK parameters of kanamycin. The demographic data, study procedures, the LC/MS method of analysis and laboratory results are discussed according to standardised conditions. Research questions that were drawn from the literature review are then answered. The clinical and therapeutic implications of the results are also highlighted in this chapter. Finally, the limitations of the study are explained.

In the sixth chapter, the main findings of the study are summarised. In addition, recommendations and justification for further studies are included.

CHAPTER 2: LITERATURE REVIEW

2.1 Structure of the literature review

Chapter 2 provides a review of the literature on a number of aspects relating to the study. It consists of ten sections.

The first section provides the structure of the literature review.

The second section presents an overview of TB and gives a brief explanation of the pathogenesis of MTB, the development of active tuberculosis and the influence of active TB on the course of HIV infection, and vice versa.

The third section gives a brief definition of drug-resistant tuberculosis, the epidemiology of MDR-TB, the mechanism of drug resistance, the causes of drug resistance, the laboratory identification of MDR-TB strains, the diagnosis of MDR-TB and the chemotherapy of MDR-TB.

The fourth section gives an overview of the definition and purpose of PK studies, PK drug interactions, the influence of co-existing diseases on the PK of kanamycin, and drugs with side effects that may affect the PK of anti-TB drugs.

The fifth section discusses drug interactions between ARVs and anti-TB drugs, with emphasis on the interaction between tenofovir and kanamycin. The sixth

section gives an overview of kidney and liver function tests and the risk factors for kidney and liver impairment.

The seventh section describes the PK and pharmacology of kanamycin. It also presents published findings on the PK of kanamycin in healthy volunteers and in MDR-TB patients, clinical uses, drug interactions, side effects and the PK–PD relationship. This section also discusses how therapeutic drug monitoring (TDM) may help to optimise kanamycin dose.

The eighth section presents a description of the methods used for the determination of the plasma concentration of anti-TB drugs, a description of the LC/MS method of analysis and previously used methods for the analysis of kanamycin. Lastly, it gives a review of published methods previously used to determine kanamycin plasma concentrations.

The ninth section outlines the hypothesis of the thesis, while the tenth section presents the research questions and objectives of the study.

2.2 Overview of tuberculosis

2.2.1 Pathogenesis of Mycobacterium tuberculosis infection

Understanding the process in the development of TB is important so as to understand the risk TB infection poses if it is not treated effectively. MTB is acquired by the inhalation of infectious airborne particles small enough (1–5 microns) to reach alveolar air spaces (Shafer and Edlin, 1996). The possibility of

MTB infection depends on the intensity of MTB exposure and probably also on the effectiveness of innate host defences.

Alveolar macrophages act as phagocytes of the innate immune system. Alveolar macrophages in some individuals might have a degree of innate mycobacterial resistance and in some other individuals the bacilli are presumably destroyed before infection is established. Furthermore, active TB disease following infection with MTB is not developed in some individuals because cell-mediated immunity is developed within two to ten weeks after initial infection (Weyer, 1999).

2.2.2 Development of active tuberculosis

The immunologic response to MTB infection does not always eliminate susceptible bacteria, since dormant organisms often reactivate latent infection. It was reported that in immuno-competent individuals, 20–50% may become infected after exposure for a prolonged period (hours/days rather than minutes) to MTB infection. In 90% of infected individuals MTB may become latent, while a few (5%) may develop TB early (typically within two years of infection) and an additional few (5%) may develop TB afterwards due to an impaired immune system (Weyer, 1999).

2.2.3 Human immunodeficiency viral infection and reactivation of latent mycobacterium tuberculosis infection

The risk of reactivating dormant MTB infection is greatly increased in HIV-infected individuals as a result of HIV. It is known that CD4+ T-lymphocytes are

involved in the processes of the immune response to MTB, including binding to processed antigen, secreting cytokines and killing mycobacteria-infected cells (Shafer and Edlin, 1996).

HIV increases the risk of advanced TB disease by inducing CD4+ T-lymphocyte depletion, leading to an ineffective immunologic response to MTB infection. It has been suggested that HIV co-infection with dormant TB results in a 50 times greater risk of the victim acquiring active TB disease and becoming infectious in the HIV population as compared to the non-HIV population **(**Bauer et al., 2008). Furthermore, the development of active TB following the activation of MTB infection in HIV-infected individuals occurs at higher CD4 counts (300–400 cells/mm^3) than other HIV-related illnesses. This is because HIV-infected individuals are more prone to developing active TB when newly infected or reinfected with MTB.

2.2.4 Impact of active tuberculosis on the course of human immunodeficiency viral infection

Active TB also plays a role on the course of HIV infection by depleting the number of CD4+ T-lymphocytes, which increases HIV replication (Taylor, 1997; Perrone, 1999). TB also induces immune stimulation via the increased production of cytokines, such as tumour necrosis factor cellularly, which could be deleterious in HIV-infected individuals (Wallis et al., 1993; Shafer and Edlin, 1996). Furthermore, opportunistic infections and death are considered to be a greater threat in HIV-infected individuals co-infected with TB than in HIV-infected

patients with similar CD4+ cell counts without active TB (Shafer and Edlin, 1996). This is due to the impaired immune system in HIV-infected individuals as a result of active TB.

A study was done by Whalen et al. (1995) to compare the incidence of opportunistic infection in HIV-infected persons with active TB (comprising the cases) and in HIV-infected persons without active TB (comprising the control). The CD4+ counts were similar for both groups of patients and the result showed that the incidence rate of new cases of AIDS-defining opportunistic infections in patients with HIV and active TB were higher than in control subjects. It was recorded that there were 4.0 infections per 100 persons for the cases, compared to 2.8 infections per 100 persons for control subjects. In addition, cases had shorter overall survival times as a result of active TB than control subjects. Therefore, it is probable that the increased burden of HIV co-infection with TB increases susceptibility to MTB infection, as well as increasing the risk of relapse of TB and the development of MDR-TB (Aaron et al., 2004).

2.3 Drug-resistant tuberculosis

2.3.1 Epidemiology of multidrug-resistant tuberculosis

The rise of MDR-TB cases in the world poses a risk to TB control, and once again sub-Saharan Africa is considered to have the worst epidemic. An estimated 489,139 new cases, or approximately 5% of new TB cases diagnosed in the world in 2006, were believed to be MDR-TB (Mitnick et al., 2008), and in South Africa there should be at least 2,000 newly active cases of MDR-TB each year (Budha et

al., 2008). According to the WHO global report on TB, the number of MDR-TB cases in South Africa has more than tripled, from 2,000 cases in 2005 to 7,350 in 2007 (WHO, 2009).

2.3.2 Mechanisms of tuberculosis drug resistance

Mutations in genes encoding the targets of anti-TB therapy are primarily responsible for the resistance of MTB to anti-TB drugs (Shafer and Edlin, 1996). These mutations lead to an altered target of anti-TB agents, for example, RNA polymerase and catalase-peroxidase in RIF and INH resistance, respectively (Rattan et al., 1998). These alterations occur with a predictable frequency of one in 10^5–10^8 organisms (Shafer and Edlin, 1996).

Drug resistance is categorised into primary, acquired or initial types based on patients' history of prior TB treatment. Primary resistance to anti-TB drugs occurs in patients with no history of previous TB treatment. According to WHO, drug resistance among new cases is characterised by the presence of resistant strains of MTB in patients who have never received TB drugs or who have received TB drugs for less than one month of treatment (Chaulet and Maher, 1997).

Acquired resistance refers to resistance to anti-TB drugs in patients with previous TB treatment. By definition, acquired resistance can only be established in patients who have received at least four weeks of anti-TB chemotherapy (Loddenkamper et al. 2002).

Initial resistance refers to drug resistance in patients with new cases of TB, as well as those with undisclosed acquired resistance (Loddenkamper et al., 2002). According to WHO, in cases where previous TB treatment in patients is unknown, this form of resistance is called initial resistance (Chaulet and Maher, 1997).

2.3.3 Causes of drug resistance

The development of resistance to anti-TB treatment is often thought to be commonly caused by non-adherence to anti-TB treatment; however, studies have shown that the organisational failure of TB control programmes, the lack of available drugs and clinical errors are responsible for many of the MDR-TB problems existing today (Loddenkamper et al., 2002; Wells et al., 2007). Common clinical errors include inadequate primary regimen, failure to provide DOT and failure to recognise existing drug resistance.

Furthermore, based on epidemiology studies, the relationship between HIV infection and MDR-TB has not been established in sub-Saharan Africa (Wilkinson et al., 1996; Kenyon et al., 1999; Churchyard et al., 2000; WHO, 2004). Notwithstanding, clinical evidence has shown that there is a link between HIV infection and the development of resistance to anti-TB drugs (Patel et al., 1995; Choudri et al., 1997; Peloquin, 2002; Gurumurthy et al., 2004; Tappero et al., 2005).

It has been reported that HIV-positive patients being treated in hospitals for drug-susceptible TB have been infected with MDR-TB (Wells et al., 2007). In South

Africa, high HIV prevalence and the lack of ventilation measures in hospital wards have contributed to the spread of drug-resistant organisms among HIV-positive individuals (Andrews et al., 2007). Hence, the sub-optimal control of TB infection and the increasing epidemic of HIV lead to the development of MDR-TB.

2.3.4 Laboratory identification of multidrug-resistant tuberculosis strains

Laboratory identification of MDR-TB strains can only be established through culture and drug-susceptibility testing of the organism (Chaulet and Maher, 1997). Proportion method is commonly used for determining the drug susceptibility of MTB isolates in the laboratory. When 1% or more of the bacterial population becomes resistant to the critical concentration, MTB isolate is regarded as resistant to that drug (Chaulet and Maher, 1997).

2.3.5 Diagnosis of multidrug-resistant tuberculosis

MDR-TB is suspected in patients with persistent acid-fast bacilli smears or cultures, despite well-documented TB treatment adherence (Weyer, 2005). MDR-TB can only be diagnosed by in-vitro confirmation of resistance. Inadequate clinical response to TB treatment is usually characterised in patients by acid-fast bacilli smears being positive at two months, which necessitates doing culture and susceptibility tests against INH and RIF (Loddenkamper et al., 2002; Weyer, 2005).

2.3.6 Chemotherapy of multidrug-resistant tuberculosis

The treatment for tuberculosis involves the use of first-line drugs, namely, INH, RIF, ethambutol and pyrazinamide, for a minimum of six to nine months (Chaulet and Maher, 1997; Budha et al., 2008). These drugs are used in the standardised treatment regimen, with an initial, intensive phase of two months and a continuation phase for four to six months (WHO, 2008). This anti-TB regimen does not always achieve elimination of MTB, as confirmed by treatment failure in some treated individuals.

MDR-TB therapy is usually more expensive than first-line anti-TB drugs. This is because treatment of MDR-TB is for a minimum of 18–24 months rather than a minimum of 6–12 months for drug-sensitive TB. Furthermore, second-line anti-TB drugs are more toxic than first-line drugs (Davies, 2001; Loddenkemper et al., 2002; Budha et al., 2008). The second-line drugs that are available for the treatment of MDR-TB infection, in order of decreasing activity, include an aminoglycoside (amikacin or kanamycin), a thioamide (ethioamide or prothioamide), pyrazinamide, a fluoroquinolone (ciprofloxacin or ofloxacin), ethambutol, terizidone or cycloserine and para-aminosalicyclic acid (Weyer, 2005).

In developed countries, treatment of MDR-TB is based on drug susceptibility tests. This means that MDR-TB treatment is delayed until susceptibility tests are available or patients are started on standardised regimen while awaiting drug susceptibility tests. However, in resource-poor countries, the WHO treatment

guidelines are used in addition to DOT-based regimens. In South Africa, the regimen consists of a six-months daily intensive phase with five drugs: kanamycin, pyrazinamide, ofloxacin, ethionamide and either terizidone or ethambutol, followed by a continuation phase of 12–24 months with three drugs: ofloxacin, ethionamide and either ethambutol or terizidone (Weyer, 2005).

The drugs used in the intensive and continuous phases and the dosages of these drugs are given in Tables 2.1 and 2.2.

Table 2.1: Second-line anti-TB drugs used in the intensive phase (6 months) in South Africa

MDR-TB drug	**Daily dosage (mg)**
Kanamycin	750–1,000
Ethionamide	500–750
Pyrazinamide	1,000–1,500
Ofloxacin	600–800
Ethambutol	800–1,200

Table 2.2: Second line anti-TB drugs used in the continuation phase (12–24 months) in South Africa

MDR-TB Drug	**Daily dosage (mg)**
Ethionamide	500–750
Ofloxacin	600–800
Terizidone	750

Ethambutol (800–1,200mg) is used if the MDR-TB strain is sensitive to ethambutol.

2.4 Pharmacokinetics

2.4.1 Overview

The term ‘pharmacokinetics’ describes the kinetics of drug absorption and disposition. Drug disposition involves processes of distribution and elimination (metabolism and excretion) (Budha et al., 2008).

The purpose of PK studies in healthy volunteers or patients is usually to ascertain the association among the dose, dosage form and plasma concentration, and to gain knowledge on drug absorption and disposition (Derendorf et al., 2000). PK studies rely on the measurement of active drug ingredient in a biological fluid such as blood, plasma or urine. From these assays, concentration-time curves may be constructed and PK measures and parameters such as area under the concentration-time curve (AUC), maximum concentration (C_{max}), clearance (Cl), volume of distribution (V_d) and half-life ($t_{1/2}$) may be calculated (Derendorf et al., 2000).

2.4.2 Pharmacokinetic parameters

Understanding PK parameters is important in determining the time course of an antimicrobial effect and in determining the optimum dose of an antibiotic. The important PK parameters used in the study are as follows:

- **C_{max}** is the maximum concentration a drug achieves in blood plasma.
- **T_{max}** is the time taken to reach C_{max} after drug administration.
- **$T_{1/2}$** is the plasma half-life. It is the time taken for the drug plasma concentration levels to fall by 50%.
- **AUC** is the area under the plasma concentration-time curve. It is the overall amount of drug in the blood stream after a dose.
- **K_e** is the drug's elimination rate constant.
- **K_a** is the drug's absorption rate constant.
- **Cl** is the drug clearance, that is, the measure of the ability to eliminate a drug. The total body systemic clearance (Cl_{tot}) of a drug is the sum of the clearances of the drug by various organs and tissues of the body.
- **V_d** is the apparent volume of distribution of the drug. It is the measure of the available apparent space in the body that is available to contain the drug.
- **MRT** is the mean residence time of the drug. It is the average time that the drug molecules spend in the body.

2.4.3 Pharmacokinetics of commonly used anti-retroviral drugs

2.4.3.1 Stavudine

The bioavailability of stavudine in the adult population infected with HIV is 82–99% (Lea and Diana, 1996). Food considerably lowers the rate of absorption. The C_{max} of stavudine is concentration dependent and T_{max} is reached within 0.5–0.75 hours after oral administration. The steady state volume of distribution is 47.3–68.9 L in adults and the $t_{1/2}$ of stavudine-5-triphosphate is 3.5 hours (Lea and Diana, 1996).

2.4.3.2 Lamivudine

After oral administration, lamivudine is rapidly absorbed, with C_{max} usually attained 0.5–1.5 hours after the dose (Johnson et al., 1999). Absolute bioavailability was recorded as 82% and 68% in adults and children, respectively. Food intake does not lower the rate of absorption of lamivudine. The elimination $t_{1/2}$ of lamivudine is approximately 5–7 hours (Johnson et al., 1999).

2.4.3.3 Efavirenz

Oral clearance was recorded as 9.4 L/hr, oral volume of distribution was 252 L and absorption rate constant was 0.3 hr^{-1} (Almond et al., 2005).

2.4.4 Pharmacokinetic drug interactions

The administration of two or more drugs at the same time to the same patient can alter the absorption, distribution, hepatic metabolism and renal elimination of the drugs involved.

2.4.4.1 Drug interaction during absorption

Drug absorption is the movement of drug molecules from the site of administration into systemic circulation (Peloquin, 2002). Interactions between two drugs or between food or drinks and drugs can happen when they interfere with each other's absorption. The absorption rate of a drug may be increased or decreased by the pH of the medium around the site of absorption and the acid dissociation constant (pka) of the drug. For example, the absorption rate of weak acidic drugs will be higher in the stomach where the pH is acidic, as opposed to

the alkaline pH in the small intestine. The absorption rate of a weak basic drug will be increased in the small intestine, where the pH is alkaline.

Food can affect the rate of absorption of a drug taken concurrently, thereby affecting the bioavailability of the drug. Grapefruit has been found to interfere with drugs that undergo CP450 oxidative metabolism in the intestinal wall or liver. It interferes with these drugs by binding to the iso-enzyme, CYP3A4 as a substrate and impairs first-pass metabolism through the inhibition of CYP3A4; the result is an increase in the bioavailability of these drugs and an increased risk of serious side effects (Leucuta and Nlase, 2006).

The poor absorption of anti-TB drugs results in sub-therapeutic plasma concentration levels and treatment failure of such drugs, with increased risk of acquired drug resistance (Gurumurthy et al., 2004). Therefore, an understanding of the PK parameters of anti-TB drugs will be effective in the rational treatment of patients infected with TB and in the prevention of drug resistance to anti-TB therapy.

2.4.4.2 Hepatic drug metabolism

Drug metabolism mediated by the CP450 system mainly occurs in the liver. Therefore, any dysfunction of the liver may lead to changes in drug metabolism. Drugs metabolised by the CP450 system can be classified as cytochrome P450 substrates, inhibitors or inducers. Efavirenz, an ARV, is metabolized by CYP450. It is a 3A4 inducer and inhibitor and therefore interacts with other drugs

administered concomitantly (Almond et al., 2005). Inhibition of CP450 is usually reversible and competitive, such that the substrate and inhibitor compete for the same site on the enzyme. Drugs that inhibit CP450 can cause reduced clearance and increased plasma concentration of drugs metabolised through the CP450 enzyme system. Similarly, drugs that induce the CP450 system increase the rate of liver metabolism. Induction of the CP450 pathway then leads to increased clearance and a subsequent fall in the plasma concentration of drugs metabolised by the pathway (Tang et al., 2005).

2.4.4.3 Drug interaction during distribution

The ability of a drug to produce the required pharmacological effect may be influenced by the extent to which the drug binds to proteins within the blood plasma. Drug–protein binding is reversible and equilibrium is established between the bound and unbound drug, regardless of the extent of protein binding. The less bound a drug is, the more effective it is, since it can cross cell membranes. Normally, it is the unbound fraction of a drug that exhibits pharmacological effects.

If both drug A and drug B are highly protein bound (>90) and are given at the same time, they will both compete for protein-binding sites. If drug A is more competitive, it might displace drug B from the binding sites, increasing drug B's unbound fraction. This may increase the effects of drug B. This change in pharmacological effect could have adverse effects in the patient.

2.4.4.4 Renal elimination

Drug–drug interactions at the renal level can involve several mechanisms. For example, reduced plasma binding (competitive or non-competitive binding displacement) leads to an increase in the excretion of highly bound drugs through an increase in glomerular filtration (Bonate et al., 1998).

In addition, the pH of urine and pka of the drug can influence the extent of the passive reabsorption of the drug. This mechanism is clinically significant for drugs that are excreted unchanged and have a narrow therapeutic index, such that changes in urine pH can interfere with plasma concentration. For example, the acidification of urine pH can increase the rate of passive reabsorption of weak acidic drugs, resulting in an increase in the half-life of the drug and therefore increased risk of drug interaction. Similarly, the alkalinisation of the urine pH can increase the rate of passive reabsorption of weak basic drugs, resulting in the increased plasma concentrations of the drug and consequently increased risk of drug interactions.

The most notable mechanism of renal drug interaction is alteration of tubular active transport. Many drug transporters are actively responsible for the secretion and reabsorption of drugs in the kidney (Keith, 2006). Transporters are specific and are susceptible to competitive drug–drug interactions.

Competition between two drugs for the same transporter protein inhibits the renal clearance of one of the drugs and this result in the increased plasma concentration

of the other drug that is a substrate of the transporter. In several cases, this increased plasma concentration can lead to increased toxic levels of the drug. For example, the inhibition of transporter organic anion transporting polypeptide by trimethoprim decreases lamivudine excretion into the proximal tubule, resulting in the increased plasma exposure of lamivudine (Taft, 2009).

2.4.5 Influence of diseases on the pharmacokinetics of anti-tuberculosis drugs

Drugs are developed to treat diseases, but sometimes these disease conditions can impair the PK of these drugs and affect patient response to drug therapy. Conditions affecting the gastro-intestinal tract, liver, heart and kidneys may increase or decrease the PK of drugs used for the treatment of MDR-TB by altering their absorption, distribution, metabolism and elimination. Some of these conditions are gastro-enteritis, diarrhoea, malabsorption syndromes, hepatitis, cirrhosis of the liver, congestive cardiac failure, renal failure and HIV infection.

2.4.5.1 Gastro-enteritis

Gastro-enteritis is the inflammation of the stomach and intestine inner layers as a result of infection by bacteria or viruses. The most common symptoms of gastro-enteritis are diarrhoea and vomiting. Therefore, gastro-enteritis may reduce the absorption of anti-TB drugs as a result of decreased contact time between the drug and the intestinal microvilli.

2.4.5.2 Diarrhoea

Diarrhoea is the passing of frequent, loose and watery stools, and it affects the absorption of drugs by enhancing gastro-intestinal motility, which reduces the resident time of drugs in the intestines and hence the absorption of drugs is reduced in general. Ethionamide, one of the drugs used in treatment of MDR-TB, produces intense gastrointestinal effects such as diarrhoea, nausea and vomiting (Arbex et al., 2010). Diarrhoea, as stated above, can increase the gastric motility of MDR-TB drugs, leading to reduced resident time in the intestine, decreasing their absorption.

2.4.5.3 Malabsorption syndrome

Malabsorption syndrome refers to difficulty in digesting nutrients from food as a result of shrinkage of the intestinal villi. Selective malabsorption of more than one anti-TB drug used in the treatment regimen could promote drug resistance.

2.4.5.4 Hepatitis and cirrhosis of the liver

Hepatitis, liver failure and cirrhosis of the liver can alter the PK of drugs biotransformed by the liver. Hepatic blood flow and liver enzyme activity, which are the primary determinants of the ability of the liver to metabolise drugs, can be affected by liver disease. For drugs susceptible to first-pass effect, the higher the blood flow to the liver, the greater the proportion of drugs metabolised by the liver and the lower the concentration of drugs in circulation, leading to reduced bioavailability of the drug. In addition, liver disease is characterised by decreased

protein binding and occurs with drugs that are highly bound to plasma proteins (Bremier, 1987).

Hepatitis is defined by the presence of inflammatory cells in the tissue of the liver. Triggers of inflammation of the liver include viruses, toxic substances (such as alcohol, certain medications and chemicals), infections and autoimmune disease. Anti-TB drugs that can cause hepatitis include pyrazinamide, RIF and INH (Thompson et al., 1995). In acute viral hepatitis, symptoms are usually serious and last for a period of three to four weeks. Here, changes in drugs PK are less than in chronic hepatitis, and hepatic elimination returns to normal as the disease resolves (Bremier, 1987). In chronic hepatitis, which normally lasts longer than six weeks, there is increased hepatic clearance of highly metabolised drug as a result of decreased serum albumin concentration.

Cirrhosis of the liver arises as a result of chronic liver disease and is characterised by scarring of the liver tissue, fibrosis and regenerated nodules, leading to liver dysfunction. Cirrhosis of the liver is generally caused by viruses, hepatitis B and C, alcoholism and fatty liver disease. In severe cases, decreased protein binding may occur due to reduced serum concentration of albumin. Thus, decreased protein binding may give rise to an elevated volume of distribution and this may prolong the elimination $t_{1/2}$ of the drug bounded to the plasma protein (Bremier, 1987).

Although high systemic bioavailability of a drug administered orally can occur due to reduced enzyme activity, it has also been suggested that increased bioavailability of an orally administered drug can arise as a result of the presence of portal-systemic shunting in cirrhosis of the liver (Bremier, 1987). Portal-systemic shunting is characterised by a partial bypass of the liver of blood containing absorbed drug. In patients with extensive shunting and severe liver dysfunction, this partial bypass of the liver could result in almost complete bioavailability of the administered drug (Bremier, 1987). Furthermore, hepatic blood flow may be reduced in patients with cirrhosis of the liver as a result of scarring within the cirrhotic liver. The consequence is the reduced systemic clearance of drugs highly metabolised by the liver (Bremier, 1987).

Liver failure is the deterioration of liver function as a result of liver damage, which can alter the binding of a drug to plasma proteins. Here, the amount of plasma protein is decreased, which would lead to a higher fraction of unbound drug and consequently a greater therapeutic effect.

Drug-induced hepatotoxicity reduces the metabolic rates of most drugs due to damage to liver cells and liver microsomal enzymes, leading to increased bioavailability of drugs and possibly toxic effects of drugs (Rodghiero, 2000). Hepatotoxicity caused by ethionamide, for example, influences its metabolism by preventing microsomal enzymes, leading to a high fraction of the administered dose that reaches systemic circulation (Weyer et al., 2004).

2.4.5.5 Congestive cardiac failure

Congestive cardiac failure is a disease characterised by a decrease in cardiac output and a decrease in renal and hepatic blood flow and as a result, renal and hepatic insufficiencies. In case of renal failure, the decrease in glomerular filtration rate (GFR) can cause the accumulation of drugs that depend on renal elimination for clearance. The reduced renal excretion of drugs may lead to the accumulation of drug in plasma and consequently to having drugs toxic effects. Tubular secretion or active transportation into the urine contributes to the elimination of many anti-bacterials such as B-lactam antibiotics. The blockage of the active transportation of anti-bacterials as a result of competition may prolong their half-life ($t_{1/2}$). Congestive cardiac failure can also impair hepatic drug metabolism by altering the metabolic capability of liver and influencing the rate of drug delivery to the liver via hepatic blood flow. Furthermore, congestive cardiac failure may also decrease the absorption of drugs by reducing gastric emptying or decreasing blood flow to the site of drug absorption in the small intestine. This will lead to the decreased plasma concentration of drugs.

2.4.5.6 Renal disease

Some diseases affecting the kidney alter plasma protein levels and contribute significantly to changes in drug-protein binding. These diseases can be HIV induced and include nephritic syndrome and HIV-associated glomerular-nephritis. Renal disease can be drug induced or caused by conditions such as shock, pyelonephritis, diabetes, urinary tract obstruction or hypertension.

Renal dysfunction has an effect on the metabolism of drugs. Most drugs are first transformed into metabolites before being excreted. Renal failure may not only prevent the excretion of these metabolites, but in some cases alters the metabolic clearance of drugs.

In general, impaired renal function has been reported to alter the plasma-protein binding and tissue-protein binding of drugs. The resultant effect is the altered bioavailability of drugs that are susceptible to plasma or tissue binding.

2.4.5.6.1 Plasma-protein binding

In addition to the altered bioavailability of drugs that are susceptible to plasma or tissue binding, impaired renal function is associated with notable changes in the plasma-protein binding of some drugs. According to a study by Rodighiero (2000), it was found that for every acidic drug studied, serum-protein binding in uremic patients was decreased. The reason for the decreased binding was suggested to be as a result of acidic drugs binding to albumin, which plays a role in the distribution volume of acidic drugs.

The decreased protein binding that arises in a case of renal impairment has been attributed to reductions in the level of serum albumin, structural changes in binding sites or the competitive displacement of drugs from albumin binding sites by organic molecules, resulting in increased V_d of these drugs (Atkinson, 2000). The increased V_d of these drugs might lead to unwanted side effects in patients.

2.4.5.6.2 Tissue binding of drugs

Impaired renal function might cause an alteration in the tissue binding of drugs, which may affect the PK of drugs, especially those with a high apparent V_d (Atkinson, 2000). The reduced tissue binding of drugs might lead to a high volume of distribution of drugs and the increased clearance of drugs susceptible to tissue binding. The resultant effect is reduced therapeutic benefit of the drugs.

2.4.5.7 Human immunodeficiency viral infection

HIV-infected patients have been noted as having as much as a 70% decrease in serum drug concentration when compared with controls, although non-absorbers have also been identified within non-HIV patients (Douglas and Mcleod, 1999).

In a study conducted by Peloquin et al. (1993), low CD4 count was indicative of malabsorption, but this was not seen in patients with AIDS who were co-infected with TB (Peloquin et al., 1993), although in a later study by Peloquin et al. (1996) it was found that serum peak concentrations of anti-TB drugs were lower in TB patients co-infected with HIV and having a CD4 cell count of <200 cell/mm^3 (Peloquin et al., 1996; Budha et al., 2008).

When there is poor absorption of one or more drugs in a TB treatment regimen, sub-therapeutic concentration could enhance the possibility of resistance developing to other drugs in the regimen of TB therapy (Peloquin, 2002). Using serum-concentration response data, it was confirmed that the reduced bioavailability of anti-mycobacterial drugs could have serious consequences, such

as delayed or incomplete response to treatment or the selection of drug resistance for HIV-positive patients co-infected with TB (Choudri et al., 1997). Furthermore, second-line anti-TB drugs such as pyrazinamide and terizidone are less effective than INH and RIF; hence, patients on MDR-TB therapy may be at a greater risk of poor treatment response if malabsorption occurs.

It has also been suggested that patients infected with HIV with or without diarrhoea may not sufficiently absorb anti-TB drugs. The study published by Gurumurthy et al. (2004) reported that HIV-infected patients with or without TB had lower plasma concentrations of RIF and INH well below the therapeutic range. In addition, it was stated that malabsorption of pyrazinamide and ethambutol was evident in HIV-positive patients co-infected with TB when compared to HIV-infected patients without TB (Gurumurthy et al., 2004).

Some studies have suggested that malabsorption of anti-mycobacterial drugs occurs in HIV-infected patients with advanced HIV infection (Patel et al., 1995; Berning et al., 2003; Gurumurthy et al., 2004). This resulting lower anti-TB drug exposure may result in acquired drug resistance and the reduced efficacy of anti-TB therapy in patients infected with HIV (Gurumurthy et al., 2004).

2.4.6 Drugs with side effects that may affect the pharmacokinetics of anti-tuberculosis drugs

ARVs with gastro-intestinal side effects may potentiate similar side effects in patients receiving MDR-TB treatment. The selective malabsorption of more than

one anti-TB drug used in the treatment regimen could promote drug resistance. Ethionamide produces intense gastrointestinal effects such as diarrhoea, nausea and vomiting. Diarrhoea, as stated above, can increase the gastric motility of ethionamide, leading to reduced resident time in the intestine, decreasing its absorption. In addition, diarrhoea is the major side effect of protease inhibitors that can reduce the bioavailability of anti-TB agents.

Nephrotoxicity as a result of the prolonged administration of nephrotoxic drugs such as vancomycin, and the co-administration with other toxic drugs, especially aminoglycosides, may result in the accumulation of anti-TB drugs in kidney tubules, leading to a longer half-life, which could be deleterious in patients.

Hepatotoxicity induced by ethionamide can cause liver injury. When major liver damage occurs, as evidenced by jaundice, with a prolonged elevation of transaminases during a period of six to eight weeks, ethionamide administration should be stopped. Ethionamide should be carefully monitored in patients with liver disease, diabetes, alcoholism or mental instability. (Weyer, 2004).

2.5 Interaction between anti-retroviral drugs and anti-tuberculosis drugs

Due to the susceptibility of HIV-infected individuals to numerous opportunistic infections, they usually receive a wide variety of drugs in addition to their ARV therapy (De Maat et al., 2003). HIV-positive patients taking ARVs and co-infected with TB have an additional problem of drug–drug interactions. For example, non-nucleoside reverse transcriptase inhibitors and protease inhibitors

are metabolised by CP450 and there is a significant PK drug interaction when they are administered concurrently with other drugs metabolised via the same enzyme pathway (Burman et al., 1999; De Maat et al., 2003). TDM should therefore be used to establish the adequacies of anti-TB drug doses and ARV drug doses, particularly protease inhibitors (PIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) (Peloquin, 2002).

Furthermore, HIV-infected patients tend to be more susceptible to adverse reactions such as peripheral neuropathy, cutaneous reactions, renal toxicity and gastro-intestinal disturbances (Weyer, 2005). Diarrhoea is one of the most common gastro-intestinal disturbance symptoms reported in HIV-positive patients and may result in drug malabsorption (Gurumurthy et al., 2004). The malabsorption of MDR-TB drugs has been reported in patients with HIV-related enteropathology (Weyer, 2005).

Studies demonstrating interactions between second-line drugs that are used in MDR-TB treatment regimens and ARVs are still very few in numbers, although the potential for adverse interactions is considerable. TDM of drugs used in TB management is supported as TDM helps in better control of TB treatment, particularly in patients co-infected with HIV and in managing anti-TB drugs interactions (Peloquin, 2002).

2.5.1 Interaction between tenofovir and kanamycin

The South African public sector ARV treatment guidelines have recently been changed to include tenofovir in the first-line regimen (National Department of Health, 2010). Drugs from the aminoglycoside class such as kanamycin are part of the MDR-TB treatment regimen in the South African tuberculosis programme. The aminoglycosides are potent nephrotoxins, in part due to the high concentration they attain in proximal convoluted tubular cells. Like aminoglycosides, tenofovir also attains high concentrations in proximal convoluted tubular cells as a result of active uptake into these cells (Kenyon et al., 2011). According to Kenyon et al. (2011), a substantial proportion of patients taking tenofovir may develop certain of the features of Fanconi's syndrome (a proximal tubular wasting syndrome as a result of proximal convoluted tubular cells dysfunction).

Aminoglycosides are nephrotoxic, producing renal impairment. Patients taking amikacin are more likely to develop nephrotoxicity than patients taking streptomycin (Arbex et al., 2010). Tenofovir may also cause renal failure. Reports show that tenofovir is associated with mild decreases in GFR when compared with patients on other ARVs (National Department of Health, 2010).

2.5.2 Interaction between alcohol and drugs

Alcohol can interact with many medications, causing reduced effectiveness of administered medications and increased risk of illness, injury or death by altering the bioavailability of medications. Acute consumption of alcohol inhibits the

metabolism of a drug by competing with the drug for the same metabolising enzymes. This interaction increases the drug's bioavailability and could potentiate the risk of unwanted side effects of the drug administered. However, a chronic dose of alcohol may induce a drug's metabolising enzymes, thus decreasing the bioavailability of the drug and diminishing its effects. Even in the absence of alcohol, the enzymes activated by alcohol are available in the blood stream and impair the metabolism of susceptible drugs.

2.6 Overview of laboratory findings on renal and liver function

2.6.1 Renal function tests

Renal or kidney function is generally assessed by measuring serum creatinine, urea concentration levels, or circulation of the GFR (Lamb, 2007). The GFR is widely used as the best indicator of kidney function because it makes possible the detection and classification of chronic kidney disease (Stevens et al., 2006; Lascano et al., 2010). In practice, the GFR is difficult to measure, therefore serum creatinine is used by clinicians to determine it (Levey et al., 1999). Kidney function ranges from normal GFR, >90 ml/min, to renal failure, GFR <15 ml/min (DiPiro et al., 2008).

Creatinine is a by-product of muscle breakdown and is filtered out of the blood by the kidney. There is almost no reabsorption of creatinine by the renal tubules, so any dysfunction in the ability of the kidney to filter results in a build-up of creatinine in the blood. Creatinine levels in the blood can be used to determine creatinine clearance, which indicates the GFR. The use of serum creatinine alone

presents some limitations, and in order to overcome them, equations for estimating the GFR take into account variables such as gender, age, race and body weight (Levey et al., 1999; Stevens et al., 2006).

The Cockcroft-Gault formula for estimating the GFR is commonly used and takes into account serum creatinine, body weight and an adjustment factor for gender (Simon et al., 2011). In addition, the Cockcroft-Gault formula over-estimates the GFR due to the active secretion of creatinine in the renal tubules (Stevens et al., 2006). Furthermore, this formula does not give room for the adjustment of body surface area, but relies solely on individual body weight, which could be misleading.

Another method for estimating the GFR is using the modification of diet in renal disease (MDRD) method. The MDRD formula adjusts for body surface area (1.73 m^2), age, gender and race. This is because the muscle mass of women is lower compared to that of men. Also, the black race tends to have an increased muscle mass when compared to the white population. The major limitation of the MDRD equation is that it is likely to under-estimate GFR at higher ranges (>60 ml/min/1.73 m^2) of kidney function (Stevens et al., 2006; Simon et al., 2011). However, the MDRD formula is a widely accepted and preferred method in evaluating the GFR as compared to previously used methods (Stevens et al., 2006; Lascano et al., 2010; Aggarwal et al., 2011).

2.6.1.1 Risk factors that might impair kidney function

Several factors contribute to the loss of kidney function in patients. They include drug-induced renal toxicity, HIV and co-existing diseases. The epidemiology of chronic kidney disease is rising in the world, and sub-Saharan Africa is considered to have the highest burden (Retnakaran et al., 2006). The rise of chronic kidney disease in South Africa is associated with the surge of diabetes and hypertension, which are potential triggers of the disease, together with the burden of HIV. Hypertension is believed to be a trigger of end-stage renal disease in 34.6% of blacks, 4.3% of whites, 20.9% of mixed-race (coloured) people and 13.9% of Indians in South Africa (Katz, 2005). In addition, diabetes is considered to occur in 10–16% of South Africans (Katz, 2005).

2.6.1.1.1 Human immunodeficiency virus-related kidney dysfunction

The introduction of highly active ARV therapy has helped in reducing the epidemiology of kidney disease in HIV-positive patients; however, kidney disease still remains a major cause of mortality and morbidity among the HIV-infected population (Cianflone et al., 2010).

HIV can infect the kidneys, as presented by HIV-associated nephropathy, which is evident by a rapid decline in renal function, lower CD4 count and proteinuria (Jeremy et al., 2008) due to HIV directly infecting renal cells (Gerntholtz et al., 2006). A study done in Soweto, South Africa, reported that HIV-positive patients exhibited HIV-associated nephropathy at any stage of HIV infection and had varying degrees of GFR and proteinuria (Gerntholtz et al., 2006). In addition,

acute kidney failure is known to be a complication of HIV infection in patients taking highly active ARV therapy. Acute kidney failure is defined as a rapid decrease in GFR in patients with previously normal kidney function or in patients with pre-existing chronic kidney disease (Roe et al., 2008). It was reported that acute kidney failure is manifested within three months after the commencement of highly active ARV therapy, commonly in patients with advanced HIV infection and in those with co-existing opportunistic infections (Roe et al., 2008).

2.6.1.1.2 Antiretroviral-induced nephrotoxicity

The adverse effects of ARVs account for 14% of acute kidney injury occurring after three months of commencing highly active ARV therapy and are usually reversible once the offending drug is discontinued (Roe et al., 2008; Kalyesubula and Parazella, 2011). Nucleoside reverse transcriptase inhibitors (NRTIs), such as zidovudine, didanosine, zalcitabine, stavudine, lamivudine, abacavir and emtricitabine, cause kidney toxicity, which is exhibited as lactic acidosis. Didanosine might cause electrolyte imbalance, which is manifested as hypokalaemia, hyponatremia, hypermagnesemia and hyperuricemia, and stavudine may cause hyperuricemia (Kalim et al., 2008). Tenofovir is an NRTI and has a potent tendency to cause kidney dysfunction.

Female HIV-positive patients with pre-existing kidney disease, undergoing prolonged treatment with ARVs, experiencing elevated serum creatinine concentration and co-administration of other nephrotoxic drugs, and with a CD4 count of <200 cells/mm^3 are more likely to present with tenofovir-related kidney

toxicity (Roe et al., 2008; Cianflone et al., 2010; Kalyesubula and Parazella, 2011). PIs such as indinavir are potent inducers of acute kidney injury. Ritonavir, which is in the same class of ARV as indinavir, has the potential to cause kidney injury. Reports show that about 70% of tenofovir-related cases of induced nephrotoxicity are observed with the co-administration of low doses of ritonavir (Roe et al., 2008; Kalyesubula and Parazella, 2011). Among the NNRTI class of ARVs, nevirapine has the most potential to cause kidney toxicity, which presents as lactic acidosis.

Furthermore, older age was established as an independent risk factor in causing a reduction in serum creatinine concentration among HIV-infected individuals (Cianflone et al., 2010). It was also published that pre-existing AIDS and a history of a low CD4 count are potential factors in the alteration of kidney function (Cianflone et al., 2010).

2.6.1.1.3 Co-existing diseases

Previous studies have defined co-existing diseases as an important risk factor for developing kidney dysfunction in patients infected with or without HIV. Hepatitis C virus co-infection has been confirmed as contributing to kidney disease (Roe et al., 2008). Hypertension is a leading cause of kidney disease and kidney failure. It can damage blood vessels in the kidney, making the elimination of wastes and fluids from the body difficult. In addition, kidney dysfunction is common in patients with type-2 diabetes. Loss of kidney function in these patients occurs at the early stage of diabetes due to micro-albuminuria (Retnakaran et al., 2006).

Micro-albuminuria is characterised by small amounts of protein albumin in the urine and is clinically used as an indicator of kidney dysfunction (Retnakaran et al., 2006).

2.6.1.1.4 Co-administered drugs

Certain medications are implicated in kidney dysfunction. Some examples include aminoglycoside antibiotics, acyclovir, amphotericin B, amitriptyline, fluoxetine, non-steroidal anti-inflamatory drugs and anti-depressants, among others. Risk factors for drug-induced nephrotoxicity include older age, pre-existing renal insufficiency, sepsis, volume depletion and diabetes (Roe et al., 2008). Nephrotoxicity due to drugs is usually reversible once the offending drug is withdrawn.

2.6.2 Liver function tests

The primary function of the liver is drug metabolism. The healthy liver transforms drugs to biologically active forms via the CP450 enzyme pathway (Aaron et al., 2004; Puoti et al., 2009). Also, the liver detoxifies the blood of damaging chemicals and metabolites (Kaplowitz, 2004; Puoti et al., 2009). Alanine aminotransaminase (ALT) and aspartate aminotransaminase (AST) are generally used as biomarkers of liver injury (Puoti et al., 2009; Aragon et al., 2010). ALT has the highest concentration in the liver when compared to other organs in the body, which makes it the most specific indicator of liver injury. AST is present in many tissues of the body, such as muscles and the brain. Gamma glutamyl

transpeptidase is found in liver and biliary epithelial cells and is the most specific marker of hepatobilary disease.

Another method used to determine the severity of liver disease is Child-Pugh score. The Child-Pugh scoring system has been a common application in evaluating the risk of developing liver disease and the chances of recovery or survival. It employs a grading scale to classify liver disease. Based on this classification, mild liver disease (class A) falls in the range of 5-6 points, moderate (class B) falls between 7 and 9 points and severe (class C) is between 10 and 15 points (Chatzicostas et al., 2003).

2.6.2.1 Risk factors that might elevate the level of liver enzymes

Biomarkers of liver injury such as ALT and AST are elevated in certain diseases, while certain medications have the potential to raise the serum level of liver enzymes. HIV-positive patients taking ARVs might have an elevation in their liver enzymes (Gisolf et al., 2000), and this is the common cause of mortality and morbidity in patients infected with HIV (Soriano et al., 2008).

2.6.2.1.1 Human immunodeficiency virus-related liver disease

For patients with HIV infection, liver disease contributes to greater morbidity, higher costs of care, and increased risk of liver failure and death (Puoti et al., 2009). Lymphoma, Kaposi's sarcoma and bilary duct disease are examples of liver diseases and intrahepatic opportunistic infections that occur commonly in

HIV-infected patients (Leucuta and Nlase, 2006) and they can alter the PK of administered drugs.

Liver failure and liver-related death have been described mainly in patients with higher liver enzyme elevation. HIV-positive patients take a combination of ARVs and almost every ARV has been implicated in liver enzyme elevations (Soriano et al., 2008; Puoti et al., 2009). Nevirapine and, less frequently, efavirenz have been found to increase gamma glutamyl transpeptidase serum levels (Clarke et al., 2002; Soriano et al., 2010)

2.6.2.1.1 Antiretroviral disease-induced hepatotoxicity

The risk of ARV-induced liver injury increases with NNRTIs, which have the highest incidence, followed by PIs and NRTIs (Puoti et al., 2009). NNRTIs such as nevirapine, efavirenz and enfuvirtide induce liver injury by provoking allergic reactions in HIV-positive patients. Symptoms of allergy are evident within a few days to eight weeks after the commencement of treatment and they include a rash, fever, fatigue and gastro-intestinal symptoms such as nausea, vomiting, abdominal pain and diarrhoea. Protease inhibitors are less implicated in serious liver-related side effects (Puoti et al., 2009). Ritonavir has been found to be more hepatotoxic when administered in full dose compared to other drugs in the same class (Soriano et al., 2008).

NRTIs have been found to cause mitochondrial toxicity that could result in lactic acidosis and hepatic steatosis (Clark et al., 2002, Soriano et al., 2008).

Mitochondrial toxicity occurs after prolonged NRTI treatment and result when drugs in the NRTI class interfere with the synthesis of mitochondrial DNA. NRTIs that are associated with mitochondrial toxicity include zalcitabine, didanosine, stavudine, zidovudine and abacavir, in descending order of potency (Soriano et al., 2008; Heil et al., 2010).

2.6.2.1.3 Co-existing diseases

Apart from HIV, other co-existing diseases in patients might elevate serum liver enzymes. Some of these diseases include alcohol-related liver disease and chronic viral hepatitis. Alcohol-related liver disease is characterised by a rise in ALT and AST levels, that is, if the AST level is two to three times higher than the normal value, together with a history of excessive alcohol use in patients. Chronic viral hepatitis is characterised by a minimal rise in ALT and AST levels, that is, two to three times higher than the upper limit (Aragon et al., 2010).

2.6.2.1.4 Co-administered drugs

Drug-induced liver injury includes the induction of hepatic enzymes, such as in the case of anti-epileptics, hypersensitivity reactions, idiosyncratic reactions and auto-immunity. Non-steroidal anti-inflammatory drugs and penicillin-derived antibiotics are also implicated in drug-induced liver injury. In patients with liver failure, aminoglycosides can be administered at full doses; however, patients with severe liver failure should be screened for co-existing hepatorenal syndrome (Arbex et al., 2010).

2.7 Kanamycin pharmacology and pharmacokinetics

2.7.1 Overview

Kanamycin is a bactericidal agent and belongs to the aminoglycoside antibiotic group. The structure of aminoglycosides consists of two or more aminosugars linked by glycosidic bonds to an aminocyclitol component. They are highly water soluble because of the many amino and hydroxyl groups in their chemical structure.

2.7.2 Kanamycin anti-microbial spectrum of activity and mechanism of action

Kanamycin exerts its effect by binding to the 30S sub-unit of bacterial ribosomes, thus leading to a reduced mRNA reading and, finally, impaired protein synthesis (Perri and Bonora, 2004). Bactericidal activity has been categorised as either concentration dependent or time dependent. Kanamycin has a concentration-dependent bactericidal activity because its rate of bacterial killing increases as its concentration increases over a wide range of concentration (Nuermberger and Grosset, 2004). Kanamycin exhibits non-saturable concentration-dependent killing (Arbex et al., 2010), which means that it is effective even when its serum concentrations are below the minimum inhibitory concentration (MIC).

2.7.3 Activity against Mycobacterium tuberculosis

Kanamycin has potent bactericidal activity against rapidly dividing mycobacteria and has little or no activity against non-replicating bacilli in the stationary or sterilising phase of growth (Perri and Bonora, 2004). Kanamycin is not effective against intracellular bacteria because it does not penetrate mammalian cells and,

as a result, it is used only in the bactericidal phase. For a drug to be used in the stationary or sterilising phase, it must be active against intraphagocytic and slowly dividing mycobacteria. This is the rationale of using kanamycin only in the bactericidal phase, when a large number of replicating mycobacteria are present at extra-cellular level (Perri and Bonora, 2004).

Kanamycin exerts bacterial activity against MTB at a concentration of about 6 µg/ml in vitro and shows rapid inhibitory effect at concentration above its MIC (Nuermberger and Grosset, 2004). Kanamycin's activity relies on a high C_{max}:MIC ratio (Douglas and Mcleod, 1999). For optimal anti-bacterial effects with kanamycin, a high C_{max} in relation to MIC or a high extent of anti-microbial exposure as determined by the AUC of time versus concentration above the MIC is needed (Douglas and Mcleod, 1999).

2.7.4 Pharmacokinetics of kanamycin

Understanding the PK of kanamycin is important in determining the time course of anti-mycobacterial action and in optimising the dose of kanamycin in patients with MDR-TB and in those with MDR-TB and co-infected with HIV.

2.7.4.1 Absorption

Kanamycin is inadequately absorbed from the gastro-intestinal tract. When kanamycin is administered orally or rectally, less than 1% of its dose is absorbed (Budha et al., 2008). The oral absorption of aminoglycosides is minimal because aminoglycoside antibiotics are very hydrophilic and poorly lipid soluble

compounds. Due to kanamycin's polar nature, it is not used in the maintenance phase of MDR-TB treatment because its penetration into cells is poor and its distribution limited to extra-cellular spaces (Budha et al., 2008). As a result, it is administered parenterally (Coyne et al., 2009). The absorption of kanamycin is complete when administered intramuscularly and the serum levels peak within 30–90 minutes (Budha et al., 2008).

2.7.4.2 Distribution

Aminoglycosides exhibit low protein binding (approximately 10%) (Pechere and Dugal, 1979; Arbex et al., 2010). The distribution of aminoglycosides is limited to extracellular spaces, which makes them to act only on extra-cellular bacilli.

2.7.4.3 Metabolism and elimination

Most of the enzymes that catalyse the metabolism of foreign compounds in the body are intracellular and since aminoglycosides do not penetrate most mammalian cells, they do not undergo significant metabolism.

The elimination of aminoglycosides is primarily by the kidneys (Arbex et al., 2010). About 80–98% of kanamycin is excreted unchanged by the kidneys over a 24-hour period with a serum half-life of about 4 hours (Coyne et al., 2009): 1% is excreted in bile and 1% in faeces (Arbex et al., 2010). This means that kanamycin's excretion may be prolonged in renal-impaired patients (Budha et al., 2008): its elimination half-life increases from 107 minutes in younger individuals to 282 minutes in elderly populations (Holdiness, 1984).

2.7.5 Kanamycin drug interactions

There is a possible risk of drug interactions when administering amphotericin B, vancomycin, cephalosporin, cisplatin and loop diuretics such as furosemide concurrently with aminoglycosides (Arbex et al., 2010). In addition, concomitant administration of aminoglycosides and neuromuscular blocking agents can cause respiratory depression, and patients with conditions such as myasthenia gravis, hypocalcemia, severe hypokalemia or hypomagnesemia are susceptible to such side effects (Arbex et al., 2010).

2.7.6 Side effects of kanamycin and precautions

Ototoxicity and nephrotoxicity are the most important clinical side effects of aminoglycosides. These effects are related to the duration and dosage of aminoglycosides and are most likely to occur in older individuals, dehydrated patients or those suffering from kidney diseases (Arbex et al., 2010).

Since aminoglycosides are majorly eliminated by the kidneys, they produce kidney toxic effects due to their accumulation in the kidney tubules (Arbex et al., 2010). Such effects are common in patients with a history of kidney disease and in elderly patients. Therefore, in patients with a creatinine clearance of <30 ml/min, the dose of kanamycin should be adjusted to 12–15 mg/kg/day administered two to three times a week (Bennett et al., 1977; Arbex et al., 2010). Thus, TDM, which involves the measurement of drug concentration in plasma, can enable dosage adjustment in patients with renal insufficiencies. By measuring drug levels

in the blood at intervals, TDM maintains TB drugs' plasma concentration within the therapeutic range (Taft, 2009).

The most severe side effect caused by aminoglycosides is ototoxicity (Arbex et al., 2010). The risk is high in the elderly, in patients undergoing prolonged periods of treatment and those receiving high doses of kanamycin. Other side effects of kanamycin include hypersensitivity, respiratory depression and neurotoxicity.

2.7.7 Clinical uses

Kanamycin is an antibiotic with a broad spectrum of activity against some gram-positive and gram-negative organisms. Kanamycin is active against aerobic bacilli, but not against anaerobic organisms, because oxygen pathway is necessary for the activity of kanamycin.

2.7.8 Resistance to aminoglycosides

The presence of sub-optimal concentrations of a drug tends to select for drug-resistant organisms. Under these circumstances, mutant strains that possess a degree of resistance are favoured and tend to dominate the bacteria population. Therefore, in order to prevent the development of resistance, it is best to pay careful attention to dosing. For aminoglycosides, a high C_{max}:MIC ratio correlates best with anti-bacterial activity (Nuermberger and Grosset, 2004).

Since the mechanism of action of aminoglycosides is the binding to the 30S sub-unit of bacterial ribosomes, any changes in ribosomal proteins either via mutations

or enzymatic modification would reduce aminoglycoside binding and therefore enhance the rapid selection of resistance within the bacteria population (Davies and Wright, 1997). Another mode of resistance of aminoglycosides is reduced uptake and accumulation of aminoglycosides in bacteria.

2.7.9 Pharmacokinetic parameters of Kanamycin

Although several studies have reported the PK parameters of kanamycin in healthy volunteers, studies on the plasma PK of kanamycin in patients infected with MDR-TB are still very scarce, particularly in South Africa.

2.7.9.1 Pharmacokinetics in healthy volunteers

Many studies have described the PK of kanamycin in healthy volunteers (Cronk and Naumann, 1959; Cabana and Taggart, 1973; Doluisio et al., 1973; Clarke et al., 1974; Kirby et al., 1976; Coyne et al., 2009) using both intravenous and intramuscular routes of administration and different doses. These studies show that the PK parameters of kanamycin are independent of the route of administration and are fairly reproducible in the same healthy individuals at different times (Pechere and Dugal, 1979). However, intramuscular administration of kanamycin is preferred, since the repeated high peaks obtained after intravenous administration are avoided (Pechere and Dugal, 1979).

The previous studies on kanamycin in healthy volunteers shows that its PK vary with age and renal function (Douglas and Mcleod, 1999), and its maximum plasma concentration does not increase with increasing age and is not influenced

by birth weight; however, clearance is directly dependent upon birth weight and post-natal age (Holdiness, 1984). Kanamycin peak serum concentration occurs within 1 hour of an intramuscular dose (Douglas and Mcleod, 1999), because a dose of 7.5 mg/kg results in peak serum levels of 20–35 µg/ml (Budha et al., 2008).

Table 2.3: Kanamycin pharmacokinetic parameters in healthy volunteers

Dose	Subjects	C_{max} (µg/ml)	T_{max} (hr)	$T_{1/2}$ (hr)	AUC (µg/ml/ hr)	K_e (hr^{-1})	V_d (L)	Cl_{tot} (l/hr)	Cl renal (l/hr)	F	Reference
539 mg	10	N/A	N/A	2.3 +/- 0.1	92	0.30 +/- 0.02	22.4 +/-2.7	6.4	4.4	0.68 +/- 0.07	Doluisio et al. (1973)
500 mg	10	N/A	N/A	2.2	105	0.32	18.5	5.9	5.3	0.90	Cronk and Naumann (1959)
1,000 mg	10	N/A	N/A	2.7	192	0.26	20.6	5.3	4.5	0.84	Cronk and Naumann (1959)
2,000 mg	20	N/A	N/A	2.4	395	0.29	17.7	5.1	4.3	0.83	Doluiso et al. (1973)
15 mg/kg	N/A	22.5	N/A	4	N/A	N/A	N/A	N/A	N/A	N/A	Coyne et al. (2009)
250 mg	12	11.9	0.80	2.1	44.2	N/A	21.7	N/A	7.9	0.84	Cabana and Taggart (1973)
500 mg	12	20.6	1.0	2.3	90.0	N/A	23.6	N/A	7.13	0.77	Cabana and Taggart (1973)
614 mg	4	18.0	2	2.0	N/A	N/A	N/A	N/A	N/A	N/A	Clarke et al. (1974)

As indicated in Table 2.3, F = bioavailability

2.7.9.2 Pharmacokinetic parameters in patients with multidrug resistant tuberculosis

Yew et al. (1999) presented a study on the serum PK of anti-mycobacterial drugs in HIV-negative patients with MDR-TB during therapy. In this study, serum samples of 13 patients with MDR-TB were taken 0, 1, 2, 4 and 8 hours after the administration of anti-mycobacterial drugs for assay of levels in order to gain insight into their basic PK. The drugs assessed were amikacin, kanamycin, ofloxacin, para-aminosalicyclic acid, prothionamide, cycloserine, pyrazinamide and ethambutol. The results from fluoroimmuno assay of kanamycin showed that after a dose of 15 mg/kg, C_{max} was achieved at 30.94 +/-7.72 mg/L (mean +/-SD), T_{max} was recorded at 0.5–1.5 hours, $t_{1/2}$ was estimated to be 3.7 hours, the C_{max}:MIC ratio was recorded as 8–16 and MIC was achieved within a range of 2–4 mg/L (Yew, 1999).

2.7.9.3 Pharmacokinetic-pharmacodynamic relationship of kanamycin

Aminoglycosides have concentration-dependent bactericidal activity, therefore the goal of anti-mycobacterial therapy is to maximise the concentration of these antibiotics. PK parameters such as the AUC_{0-24}: MIC ratio and C_{max}: MIC ratio correlates best with the rate of bacterial killing (Nuermberger and Grosset, 2004). For kanamycin, the ideal dosing regimen would maximise concentration, because the higher the concentration, the higher the extent of bacterial killing. In order to optimise drug therapy, dosing strategies for kanamycin include the highest possible dose that does not lead to toxicity in order to maximise drug

concentrations and therefore the rate of killing at site of mycobacterial infections. For aminoglycosides, it is best to have a C_{max}: MIC ratio of at least 8–10 to prevent resistance (Nicolau, 2003).

2.7.9.4 How therapeutic drug monitoring may optimise the dose of kanamycin

A standard dose of 15 mg/kg intramuscularly produce C_{max} values of 35–45 µg/kg (Peloquin, 2002). According to Peloquin (2002), it is probable that repeated intramuscular injection may harden the tissue and alter the absorption of kanamycin over time, which may interfere with C_{max}. Furthermore, kanamycin excretion is dependent on renal elimination. Thus, the adequacy of C_{max} and the capability of the kidneys in excretion can be detected using the two- and six-hour sampling strategy. A dose increase is expected if peak concentration is less than 75% of desired range (Peloquin, 2002). TDM may be useful to prevent further resistance in patients with MDR-TB and in those susceptible to drug–drug interactions that may complicate clinical situation (Peloquin, 1997).

2.8 Overview of methods used for the determination of plasma concentrations of anti-tuberculosis drugs

Several methods have been suggested by previous studies for the determination of concentrations of anti-mycobacterials in biological fluids. These methods could be qualitative, such as nuclear magnetic resonance spectroscopy, mass spectrometry and x-ray crystallography, or quantitative, like radio-chemical assay, microbiological assay, radio-immuno assay and enzyme-immuno assay.

Although X-ray crystallography, microbiological assay and enzyme-immuno assay are easy to use, they are not specific (La and Feng, 2007). HPLC methods are reliable and are widely utilised for the analysis of anti-mycobacterials. HPLC combined with MS analysis of anti-TB agents is rapid, sensitive and specific and could successfully be applied to TDM in TB patients (La and Feng, 2007).

2.8.1 Liquid chromatography coupled with mass spectrometry

The use of LC/MS has been developed and approved for the instantaneous quantification of anti-TB agents in human plasma (Brewer and Henion, 1998). The LC/MS technique is oriented toward the detection and identification of specific chemicals in the presence of other chemicals, for example, in a complex mixture (Brewer and Henion, 1998). In comparison to ultra-violet (UV) spectrometry, mass spectrometry is used in combination with HPLC due to high sensitivity and specificity as long as the drug to be analysed can be suitably ionised and analysis time is short.

A large number of HPLC techniques coupled with UV, fluorometric or mass spectrometric for the determination of concentration of aminoglycosides and other antibiotics are contained in the literature (Niessen, 1998; Gennaro, Calvino and Abrigo, 2001; Khuhawar and Rind, 2002). Although HPLC coupled with UV and fluorescence detectors have provided satisfactory results, they have limited specificity, and thus it may be difficult to differentiate the analyte from its metabolites in a complicated mixture. Another reason why better analytical methods may be needed is that some very potent drugs are administered at low

doses and as a result are present at very low levels in biological matrices, making them difficult to detect in a complex mixture with other chemicals. Therefore, higher-specificity and -sensitivity capabilities are required, which are not provided by HPLC coupled with UV or fluorescence techniques in the analysis of drugs or their metabolites in biological mixtures.

2.8.2 Previously used methods for determining kanamycin plasma concentrations

Numerous methods have reported the analysis of aminoglycosides in a variety of biological samples such as tissue, milk, blood and urine (Kennedy et al., 1998; Brewer and Henion, 1998; Stead, 2000; Loffler and Ternes, 2003; La and Feng, 2007). These methods include microbiological, radio-immuno and enzyme-immuno assay. They are easily handled, but are not specific (La and Feng, 2007).

2.8.2.1 Microbiological assays

Microbiological assays have been widely used in the past to estimate the potency of anti-bacterials (Maitra et al., 1979; Stead, 2000). Potency is assessed by comparing a standard with the inhibition of growth of a specific micro-organism produced by known concentration of the antibiotic observed (Stead, 2000). Microbiological assays are appropriate for handling a large number of samples, while the equipment used is less sophisticated and relatively inexpensive. The basic method is the agar-diffusion assay (Maitra et al., 1979; Stead, 2000). However, due to the introduction of enzyme-multiplied-immuno assay technique (ELISA), there has been a considerable decline in the use of microbiological assays for the analysis of aminoglycoside over the past 20 years (Stead, 2000). It

was reported that there was inaccuracy in the determination of tobramycin by agar diffusion in comparison with ELISA and HPLC (Sachetelli et al., 1998). Another factor contributing to the reduction in the use of the agar diffusion method is the length of time taken before the results can be read (Stead, 2000). It takes more than six hours for results to be readable (Peng et al., 1977).

2.8.2.2 Radio-immuno assay

Radio-immuno assay is a quantitative method that has been used successfully in determining aminoglycosides in biological fluids (Maitra et al., 1979). This method involves a mixture of the free drug in the sample and a fixed amount of I-labelled drug (tracer) (Stead, 2000). Antigen-antibody complex is precipitated when a second antibody is directed against the first antibody to achieve separation of the bound and free drug. The amount of radioactivity associated with the bound drug is measured using a gamma counter. Although radio-immuno assay is highly sensitive and specific, the equipment is expensive and cross-reactions between aminoglycoside antibiotics have been reported (Peng et al., 1977; Stead, 2000).

2.8.2.3 Enzyme-linked immuno-sorbent assay

In ELISA, an antigen binds to a solid immobilised surface and then conjugates with an antibody that is specific to an enzyme. ELISA can also be competitive or non-competitive assays (Stead, 2000). In competitive ELISA, antigen in a sample (free antigen) and plate-adsorbed antigen (bound) compete for the detection antibody. Competitive ELISA is developed for low molecular weight compounds such as aminoglycosides, because they possess only one antibody-binding site. In

non-competitive ELISA there is binding of antigen in the sample to an excess of detection antibodies.

The use of ELISA to detect aminoglycosides is difficult due to low molecular weight compound adsorption to solid phases (Sachetelli et al., 1998). Also, in a study on the analysis of tobramycin it was recorded that ELISA and HPLC gave equal results, while analysis with microbiological agar diffusion was an overestimation of the initial quantity (Sachetelli et al., 1998).

Furthermore, studies done on tobramycin using ELISA were not tested on samples with complicated matrices (Sachetelli et al., 1998). Hence, according to Stead (2000), it is not known whether ELISA would be appropriate for applications involving tissue or plasma, since many molecules in the sample would compete with the analyte for the non-specific binding site (Sachetelli et al., 1998). Another potential barrier in using ELISA for plasma or tissue samples is the specificity of enzyme properties. This may affect this type of assay and in some cases normal constituents of plasma may interfere with the assay.

2.8.2.4 High-pressure liquid chromatography

In PK studies, methodologies must provide high sensitivity and exceptional specificity, because the concentration-time curve determines how well a drug is being cleared by an individual and how well the analytical system is working (Want et al., 2003). HPLC currently meets the requirements for the determination of the plasma concentration of aminoglycosides (Stead, 2000**)**. HPLC methods are

reliable and widely used for the analysis of aminoglycoside antibiotics; however, the major disadvantage of HPLC in the assay of aminoglycosides is the lack of chromophore or fluorophore, which makes them unsuitable for UV or fluorescence detectors (Stead, 2000). Therefore, it is essential that aminoglycosides are labelled with a chromophore or fluorophore tag by a chemically derivatised method, which is complex and time-consuming. Mass spectrometry is the method of choice for detecting aminoglycosides due to the lack of chromophore and fluorophore (Loffler and Ternes, 2003).

2.8.3 Separation method for High-pressure liquid chromatography

Aminoglycosides are very water-soluble compounds due to many amino and hydroxyl groups in their chemical structure (La and Feng, 2007). Due to the polarity and adsorption properties of these compounds, several challenges arise when using HPLC for chromatography differentiation of aminoglycosides (Loffler and Ternes, 2003). Since aminoglycosides are not retained by reversed-phase column because of their hydrophilic nature, a volatile agent is necessary for MS detection and this may be accomplished using perfluorinated carboxylic acids and ammonium salts (ion exchange) or heptafluorobutyric acid (ion pairing). Ion-pairing HPLC is generally preferred to ion-exchange HPLC because it is more efficient, and control over selectivity and resolution is easier (Stead, 2000).

2.8.4 Sample preparation for High-pressure liquid chromatography analysis

The purpose of sample preparation for HPLC analysis is to concentrate the analyte from the matrix, leaving behind substances that may hinder the processes of

derivatisation, isolation and detection. Aminoglycosides are highly water-soluble molecules and are immiscible in water-insoluble liquid; therefore liquid-liquid extraction with organic solvent is difficult. Solid-phase extraction is appropriate for separating polar compounds (La and Feng, 2007). A number of solid-phase extraction columns are commonly used to extract aminoglycosides from biological samples (Peng et al., 1977; Keevil et al., 2003).

In this study, LC/MS will be the preferred method in the quantification of kanamycin plasma concentration due to its high specificity and sensitivity compared to the other methods described above.

2.9 Hypothesis

2.9.1 Experimental hypothesis

- There are differences in the PK parameters between HIV-positive and HIV-negative patients
- HIV influences the PK of kanamycin.
- Changes in kidney function influence PK of kanamycin.
- Interactions between ARVs and kanamycin affect the PK of kanamycin.

2.9.2 Null hypothesis

- There is no difference in the PK parameters of kanamycin between patients infected with MDR-TB (control group) and patients co-infected with MDR-TB and HIV (experimental group).
- HIV does not influence the PK of kanamycin.

- Changes in kidney function do not influence the PK of kanamycin.
- Interactions between ARVs and kanamycin do not affect the PK of kanamycin.

2.10 Research questions

Issues important to conceiving the research question were discussed in the literature review. These issues are summarised below.

To the best of my knowledge, no previous study has investigated the PK of kanamycin in patients infected with MDR-TB and in those with MDR-TB co-infected with HIV using LC/MS analysis or any other analytical method. Therefore, it would be interesting to determine the PK parameters of kanamycin in these patients using LC/MS and to find out if HIV changes the PK of kanamycin in MDR-TB patients co-infected with HIV.

Secondly, we seek to find out whether the changes in kidney function that are common in patients suffering from MDR-TB and MDR-TB plus HIV infection have any influence on the PK of kanamycin.

Thirdly, many of the patients with MDR-TB and co-infected with HIV are on ARV therapy, therefore we would like to find out if there is any interaction between ARVs and drugs used for MDR-TB treatment in patients infected with MDR-TB and HIV.

The study therefore attempts to answer the following questions:

1. Is LC/MS a reliable method for the determination of kanamycin plasma concentrations?
2. What are the PK parameters of kanamycin in patients with MDR-TB and in patients with MDR-TB co-infected with HIV?
3. Could HIV infection influence the PK of kanamycin in MDR-TB patients?
4. To what extent do changes in kidney function influence the PK of kanamycin?
5. Do lamivudine, stavudine, efavirenz and tenofovir affect the PK of kanamycin?

2.10.1 Objectives of the study

The objectives of the present study are:

1. To determine the plasma concentration of kanamycin using LC/MS.
2. To determine the PK of kanamycin in patients with MDR-TB infection and in patients with MDR-TB co-infected with HIV;
3. To determine if HIV infection influences the PK of kanamycin;
4. To assess if kidney dysfunction affects the PK of kanamycin; and
5. To find out if there is any interaction between ARVs and kanamycin and to what extent it influences the PK of kanamycin.

CHAPTER 3: METHODS

3.1 Structure of the methods

Chapter 3 gives a detailed description of the methods used in the study. The first section provides the structure of chapter three. The second section gives a brief description of the duration of the study and the study site. In the third section the study design is described. In the fourth and fifth sections criteria for including and excluding participants in the study are highlighted. The sixth section discusses the demographic, clinical and therapeutic characteristics of patients.

The seventh section gives a detailed description of laboratory tests done, including where they were performed. The eighth section provides study procedures. The process of kanamycin administration and blood sampling are explained. The ninth section explains the processes of determining kanamycin plasma concentrations in detail. The chemicals used in the study and their sources are listed, while chromatographic and mass spectrometric conditions are also described and the preparation of kanamycin stock solution and calibration standard is explained.

The tenth section provides the determination of the PK parameters of kanamycin, as well as the definition of these parameters. The eleventh section explains the determination of the number of patients and statistical methods used in the analysis of data. The final section presents ethical processes that were considered and followed.

3.2 Study site

The study was conducted at Brewelskloof Hospital in Worcester, Western Cape Province, South Africa. This is one of the South African hospitals specialising in the treatment of MDR-TB.

3.3 Study design

The study was designed as a prospective, two-group, non-randomized PK study involving male and female HIV-positive and HIV-negative patients on treatment for MDR-TB.

3.4 Inclusion criteria

A patient was included in the study if he/she complied with all of the following:

a. Signature of an informed written consent form for the blood samplings after he/she had been given a clear explanation of the aims, procedures, advantages and disadvantages of the study in his/her first language

b. Informed consent for an HIV test

c. On kanamycin treatment for at least two weeks

d. Adult patients aged 18–65 years

e. MDR-TB sensitive to second-line anti-TB drugs.

3.5 Exclusion criteria

A patient was excluded from the study if one or more of the following criteria applied:

a. On the patient's request

b. History of congestive cardiac failure

c. Uncontrolled hypertension

d. Ischemic heart disease

e. Pregnancy or breast feeding

f. Hypersensitivity to kanamycin

g. Older than 65 years

i. Haemoglobin less than 7g%.

3.6 Demographic, clinical and therapeutic characteristics

The demographic characteristics such as age, body weight, height and gender were recorded for every patient. Clinical and therapeutic characteristics such as viral load and CD4 counts were recorded for HIV-positive patients. In addition, kidney and liver function tests were determined for each patient after inclusion in the study. These characteristics were captured on a computer spreadsheet designed for the study.

3.7 Laboratory tests

The liver function, kidney function and haematology tests were done at PathCare laboratory (PathCare Park, Neels Bothma Street, N1 City, Goodwood, Cape Town, South Africa) in order to assess liver and kidney function and to determine

the haematological profile of the patients. HIV, viral load, CD4 counts and sputum microbiology tests were done by the National Health Laboratory Services.

3.7.1 Liver function tests

Liver function was evaluated using the Child-Pugh score. In calculating this score, clinical and biochemical measurements such as hepatic encephalopathy, ascites, total bilirubin, serum albumin and prothrombin time were graded based on standardised points.

Table 3.1: Scoring system used for Child-Pugh classification

Clinical and biochemical Measurements	**Points scored for increasing abnormality**		
	1	**2**	**3**
Hepatic encephalopathy	None	1 and 2	3 and 4
Ascites	Absent	Mild	Moderate
Total bilrubin (mg/dl)	<2.0	2.0-3.0	>3.0
Serum albumin (g/dl)	>3.5	2.8-3.5	<2.8
Prothrombin time	<4 or <1.7	4-6 or 1.7-2.3	>6 or >2.3

Source: Trey et al. (1996)

The individual points were added and then graded according to the severity of liver disease, as explained in the previous chapter.

3.7.2 Kidney function tests

Kidney function was determined by calculating the GFR from serum creatinine using the MDRD formula:

GFR (ml/min/1.73m^2) = 175 x {[standardized S_{Cr} (µmol/L)/88.4]$^{-1.154}$} x {age (years)$^{-0.203}$} x (0.742 if female) x (1.210 if black).

S_{Cr}= serum creatinine

3.7.3 Haematological tests

The full blood count was determined for each patient. However, our main interest was in the assessment of the CD4 cell count, which indicates the immuno-competence level in HIV -positive patients.

3.7.4 Sputum microbiological test

The sensitivity test for MTB was identified in the sputum of patients for each second-line anti-TB drug before the start of the treatment. At Brewelskloof Hospital the second-line anti-TB drugs used were kanamycin, ofloxacin, pyrazinamide, ethionamide, ethambutol and terizidone.

3.7.5 HIV test and determination of viral load and CD4 counts

After individual pre- and post-test counselling regarding the HIV test, ELISA was conducted for every patient. Tests for viral load level and CD4 count were performed for HIV -positive patients.

3.8 Study procedures

3.8.1 Blood sampling for kanamycin plasma concentrations

On the study day, after an eight-hour overnight fast, patients received kanamycin at 7 a.m. All patients were allowed to have breakfast 30 minutes after drug administration. Blood samples were collected in appropriate tubes in the following order:

i. Five (5) milliliters for liver and renal function tests

ii. Five (5) milliliters for haematological and virological tests

iii. Five (5) milliliters for assessment of kanamycin plasma concentrations.

3.8.2 Kanamycin administration

Patients received kanamycin injection intramuscularly at different doses (500, 600, 660, 750 and 1000 mg) daily based on the patients' body weight and renal function.

3.9 Determination of kanamycin plasma concentrations

Plasma levels of kanamycin were determined using LC-MS at Stellenbosch University, Cape Town, South Africa.

3.9.1 Chemicals

The following chemicals were used in the study: analytical-grade dimethyl sulfoxide (DMSO), acetonitrile, trichloroacetic acid, phosphoric acid and HPLC-grade trifluroacetic acid (TFA). All of them were obtained from Sigma-Aldrich (Cape Town, South Africa). Kanamycin was supplied by Brewelskloof Hospital

and used as the working standard.

3.9.2 Chromatographic and mass spectrometric conditions

3.9.2.1 Column liquid chromatography

The liquid chromatography was performed with the Waters 2695 HPLC system (Waters, Microsep Pty Ltd). The column utilised was a polar C18 liquid chromatography column, Waters Atlantis™ (5 µm, 2.1 mm x 100 mm) at ambient temperature. The mobile phase used was 0.1% TFA at a flow rate of 0.20 ml/min and an injection volume of 5 µl.

3.9.2.2 Mass spectrometry setting

The instrument used was a Waters API Quattro Micro triple quadrupole mass spectrometer with electrospray ionisation (Waters, Microsep Pty Ltd*).* The ion source and desolvation temperatures were held at 120°C and 400°C, respectively. The capillary voltage was 2.8 kV. The desolvation gas was at 1000 L/h and the cone gas was at 50 L/h. The instrument was operated at multiple reaction monitoring (MRM) mode. The MRM settings for kanamycin was 485>163 at collision energy of 25 eV, and cone voltage of 20 V. Propranolol was used as internal standard and was monitored at an MRM of 260.3 > 183 at collision energy of 20eV and cone voltage of 18V. Waters Mass Lynx^{TM} software was used for data collection, integration and calibration.

3.9.2.3 Kanamycin stock solution

The stock solution of 1mg/ml was serially diluted with acetonitrile to obtain

working solutions with the concentrations 10, 5, 1, 0.5, 0.1 and 0.05 µg/ml. All these standards contained 10 ppm propranolol as internal standard.

3.9.2.4 Preparation of calibration standards

Kanamycin plasma calibration standards were prepared by spiking blank plasma with kanamycin working solution (with concentrations of 10, 5, 1, 0.5, 0.1 and 0.05 µg/ml).

3.9.2.5 Patient samples preparation

To prepare the patients' plasma samples for the LC-MS assay, trichloroacetic acid (30µl) was added to 50 µl plasma followed by 170 µl internal standard solution (10 ppm propanolol in water). The mixture was vortexed for 1 minute, followed by centrifugation at 6000 g for 5 minutes. The supernatant was injected onto the LC-MS. Blank plasma was spiked with 1 and 5 ppm of kanamycin in triplicate to determine the recoveries and repeatability. The relative standard deviation was better than 12%. This relatively high standard deviation and relatively high limit of detection and quantification is due to the fact that kanamycin elutes just after the void volume of the column. The use of a very polar C18 column is very important for this analysis

3.10 Determination of pharmacokinetic parameters

The plasma concentration-time profile for each patient was plotted manually. Kanamycin PK parameters were calculated based on the non-compartmental analysis (Mugabo, 2009) and expressed as median and range (lowest to highest)

as follows:

3.10.1 The maximum concentration and the time to reach maximum concentration

The maximum concentration (C_{max}) and the time to reach the maximum concentration (T_{max}) were obtained directly from the plasma concentration-time profile.

3.10.2 The elimination rate constant

The elimination rate constant (K_e) was determined from the terminal linear phase of the plasma concentration-time profile.

Since natural log was used, the slope was calculated as

Slope = $(\ln y_2 - \ln y_1 / x_2 - x_1)$,

K_e was obtained as – (slope)

3.10.3 The absorption rate constant

The absorption rate constant (K_a) was calculated by plotting the residual concentrations using the method of residuals and determining the slope from the straight line. K_a was then obtained from the slope of the straight line, which represents absorption phase.

K_a is equal to – (slope) = – $(\ln y_2 - \ln y_1 / x_2 - x_1)$

(Dipiro et al., 2010)

3.10.4 The half-life

The half-life ($t_{½}$) was calculated using the formula: $t_{½} = 0.693/K_e$.

3.10.5 The area under the plasma concentration-time curve

The area under the plasma concentration-time curve from zero to 24 hours (AUC_{0-24}) was calculated by the trapezoidal method manually.

The area under the plasma concentration-time curve from zero to infinity ($AUC_{0-\infty}$) was calculated using the following formula:

$AUC_{0-\infty} = AUC_{0-24} + Cp_{last}/K_e$

Where Cp_{last} is the last measurable plasma concentration.

3.10.6 The volume of distribution

The following formula was used to determine the volume of distribution (V_d):

$V_d = Dose/ (AUC_{0-\infty} \times K_e)$.

3.10.7 The total body clearance

The total body clearance (Cl_{tot}) was calculated using the following formula:

$Cl_{tot} = Dose/AUC_{0-\infty}$

3.10.8 The mean residence time

The MRT was calculated as: $MRT = AUMC_{\infty}/AUC_{\infty}$

Where AUMC∞ is the area under the momentum curve from zero to infinity and was calculated as:

$AUMC_{\infty} = AUMC + (C_{last} / K_e^2) + t_{last} \times C_{last}/K_e$

$AUMC = \frac{tici + ti\text{-}1 \times ci\text{-}1}{2}$

Where C_{last} is the last measurable plasma concentration and t_{last} is the time of the last measurable concentration.

3.11 Determination of the number of patients and statistical analysis of data

3.11.1 Determination of the number of patients

The number of patients to be included in the study was determined by using a two-sample t-test and comparing means to see if there is a possibility of detecting a difference between HIV-positive and HIV-negative patients.

3.11.2 Statistical analysis of data

Patients' data collected during the study was organised by the researcher and coded onto data collection forms anonymously before being captured into computer files. Microsoft Excel was utilised for computer management of the data, which was reported as median and range. Analysis was done using SAS version 9.0.

Basic statistics like mean, standard deviation, median and range were used to describe continuous and ordinal variables and frequency tables for nominal variables. When data was not normally distributed, the Wilcoxon Rank Sum test,

the non-parametric alternative to a paired t-test, was used and PK parameters were reported as median and range. The Kruskal-Wallis one-way analysis of variance was used in comparing more than two groups to see if they originate from the same population.

Statistical significance was assumed at the $p<0.01$ level to avoid a type 1 error (false positive).

3.12 Ethical considerations

The study was approved by the University of the Western Cape ethics committee (Ethics certificate number: 07/6/12). It was conducted according to the declaration of Helsinki and ICH guidelines. Patients were provided with information sheets about the study, as mentioned above, and all information obtained during the study was treated as confidential. Permission to conduct the study was granted by the Provincial Department of Health and the medical superintendent of Brewelskloof Hospital.

CHAPTER 4: RESULTS

4.1 Structure of the results

Chapter 4 presents the results of the study. Tables and graphs are used appropriately to describe the findings. The first section provides the structure of chapter four. The second section describes the validation of LC-MS assay as a sensitive and specific method in the analysis of the plasma concentrations of kanamycin using graphs. The third section presents the demographic data in patients that participated in the study. Following this section are tables highlighting the virological and immunological profile in HIV-positive patients who participated in the study. Similarly, there are tables presenting renal function in HIV-positive and HIV-negative patients.

The fourth section describes kanamycin plasma concentrations at different time intervals in both HIV-positive and HIV-negative patients. It also describes kanamycin PK parameters in both groups of patients.

4.2 Kanamycin liquid chromatography-mass spectrometry analysis method validation

Liquid chromatography coupled with mass spectrometry was used in the quantification of kanamycin plasma concentrations. This method was validated by determining linearity, recovery, precision and accuracy, low limit of detection, low limit of quantification, and specificity.

4.2.1 Calibration curve and linearity

As indicated in Figure 4.1, the kanamycin calibration curve was constructed by plotting responses (y-axis) against corresponding concentrations (x-axis). The calibration curve was linear over ranges of -0.1–10 µg/ml-. The correlation co-efficient (r^2) was 0.97.

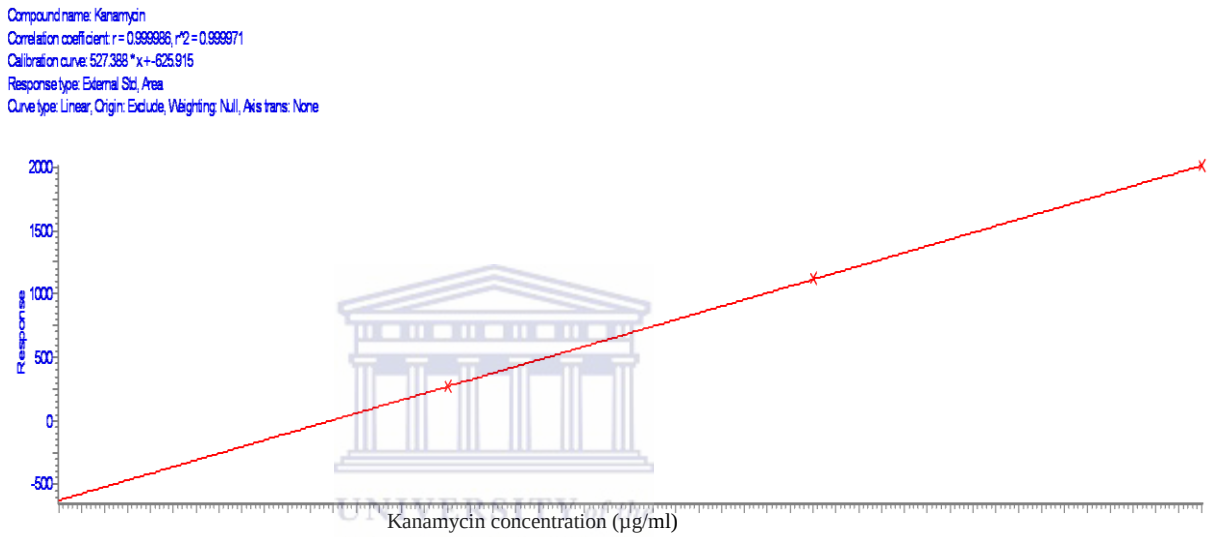


Fig 4.1: Kanamycin calibration curve

4.2.2 Precision, recovery and accuracy

The overall precision expressed as relative standard deviation (RSD%), was less than 12%. The accuracy, expressed in terms of recovery was 74 ± 9.1% and 111 ± 6.2% for 1 µg/ml and 5 µg/ml, respectively.

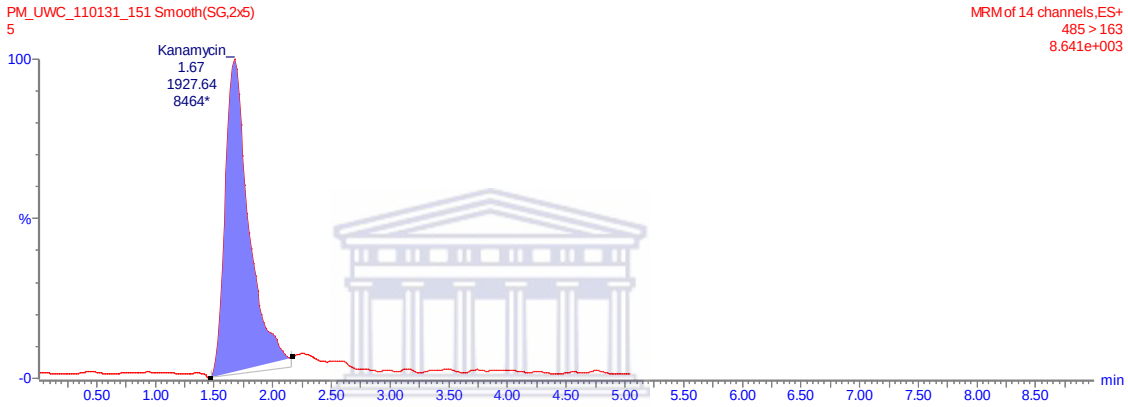
4.2.3 Lower limit of detection and quantification

The lower limit of detection of kanamycin was 0.06 µg/ml. The lower limit of quantification of kanamycin in the plasma was found to be 0.15 µg/ml.

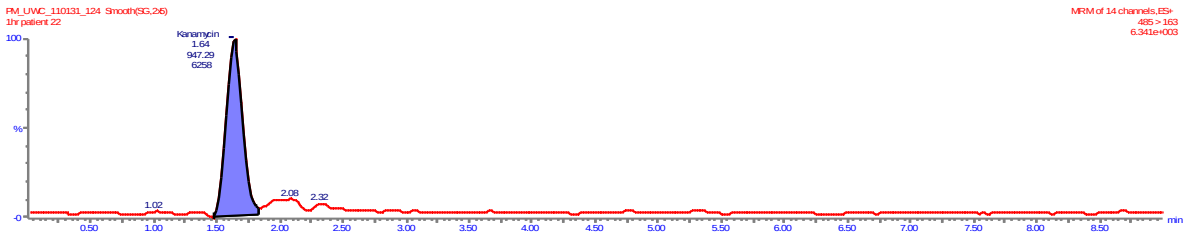
4.2.4 Specificity

There were no interfering peaks from the plasma components with kanamycin peak, which was detected at a retention time of 2.65 minutes. Chromatograms showing the separation of kanamycin standard and kanamycin plasma extract (calibration standard) in addition to the blank plasma are shown in Figure 4.2.

(A) Chromatogram of kanamycin standard drug



(B) Chromatogram of kanamycin patient plasma



C) Chromatogram of a blank plasma

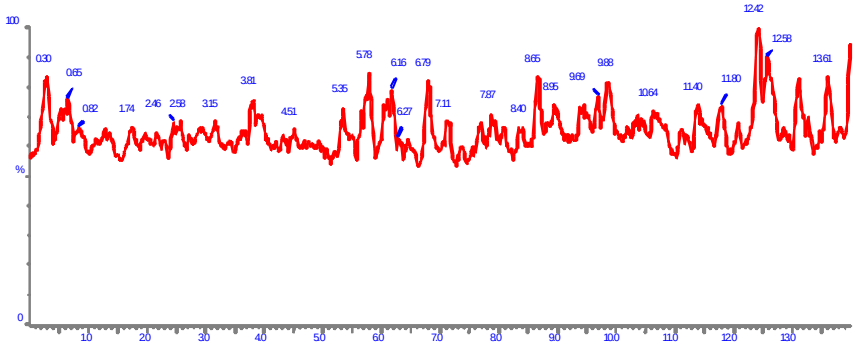


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Figure 4.2: Kanamycin chromatograms: (A) Chromatogram of kanamycin standard drug; (B) Chromatogram of kanamycin in a patient's plasma; (C) Chromatogram of a blank plasma

4.3 Patients' demographic data and characteristics

Thirty-one black and coloured (mixed race) patients (17 males and 14 females) infected with MDR-TB participated in the study. Of these 31 patients, 13 (8 males and 5 females) were infected with HIV. The median (range) age and weight of patients were 32 (18–54) years and 53.0 (41.8–90.0) kg, respectively.

4.3.1 Number of HIV-positive patients on antiretroviral treatment

Of the 13 patients infected with HIV, only ten were on ARV therapy. The patients were taking the following ARVs: stavudine (30 mg/po/bd), lamivudine (150 mg/po/bd) and efavirenz (600 mg/po/nocte).

Table 4.1: Immunological profile in HIV-infected patients

CD4 count (range) cells/mm^3	Number and gender
<60	4 (2 males and 2 females)
90–99	1 (1 male)
100–299	3 (1 male and 2 females)
300–399	3 (3 males)
400–599	2 (1 male and 1 female)

From Table 4.1, five patients infected with HIV had a CD4 count of <100 cells/mm^3. Eight HIV-positive patients had a CD4 count of >100 cells/mm^3.

Table 4.2: Virological profile in HIV-infected patients

Viral load (range) copies/ml	Number and gender
<40	10 (7 males and 3 females)
5,000–1,000,000	3 (1 male and 2 females)

Table 4.2 shows viral load in all the HIV-positive patients who participated in the study. The viral load varied among HIV-positive patients. Ten patients had a viral load of less than 40 copies/ml and three patients had a viral load of 5,000–1,000,000 copies/ml.

Table 4.3: Renal function in HIV -positive and in HIV -negative patients

GFR (ml/min/1.73 m^2)	Number of patients
Normal (>90)	5 HIV-positive and 15 HIV-negative
Mild (60–89)	8 HIV-positive and 3 HIV-negative
Moderate (40–59)	2 HIV-positive

Table 4.3 presents renal function in HIV-positive and HIV-negative patients that participated in the study. Eleven patients in total had mild renal function and only two patients had moderate renal function. The median value for the GFR in HIV-positive patients was 80 (44–88) ml/min. Similarly, the median value for GFR in HIV-negative patients was 88 (84–89) ml/min.

4.4 Kanamycin plasma concentrations and pharmacokinetic parameters

4.4.1 Kanamycin plasma concentrations

Kanamycin plasma concentrations at different time intervals after kanamycin intramuscular administration over a 24-hour period are shown in the plasma concentration-time graphs in Tables 4.4–4.5.

4.4.1.1 Kanamycin plasma concentration in HIV-positive and HIV-negative patients

Tables 4.4–4.5 illustrate kanamycin plasma concentrations in HIV-positive and HIV-negative patients.

Table 4.4: Kanamycin plasma concentration-time profile in HIV-positive patients

Patient	**Baseline (0 hr) (µg/ml)**		**Time (hours) after kanamycin administration**				
		0.5hr (µg/ml)	**1hr (µg/ml)**	**2hrs (µg/ml)**	**4hrs (µg/ml)**	**8hrs (µg/ml)**	**24hrs (µg/ml)**
1	**BDL**	**BDL**	14.47	12.95	9.98	**BDL**	**BDL**
2	**BDL**	**BDL**	21.67	17.64	13.06	8.13	**BDL**
3	**BDL**	**BDL**	14.35	12.95	9.42	8.18	**BDL**
4	**BDL**	**BDL**	**BDL**	12.71	8.06	2.70	0.66
5	**BDL**	**BDL**	5.15	8.40	5.22	2.85	0.75
6	7.80	**BDL**	21.67	20.99	14.95	13.70	9.29
7	**BDL**	**BDL**	19.36	9.82	11.11	8.37	**BDL**
8	**BDL**	**BDL**	16.15	18.19	13.77	9.85	**BDL**
9	7.46	**BDL**	19.77	19.81	13.02	9.35	**BDL**
10	**BDL**	**BDL**	27.63	16.76	9.23	3.34	5.82
11	**BDL**	12.23	11.22	7.42	5.53	2.61	**BDL**
Median	**N/A**	**N/A**	**16.15**	**12.95**	**9.98**	**8.13**	**N/A**
Range	**N/A**	**N/A**	**5.15-27.63**	**7.42-20.99**	**5.22-14.95**	**2.61-13.70**	**N/A**

BDL = below detectable level (0.05µg/ml)

N/A= not applicable.

As indicated in Table 4.4, kanamycin plasma concentrations are below detectable level at base line (0hr), 0.5hr and 24hrs. Peak plasma concentration is at 1hr.

Table 4.5: Kanamycin plasma concentration-time profile in HIV-negative patients

Patient	Baseline (0hr) (µg/ml)	Time (hours) after kanamycin administration					
		0.5hr (µg/ml)	1hr (µg/ml)	2hrs (µg/ml)	4hrs (µg/ml)	8hrs (µg/ml)	24hrs (µg/ml)
12	**BDL**	**BDL**	14.19	10.26	9.44	**BDL**	**BDL**
13	**BDL**	**BDL**	25.26	22.57	15.28	8.94	**BDL**
14	**BDL**	**BDL**	18.18	16.36	12.75	9.05	**BDL**
15	**BDL**	**BDL**	23.55	13.38	9.82	7.92	**BDL**
16	**BDL**	**BDL**	18.64	18.34	10.86	7.56	**BDL**
17	**BDL**	13.32	11.87	6.38	2.85	0.77	1.72
18	**BDL**	**BDL**	7.21	15.82	9.82	7.60	7.26
19	**BDL**	**BDL**	17.94	17.33	11.59	8.87	**BDL**
20	**BDL**	**BDL**	14.16	**BDL**	10.97	8.14	8.39
21	**BDL**	**BDL**	24.19	23.87	12.69	9.05	**BDL**
22	**BDL**	**BDL**	24.11	17.85	11.76	**BDL**	**BDL**
23	**BDL**	**BDL**	19.06	21.71	10.33	8.30	**BDL**
24	**BDL**	**BDL**	17.90	18.60	12.16	9.59	**BDL**
25	**BDL**	**BDL**	14.88	8.16	5.54	2.03	**BDL**
Median	**N/A**	**N/A**	**18.06**	**16.85**	**10.92**	**8.03**	**N/A**
Range	**N/A**	**N/A**	**7.21-25.26**	**6.38-23.87**	**2.85-15.28**	**0.77-9.59**	**N/A**

From table 4.5, it is observed that kanamycin plasma concentrations are below detectable level at baseline, 0.5hr and 24hrs. Peak plasma concentration is at 1hr.

The median plasma concentration-time profile of kanamycin in HIV-positive and HIV-negative patients is shown in Figure 4.3.

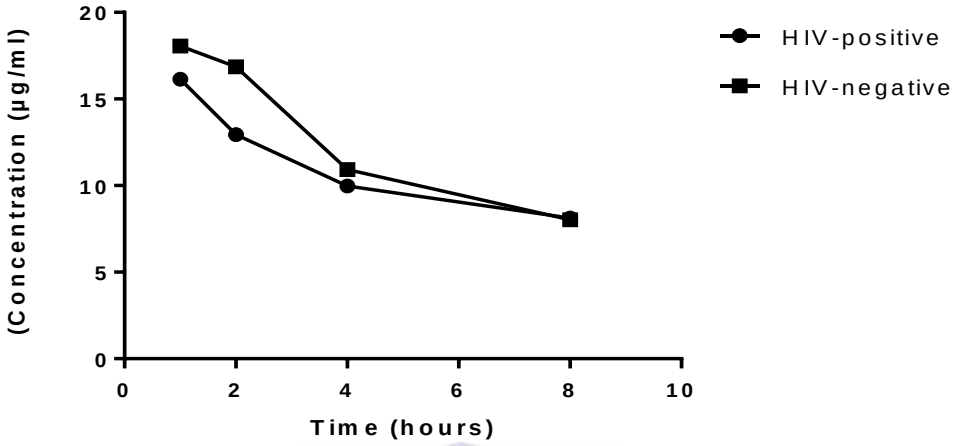

Figure 4.3: Kanamycin median plasma concentration-time profile in HIV-positive and HIV-negative patients

From Figure 4.3, it can be observed that the plasma concentration profile of kanamycin in the 11 HIV-positive and 14 HIV-negative patients are similar. There is no significant difference ($p = 0.43$) at all sampling times.

4.4.1.2 Kanamycin plasma concentrations in additional patients

Plasma concentrations in the additional six patients that were introduced into the study are shown in Table 4.6.

Table 4.6: Kanamycin plasma concentration-time profile in additional patients.

		Time after kanamycin administration											
	Baseline (0hr) (µg/ml)	**15mins (µg/ml)**	**25mins (µg/ml)**	**35mins (µg/ml)**	**45mins (µg/ml)**	**55mins (µg/ml)**	**65mins (µg/ml)**	**90mins (µg/ml)**	**2hrs (µg/ml)**	**4hrs (µg/ml)**	**8hrs (µg/ml)**	**16hrs (µg/ml)**	**24hrs (µg/ml)**
26	**BDL**	8.27	5.53	13.69	11.13	12.71	9.08	9.30	9.83	6.90	3.03	1.01	**BDL**
27	**BDL**	1.71	7.47	13.23	23.04	17.48	22.12	21.57	20.48	7.54	1.20	**BDL**	**BDL**
28	**BDL**	3.01	5.25	10.16	5.22	6.57	6.67	5.95	10.57	5.55	0.88	**BDL**	**BDL**
29	**BDL**	3.07	6.20	6.90	9.78	10.83	12.08	10.38	9.91	5.06	**BDL**	**BDL**	**BDL**
30	**BDL**	1.73	6.79	**BDL**	10.51	15.71	15.58	9.42	7.41	3.58	**BDL**	**BDL**	**BDL**
31	**BDL**	0.20	0.81	1.24	1.84	3.73	6.73	5.46	5.07	3.03	0.65	**BDL**	**BDL**
Median	**BDL**	**2.37**	**5.87**	**8.53**	**10.15**	**11.77**	**10.58**	**9.36**	**9.87**	**5.31**	**1.04**	**BDL**	**BDL**
Range	**BDL**	**0.20-8.27**	**0.81-7.47**	**1.24-13.69**	**1.84-23.04**	**3.73-17.48**	**6.73-22.12**	**5.46-21.57**	**5.07-20.48**	**3.03-7.54**	**0.65-3.03**	**N/A**	**N/A**

Table 4.6 presents kanamycin plasma concentrations at different time intervals in the additional patients. These concentrations were not added to concentrations in the previous patients because of the different blood sampling times.

As indicated in Table 4.6, at baseline (before kanamycin administration), plasma concentrations were below detectable levels (0.05 µg/ml). This could be as a result of an insufficient amount of kanamycin remaining in the body 24 hours after dosing that could be detected at baseline. Also, plasma concentration levels were not detected within 16 and 24 hours in most patients. Plasma concentration levels were detected last at 16hrs for patient 26. Patient 26 had a moderate renal impairment of 51ml/min and this resulted in the lengthening of the residence time of kanamycin.

4.4.2 Kanamycin pharmacokinetic parameters

Tables 4.7, 4.8 and 4.9 demonstrate kanamycin PK parameters in HIV-positive, HIV-negative and additional patients, respectively. Patients were on MDR-TB treatment for two weeks.

Table 4.7: Kanamycin pharmacokinetic parameters in HIV-positive patients

Patient	K_e (hr^{-1})	Dose (mg)	$T_{1/2}$ (hrs)	$AUC_{(0-24)}$ µg/ml.hr	$AUC_{(0-\infty)}$ µg/ml.hr	C_{max} (µg/ml)	T_{max} (hrs)	Ka (hr^{-1})	Absorption$T_{1/2}$ (hrs)	V_d (L)	Cl_{tot} (L/hr)	MRT (hrs)
1	0.11	1000	6.30	60.22	150.95	14.47	1.00	N/A	N/A	60.22	6.62	7.92
2	0.18	750	3.85	163.20	208.37	21.67	1.00	N/A	N/A	20.00	3.60	2.99
3	0.079	1000	8.77	140.25	243.79	14.35	1.00	N/A	N/A	51.92	4.10	8.80
4	0.27	1000	2.57	75.53	77.97	12.71	2.00	N/A	N/A	47.50	12.83	1.11
5	0.16	1000	4.33	66.63	71.32	8.40	2.00	0.19	3.65	87.63	14.02	2.02
6	0.063	750	11.00	305.86	453.32	21.27	1.00	0.16	4.33	26.26	1.65	12.96
7	0.19	1000	3.65	146.28	190.33	19.36	1.00	0.44	1.58	27.65	5.25	3.12
8	0.13	1000	5.33	179.21	254.98	18.19	2.00	0.85	0.82	30.17	3.92	4.69
9	0.22	750	3.15	178.97	221.47	19.81	2.00	0.90	0.77	15.39	3.39	2.39
10	0.33	750	2.10	153.52	171.16	27.63	1.00	0.33	2.1	13.28	4.38	2.87
11	0.22	750	3.15	68.35	80.21	12.23	0.50	0.42	1.65	42.50	9.35	1.86
Median	**0.18**	**1000**	**3.85**	**146.28**	**190.33**	**18.19**	**1.00**	**0.19**	**0.82**	**30.17**	**4.38**	**2.99**
Range	**0.063-0.33**	**750-1000**	**2.10-11.00**	**60.22-305.86**	**71.32-453.32**	**8.40-27.63**	**0.50-2.00**	**0.16-0.90**	**0.77-4.33**	**13.28-87.63**	**1.65-14.02**	**1.11-12.96**

Table 4.8: Pharmacokinetic parameters in HIV -negative patients

Patient	K_e (hr^{-1})	$T_{1/2}$ (hrs)	Dose (mg)	$AUC_{(0-24)}$ µg/ml.hr	$AUC_{(0-\infty)}$ µg/ml.hr	C_{max} (µg/ml)	T_{max} (hrs)	K_a (hr^{-1})	Absorption $T_{1/2}$ (hrs)	V_d (L)	Cl_{tot} (L/hr)	MRT (hrs)
12	0.34	2.04	750	54.36	82.12	14.19	1	N/A	N/A	26.86	9.13	2.43
13	0.15	4.62	750	188.04	247.64	25.26	1	N/A	N/A	20.19	3.03	3.58
14	0.11	6.3	750	166.93	249.2	18.18	1	N/A	N/A	27.36	3.01	5.68
15	0.29	2.39	750	146.4	173.71	23.55	1	N/A	N/A	14.89	4.32	1.87
16	0.12	5.78	750	149.67	212.67	18.64	1	N/A	N/A	29.39	3.53	4.88
17	0.35	1.98	750	55.15	60.06	13.32	0.5	N/A	N/A	35.68	12.49	2.21
18	0.12	5.78	1000	192.68	253.18	15.82	2	0.13	5.33	32.91	3.95	4.87
19	0.096	7.22	1000	162.93	255.33	17.94	1	N/A	N/A	40.8	3.92	6.7
20	0.081	8.56	1000	192.05	295.63	14.16	1	N/A	N/A	41.76	3.38	12.76
21	0.14	4.95	1000	182.52	247.16	24.19	1	N/A	N/A	28.9	4.05	4.01
22	0.23	3.01	1000	80.14	131.27	24.11	1	N/A	N/A	33.12	7.62	3.34
23	0.12	5.78	1000	160.86	230.03	21.71	2	0.26	2.67	36.23	4.35	4.95
24	0.084	8.25	1000	173.61	287.78	18.6	2	0.94	0.74	41.37	3.47	7.93
25	0.33	2.1	1000	60.32	66.47	14.88	1	N/A	N/A	45.59	15.04	1.13
Median	**0.13**	**5.37**	**1000**	**161.9**	**236.6**	**18.39**	**1**	**N/A**	**N/A**	**33.02**	**4**	**4.44**
Range	**0.081-0.35**	**1.98-8.56**	**750-1000**	**54.36-192.68**	**60.06-295.63**	**13.32-25.26**	**0.50-2.00**	**N/A**	**N/A**	**14.89-45.59**	**3.01-15.04**	**1.13-12.76**
Result from published studies	**0.29-0.30**	**4**	**500-2000**	**N/A**	**N/A**	**20.00-35.00**	**1.00-2.00**	**N/A**	**N/A**	**17.00-23.00**	**5.00-6.00**	**N/A**

Ranges of pharmacokinetic parameters of kanamycin in HIV -negative patients from previous published studies are shown in the last row of Table 4.8.

As indicated in table 4.7 and 4.8, K_a and absorption $t_{1/2}$ were not calculated for patients in whom there was no absorption phase of kanamycin.

Table 4.9: Pharmacokinetic parameters in additional patients

Patient	K_e (hr^{-1})	$T_{1/2}$ (hrs)	Dose (mg)	$AUC_{(0-24)}$ µg/ml.hr	$AUC_{(0-\infty)}$ µg/ml.hr	C_{max} (µg/ml)	T_{max} (hrs)	K_a (hr^{-1})	Absorption $T_{1/2}$ (hrs)	V_d (L)	Cl_{tot} (L/hr)	MRT (hrs)
26	0.16	4.33	1000	71.52	77.75	13.69	0.58	0.35	1.98	80.39	12.86	1.78
27	0.44	1.58	660	82.12	84.85	23.04	0.75	1.55	0.45	17.68	7.78	0.33
28	0.27	2.57	1000	44.58	47.84	10.57	2.00	1.95	0.36	77.42	20.90	0.79
29	0.25	2.77	1000	41.72	61.96	12.08	1.08	3.84	0.18	64.56	16.14	2.61
30	0.32	2.17	660	34.50	45.69	15.71	0.92	2.29	0.30	45.14	14.45	1.74
31	0.19	3.65	1000	25.07	28.49	6.73	1.08	1.89	0.37	184.74	35.10	1.60
Median	**0.26**	**2.67**	**1000**	**43.15**	**54.90**	**12.89**	**1.00**	**1.92**	**0.37**	**70.99**	**15.30**	**1.67**
Range	**0.16-0.44**	**1.58-4.33**	**660-1000**	**25.07-82.12**	**28.49-84.85**	**6.73-23.04**	**0.58-2.00**	**0.35-3.84**	**0.18-1.98**	**17.68-184.74**	**7.78-35.10**	**0.33-2.61**

In table 4.10, the GFR and PK parameters in HIV -positive and HIV -negative patients are compared using the Wilcoxon rank sum test to see if there is any statistically significant difference.

Table 4.10: p values for Wilcoxon non-parametric test

Glomerular filtration rate and Pharmacokinetic parameters	**p value**
GFR	0.013
K_e	1.00
$T_{1/2}$	1.00
AUC_{0-24}	0.61
$AUC_{0-\infty}$	0.43
C_{max}	0.47
T_{max}	0.63
V_d	0.77
Cl_{tot}	0.51
MRT	0.50

As indicated in table 4.10, there is no significant difference in the PK parameters of kanamycin in HIV positive and HIV negative patients except for the GFR which has a p value of 0.013.

CHAPTER FIVE: DISCUSSION

5.1 Structure of the discussion

Chapter 5 discusses the study design, study procedures, the determination of kanamycin plasma concentrations, the statistical methods and results obtained from the study. It consists of 16 sections.

The first section presents the structure of chapter five. The second section discusses the demographic characteristics of patients who participated in the study. The power calculation used to determine the number of patients needed for the PK study is also discussed. The third section discusses the procedures used in the study. In this section, the blood sampling of patients is explained.

The fourth section discusses the LC-MS method of analysis. In this section LC/MS is compared to previously used methods to determine kanamycin plasma concentrations. The fifth and sixth section explains kidney and liver function tests in HIV -positive and in HIV -negative patients.

The seventh section explains the virological and immunological profile in HIV-positive patients. The eighth section discusses the statistical methods used to analyse kanamycin PK parameters. In the ninth, tenth and eleventh sections, research questions in the study are extensively answered based on the results given in the previous chapter.

The twelfth section deals with abnormal PK parameters in HIV-negative patients compared to previously published studies. The thirteenth section discusses kanamycin plasma PK in the

six additional patients. The PK parameters of kanamycin in previously published studies are discussed in the fourteenth section.

The fifteenth section discusses the therapeutic implications of the study. It presents the two PK parameters associated with the clinical efficacy of kanamycin in patients and explains their significance. Finally, the sixteenth section explains the limitations of the study.

5.2 Study design

5.2.1 Demographic characteristics

The 31 (17 males and 14 females) patients who participated in the study were sufficient to describe the PK of kanamycin in patients with MDR-TB. The number of patients in HIV-positive and HIV-negative groups was sufficient to give a power of about 80% in detecting a statistical significant difference in the means of about 1.2 standard deviations based on our power calculations. Also, the number of patients who participated in previously published studies was similar to the number of patients in our study.

In the study on serum PK of anti-mycobacterial drugs in HIV -negative patients with MDR-TB during therapy, 13 patients participated in the study (Yew et al., 1999). Furthermore, a sample size of ten patients was seen in studies of kanamycin in healthy volunteers (Cronk and Naumann, 1959; Doluiso et al., 1973; Clarke et al., 1974). Twelve patients participated in a study on the comparative PK of BB-K8 and kanamycin humans (Cabana and Taggart, 1973). Based on the number of patients in previous studies, our sample size of 31 was sufficient to describe the PK of kanamycin in patients with MDR-TB and in those with MDR-TB co-infected with HIV.

Patients' age ranged from 18 to 54 years, which is an active age group and is within the inclusion criteria. From the body mass index (BMI), most patients had normal body weight. Sixteen patients had normal body weight (18.5–24.9 BMI), 11 patients were underweight (<18.5 BMI) and four patients were overweight (25.0–29.9 BMI).

5.3 Study procedures

5.*3.1 Blood sampling for kanamycin plasma concentrations*

The stability of plasma samples was maintained by storing samples at -80˚C until LC-MS analysis.

As indicated in Table 4.4, patient 6 had a concentration-time profile starting at 0 hr and ending at 24 hrs. The reason for this is because patient 6 had a moderate renal impairment of 44ml/min, which contributed to the reduced Cl_{tot} of 1.65 L/hr and the long $t_{1/2}$ of 11.00 hrs. Patient 10 and 4 also had concentration values ending at 24hrs, although there were no detectable plasma concentrations at 0 hour. Patients 10 and 4 both had normal renal function of 90ml/min.

From Tables 4.4 and 4.5, plasma concentrations were not detected in most patients at baseline (0 hour). This suggests that there was insufficient amount of kanamycin remaining in the body 15 to 20 hours after kanamycin administration that could be detected at baseline (24 hours after dosing). In addition, there were no plasma concentration values in most patients within 30 minutes after kanamycin intramuscular administration. These concentrations are below detectable level.

An additional kanamycin plasma concentration of six patients were analysed at time intervals of 15, 25, 35, 45, 55, 65 and 90 minutes and 2, 4, 8, 16 and 24 hours; this was in addition to our previous 25 patients. This was done in order to be able to see if we could detect the C_{max} and T_{max} of kanamycin between 0.25 and 1 hour and to find out if it differs from our previous patients.

5.4 The Liquid chromatography-mass spectrometry method of analysis

The LC-MS method is a proven method and has been widely used in the quantification of anti-TB drugs (Peng et al., 1977; Maitra et al., 1979; Niessen, 1998; Mugabo et al., 2009). The plasma levels of kanamycin were determined using LC-MS method and analysis was conducted according to scientific standards.

5.4.1 Validation of liquid chromatography-mass spectrometry

To validate the assay, linearity, lowest limit of detection, lowest limit of quantification, specificity, precision, recovery and accuracy were determined in the previous chapter. All chemicals used were prepared and standardised regularly. Chemicals were obtained from a reputable source (Sigma-Aldrich, Cape Town). Separation of kanamycin was achieved at a retention time of 1.50 minutes with a high recovery percentage of 92.5%.

In summary, previously published studies have used microbiological and fluoroimmuno assay in the quantification of kanamycin (Cronk and Naumann, 1959; Doluiso et al., 1973; Clarke et al., 1974; Yew et al., 1999). However, our validation results demonstrate that the LC-MS analysis method is highly successful and accurate in the detection and quantification of kanamycin in plasma.

5.5 Kidney function tests

Kidney function was assessed by evaluating GFR calculated from the MDRD formula. The MDRD formula was preferred in evaluating GFR because it allows for adjustment for body surface area. Normal renal function is equal or greater than 90 ml/min. Impaired renal function includes mild (60–89 ml/min), moderate (40–59 ml/min) and severe renal impairment (<15 ml/min).

Eleven patients (eight HIV-positive and three HIV-negative) had mild renal impairment and two HIV-positive patients had moderate renal impairment. The median GFR for patients with normal GFR was >90 ml/min, for patients with mild renal impairment it was 84 ml/min and for those with moderate renal impairment it was 48 ml/min. The median GFR for HIV-positive patients with a CD4 count less than 100 cells/mm^3 and a CD4 count greater than 100 cells/mm^3 was 81 and 88 ml/min respectively. This indicates that there was no significant change in the GFR in HIV positive patients according to the level of HIV infection.

5.6 Liver function tests

Liver function was evaluated using the Child-Pugh score. The score was normal (< 10) in all patients.

5.7 Virological and immunological profile

Viral load and CD4 count were performed by an accredited laboratory (National Health Laboratory Services) in order to determine the immune competence of HIV-positive patients in the study. There was an unequal balance of CD4 count and viral load among HIV-positive patients. Five patients had a CD4 count of less than 100 cells/mm^3 and eight patients had CD4 counts greater than 100 cells/mm^3.

As indicated in Table 4.2, ten HIV-positive patients had a viral load of <40 copies/ml and the remaining three had a viral load of 5,000–1,000,000. The reason for this variation of viral load in HIV-positive patients is that the ten patients were on ARV therapy and the other three were not.

5.8 Statistical analysis of Pharmacokinetic parameters

PK parameters were analysed using the Wilcoxon Rank Sum test, because PK data points were not normally distributed. The Wilcoxon Rank Sum test was used because we had one categorical variable (Pos) with two variables (HIV-positive and HIV-negative groups) and one nominal variable (K_e, C_{max}, V_d, Cl_{tot}, MRT, AUC_{0-24}, $AUC_{0-\infty}$ and $t_{1/2}$, respectively).

The median and ranges were used to express the PK parameters because of the skewed distribution of PK data points. These were used because they are not generally affected by extreme values or outliers and also useful when data are not normally distributed.

The Kruskal-Wallis one-way analysis of variance is a non-parametric method and its equivalent is the one-way analysis of variance (ANOVA). The Kruskal-Wallis test was used to correlate the GFR values and PK parameters to see if there was a difference in the HIV positive and HIV negative groups of patients as a result of renal dysfunction.

A p value of 0.43 **s**uggests that there was no statistically significant difference in the PK parameters between HIV-positive and HIV-negative patients, and on this basis the null hypothesis was accepted in favour of the alternate hypothesis.

5.9 Could HIV infection influence the pharmacokinetics of kanamycin in patients infected with multi-drug resistant tuberculosis?

The rate and extent of absorption of kanamycin as represented by the C_{max} and AUC_{0-24} was similar in both HIV -positive and HIV -negative patients as indicated in Table 4.7 and 4.8. HIV positive patients were not severely affected by HIV (Table 4.1 and 4.2). Many HIV positive patients had good immunological and virological profile since eight out of thirteen patients had a CD4 count greater than 100 cells/mm^3 and ten had viral load less than 40 copies/ml. Furthermore, none of our patients had gastrointestinal diseases such as gastro-enteritis, diarrhoea or vomiting that could have influenced the absorption rate of kanamycin in HIV -positive and HIV -negative patients (Arbex et al., 2010). Also, there were no observable side effects of antiretroviral drugs or MDR-TB drugs that could have affected the absorption of kanamycin in the patients. For example, diarrhoea is the major side effect of protease inhibitors that could reduce the bioavailability of kanamycin. Similarly, ethionamide, one of the MDR-TB drugs produces severe gastrointestinal effects that could affect the PK of kanamycin (Weyer, 2005).

In addition, patients 1 and 3 had a low CD4 count of 17 and 20 cells/mm^3, respectively, which might have contributed to the low C_{max} of 14.47 and 14.35 µg/ml (Table 4.7), respectively. Some studies have suggested that there is altered absorption of anti-mycobacterial drugs in patients with advanced HIV infection and with a history of low CD4 counts (Patel et al., 1995; Berning et al., 2003; Gurumurthy et al., 2004).

A reduction in the extent of distribution of kanamycin in patients 9 and 10 with CD4 count of 382 and 336 cells/mm^3 and represented by a V_d of 13.28 and 15.39 L, respectively, might be associated with the short $t_{1/2}$ of 2.10 and 3.15 hours, respectively, that was observed. The

$t_{1/2}$ of a drug is a PK parameter dependent on both V_d and CL_{tot} and is a reflection of the extent of the distribution or elimination of a drug (Mehzar, 2004). Similarly, an increase in the V_d (60.22, 51.92, 26.26 and 30.17 L) of kanamycin in patients 1, 3, 6 and 8 resulted in the lengthening of the $t_{1/2}$ of 6.30, 8.77, 11.00 and 5.33, respectively.

The median and range for the K_a, K_e, $t_{1/2}$, AUC_{0-24}, $AUC_{0-\infty}$, C_{max}, T_{max}, V_d, Cl_{tot} and MRT of kanamycin in HIV -positive patients were 0.52 (0.16-0.90) hr^{-1}, 0.18 (0.063-0.33) hr^{-1}, 3.85 (2.10-11.00) hrs, 146.28 (60.22-305.86) µg/ml.hr, 190.33 (71.32-453.32) µg/ml.hr, 18.19 (8.40-27.63) µg/ml, 1.00 (0.50-2.00) hrs, 30.17 (13.28-87.63) L, 4.38 (1.65-14.02) L/hr and 2.94 (1.11-12.96) hrs, respectively. There was no significant difference (p=0.43) in the PK parameters of kanamycin in HIV -positive patients when compared to the median PK parameters in HIV -negative patients.

5.10 To what extent do changes in kidney function influence the pharmacokinetics of kanamycin?

There was some apparent significant difference (p=0.013) in the renal function between the HIV positive and HIV negative patients. However, there was no significant difference (p=0.31) when we correlated the GFR values and the PK parameters in the two groups of patients to see if changes in the renal function affected the PK of kanamycin. HIV could be responsible for the significant difference in the renal function between HIV -positive and HIV -negative patients. A decline in renal function and low CD4 count are associated with HIV-related nephropathy, due to HIV infecting the renal cells directly (Gerntholz et al., 2006; Jeremy et al., 2008; Roe et al., 2008). Also, as indicated in Table 4.3, we had more patients with impaired renal function in the HIV -positive group than in the HIV -negative group and this could have contributed to the statistical difference. This result is consistent with a study

done in South African patients, reporting that HIV-related nephropathy is exhibited in HIV positive patients at any stage of HIV infection and is characterised with varying degrees of GFR (Gerntholz et al., 2006).

Furthermore, individual change in PK parameters due to renal dysfunction was observed. Patient 6 had a moderate renal impairment of 44 ml/min. The decrease in GFR was associated with a decrease in Cl_{tot} (1.65 L/hr) and the result is an increase in the AUC_{0-24} (305.86 µg/ml.hr) and C_{max} (21.27 µg/ml) of kanamycin, as observed in this patient. This could be because kanamycin is primarily eliminated by the kidneys, and therefore its clearance can be prolonged in patients with renal impairment (Holdiness, 1984; Budha et al., 2008; Arbex et al., 2010).

In addition, in Table 4.7, the other HIV-infected patients (patients 5 and 11) with mild renal impairment (64 and 85 ml/min respectively) and a CD4 count of (98 and 102) cells/mm^3 had a significant increase in the clearance (14.02 and 9.35 L/hr) of kanamycin when compared to the other HIV -positive patients. This suggests that there was no relationship between mild renal impairment and the immunological profile in HIV positive patients

5.11 Do lamivudine, stavudine and efavirenz affect the pharmacokinetics of kanamycin?

Kanamycin was administered with other MDR-TB drugs to all patients. Some of the HIV positive patients were also given lamivudine, stavudine and efavirenz. None of these drugs can affect the metabolism, distribution and renal elimination of kanamycin.

5.12 Abnormal pharmacokinetic parameters in HIV-negative patients

Kanamycin exhibits low protein binding (approximately 10%) and therefore there should be a high V_d, because distribution is limited to extravascular tissues (Pechere and Dugal, 1979; Arbex et al., 2010). Patients 14, 16, 18, 19, 20 and 24 had an increase in $t_{1/2}$ of 6.30, 5.78, 5.78, 7.22, 8.56 and 8.25 hours, respectively. The increase in $t_{1/2}$ was as a result of the increase in the V_d of 27.36, 29.39, 32.91, 40.80, 41.76 and 41.37 L. This is consistent with the literature that kanamycin is highly distributed within extravascular tissues (Budha et al., 2008). Similarly, a reduction in the Cl_{tot} of kanamycin of 3.01, 3.53, 3.95, 3.92, 3.38 and 3.47 L/hr could have resulted in the lengthened $t_{1/2}$ of kanamycin in these patients.

In addition, the reduction in the rate and extent of absorption as represented by the low C_{max} of 14.19, 13.32 and 14.88 µg/ml and low AUC_{0-24} of 54.36, 55.15 and 60.32 µg/ml.hr in patients 12, 17 and 25, respectively, could be as a result of the rapid clearance of 9.13, 12.49 and 15.04 L/hr of kanamycin, respectively. The rapid elimination of kanamycin in these patients resulted in a short $t_{1/2}$ of 2.04, 1.98 and 2.10 hours, respectively. It is difficult to explain the rapid clearance of kanamycin in these patients because they had normal renal function, were not suffering from any other disease, no drug–drug interactions were observed and they were all HIV-negative.

The median and range for the K_e, $t_{1/2,}$ $AUC_{0-24,}$ C_{max}, V_d, MRT and CL_{tot} of kanamycin in HIV -negative patients were 0.13 (0.081-0.35) hr^{-1}, 5.37 (1.98–8.56) hours, 161.90 (54.36–192.68) µg/ml.hr, 18.39 (13.32–25.26) µg/ml, 33.02 (14.89–45.59) L, 4.44 (1.13–12.76) hours, 4.00 (3.01–15.04) L/hr. When compared to previously published studies (Cronk and Naumann, 1959; Cabana and Taggart, 1973; Clarke et al., 1974; Doluiso et al., 1973), there was a reduction in the rate and extent of absorption in HIV-negative patients. The $t_{1/2}$ and MRT of kanamycin were lengthened in HIV-negative patients. The V_d was also increased. K_e and Cl_{tot} were decreased. The increase in the extent of distribution, the decrease in the elimination rate constant and the decrease in the clearance of kanamycin could be responsible for the lengthened time kanamycin molecules resided in HIV-negative patients.

5.13 Additional patients' results

In addition to seeing clearly what is happening between the time intervals of 0.25 and 1 hour in the description of kanamycin PK parameters, blood samples were also collected at different time intervals because we were testing for ethionamide, which has a T_{max} around 1 hour.

Kanamycin PK was determined in the six additional patients at the following time intervals: baseline (before kanamycin administration), 15, 25, 35, 45, 55, 65, 90 minutes and 2, 4, 8, 16 and 24 hours after kanamycin administration, respectively. The PK parameters are expressed as median and range. C_{max}, T_{max} and AUC_{0-24} were 12.89 (6.73-23.04) µg/ml, 1.00 (0.58–2.00) hours, and 43.15 (25.07–82.12) µg/ml.hr, respectively. MRT and $t_{1/2}$ were 1.67 (0.33–2.61) hours and 2.67 (1.58–4.33) hours, respectively. V_d and Cl_{tot} were 70.99 (17.68–184.74) L/hr and 15.30 (7.78–35.10) L, respectively. There was similarity in the PK parameters of kanamycin when compared to HIV -negative patients in the study. Kanamycin was not

adequately absorbed in the patients as represented by the low C_{max} and AUC_{0-24}. Kanamycin was highly eliminated as indicated by the high K_e and Cl_{tot} and this resulted in the short $t_{1/2}$ and MRT.

5.14 Pharmacokinetic data of kanamycin from published studies

There is not enough data from previous studies on the PK of kanamycin in patients with MDR-TB and in those with MDR-TB co-infection with HIV. Three studies have described the PK of kanamycin in healthy volunteers (Cronk and Nauman, 1959; Cabana and Taggart, 1973; Doluiso et al., 1973) and only one described the PK of kanamycin in HIV -negative patients infected with MDR-TB (Yew et al., 1999) using immuno-assay methods. The ranges for some PK parameters from previous studies are as follows: C_{max} of 20–35 µg/ml, T_{max} of 1–2 hours, K_e of 0.29–0.30 hr^{-1}, $t_{1/2}$ of 2–4 hours, Cl_{tot} of 5–6 L/hr and V_d of 17–-23 L.

When comparing these results to PK parameters of HIV -negative patients in the study, the median C_{max} (18.39 µg/ml) was lower in this study, T_{max} (1hr) was similar, K_e (0.13hr^{-1}) was lower, $t_{1/2}$ (5.37hrs) was longer, Cl_{tot} (4.00L/hr) was lower and V_d (33.02L) was higher.

5.15 Therapeutic implications

The two common PK parameters associated with the clinical efficacy of aminoglycosides are C_{max} and AUC_{0-24} (Yew, 2001). High peak plasma concentration of aminoglycosides is associated with increased rate of survival and better therapeutic response in patients. However, due to the narrow therapeutic index of aminoglycosides and the fact that the concentration required for therapeutic effects is close to the concentration that potentially

causes toxic effects, care must be taken when administering kanamycin in patients with renal impairment.

Most of our patients had a low peak plasma concentration of kanamycin when compared to the literature (Cronk and Naumann, 1959; Cabana and Taggart, 1973; Clarke et al., 1974; Doluiso et al., 1973). The low C_{max} could result in poor response to therapy and increased risk of selection of drug-resistant organisms. It has been suggested that clinicians should aim for a C_{max} of 20–35 µg/ml and a C_{trough} (trough level) of <10 µg/ml when kanamycin is given intramuscularly (Peloquin, 2002). Based on our results, the median C_{trough} of kanamycin in HIV -positive and in HIV -negative patients was 7.8 (0.66-9.98) µg/ml and 8.6 (0.77-11.76) µg/ml respectively. This further explains that kanamycin plasma levels were not toxic in our patients.

Furthermore, AUC is associated with bioavailability and clearance. Increased total clearance results in a fall in the AUC over 24 hours, and vice versa. Also, bioavailability is an important factor that determines clinical efficacy and the therapeutic response of a drug (Burgess, 1999). It was observed in most patients that increased clearance of kanamycin resulted in a fall in the AUC_{0-24}. This means that there was a reduction in the rate and extent of absorption of kanamycin, which could potentially result in the development of drug resistance.

5.16 Limitations of the study

We had an unequal distribution of patients in the HIV-positive and HIV-negative groups. Twenty-five patients volunteered to participate in the study, of whom 11 were HIV-positive.

Also, there were very few patients with kidney dysfunction and no drug–drug interaction was observed between kanamycin and co-administered medications.

There was no balance in the CD4 count and viral load in HIV-positive patients. Five patients had a CD4 count of <100 cells/mm^3 and six had a CD4 count of >100 cells/mm^3. Some patients were in the early stage of the disease, while some had advanced HIV infection; thus, it might be difficult to conclude if HIV infection was responsible for the abnormal PK parameters seen in these patients. In addition, studies on the PK of kanamycin are very scarce, especially in sub-Saharan Africa. Thus, comparison of our results with previously published studies for similarities or differences in the PK parameters was limited. Furthermore, the cost of getting more volunteers to participate in the study would have been very expensive. This impeded the assessment of whether HIV infection, kidney dysfunction or drug interaction influences the PK of kanamycin.

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The aim of the study was to determine kanamycin plasma concentrations using LC-MS and to describe the PK of kanamycin in patients with MDR-TB infection and in patients with MDR-TB co-infected with HIV during their course of treatment. In addition, we also examined whether HIV, kidney dysfunction and drug interactions influenced the PK of kanamycin.

Based on the results obtained, we can conclude that objectives of the present study were achieved:

- The LC-MS analysis method is highly specific, highly sensitive and reproducible in the detection and quantification of the plasma concentrations of kanamycin.
- The PK of kanamycin in patients with MDR-TB and in those with MDR-TB co-infected with HIV was described.
- HIV infection does not have any influence on the PK of kanamycin.
- Changes in renal function do not influence the PK of kanamycin.

6.2 Recommendations

Based on the above mentioned limitations, the following should be considered for future studies:

- PK/PD relationship of kanamycin should be determined, because it quantifies the activity of an antibiotic and describes the time course of action of the antibiotic.

- Kanamycin-free plasma concentration should be evaluated, because the free plasma concentration is responsible for therapeutic effects.

- The minimum inhibitory concentration (MIC) of kanamycin should be determined on each patient's sputum. MIC is a good predictor of the potency of anti-bacteria activity.

- Sputum conversion for each patient should be recorded five times during the treatment. This indicates if MDR-TB therapy was successful.

- Other MDR-TB treatment outcomes (cure, treatment failure, death and defaultment) should be determined.

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APPENDIX A

Pharmacokinetics and Pharmacodynamics of second line antimycobacterial drugs in patients with multidrug-resistant tuberculosis and in patients co-infected with multidrug-resistant tuberculosis and HIV

Patient consent form (written consent only)

Patient study number:.....

Patient Statement

This study and related procedures have been explained to me to my satisfaction, I have received a copy of the patient information sheet and have been given the opportunity to ask questions which have been answered to my satisfaction. I hereby agree to voluntarily participate in this programe.

I understand that if I choose to withdraw from this study, I will need to inform my Doctor in order to enable him to evaluate my status, to review the consequences of my decision, as well as to allow him to perform procedures for an orderly termination of my participation.

....

Signature of patient Date (to be completed by patient)

Statement of randomising Doctor

I certify that I have explained to the above patient the nature, purpose, procedures, the possible risks and potential benefits of this research project and have provided the patient with the patient information sheet whose reference is ( )*

(* Insert reference of Patient Information Sheet given to the patient)

....

Signature of randomising Doctor Date

Top Copy-patient's hospital notes Second Copy - patient Third Copy-Site Working

APPENDIX B

UNIVERSITY of the
WESTERN CAPE

OFFICE OF THE DEAN
DEPARTMENT OF RESEARCH DEVELOPMENT

26 September 2012

To Whom It May Concern

I hereby certify that the Senate Research Committee of the University of the Western Cape has approved the methodology and ethics of the following research project by:
Prof P Mugabo (School of Pharmacy)

Research Project:	Pharmacokinetics and pharmacodynamics of second line antimycobacterial drugs in patients with multidrug-resistant tuberculosis and in patients co-infected with multidrug-resistant tuberculosis and HIV.
Registration no:	12/6/44

Any amendments, extension or other modifications to the protocol must be submitted to the Ethics Committee for approval.

The Committee must be informed of any serious adverse event and/or termination of the study.

Ms Patricia Josias
Research Ethics Committee Officer
University of the Western Cape

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