Ethionamide pharmacokinetics in multidrug-resistant tuberculosis patients with and without HIV-infection

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KEY WORDS

Multidrug-resistant tuberculosis

Human immunodeficiency virus
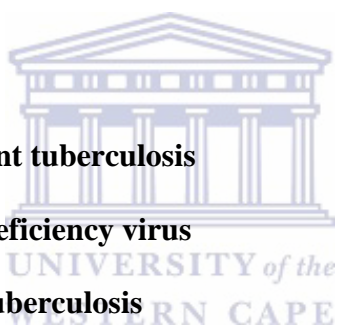
Mycobacterium tuberculosis

Antiretroviral agents

Ethionamide

Pharmacokinetics

Plasma concentrations

Liquid chromatography

Mass spectrometry

ABSTRACT

Ethionamide pharmacokinetics in multidrug-resistant tuberculosis patients with and without HIV infection.

Many studies have investigated the pharmacokinetics (PK) of anti-tuberculosis drugs in tuberculosis patients. However, currently in South Africa, no studies have been done on ethionamide (ETH) PK in adult MDR-TB patients that are infected with HIV and those without HIV infection. Therefore, the objective of this current study was firstly, to find out ethionamide plasma concentration using the LC-MS method; secondly, to evaluate and compare the pharmacokinetics of ethionamide in MDR-TB patients infected with and without HIV infection; thirdly, to examine the effects of ARVs and kidney impairment on the PK of ethionamide and fourthly, to find out the consequence of sex and age on ETH PK parameters.

The study was conducted in male and female patients, 18 to 65 years old, and MDR-TB patients that are infected with HIV and those without HIV infection, at least two weeks after starting treatment. After informed written consent was obtained, blood samples were collected for renal and liver functions, CD4 count and viral load tests. More blood samples were collected (8 times) over 24 hours post-dosing from each patient in heparinized tubes for ETH plasma concentrations determination. After centrifugation, the plasma was separated from the sediment and ETH plasma levels were determined using LC-MS. Ethionamide plasma concentration-time profile was constructed and pharmacokinetic parameters (AUC, Ka, Ke, $T_{1/2}$, T_{max}, C_{max}, Vd, CL, and MRT) were calculated.

The LC-MS method used was simple, highly sensitive, specific, and its chromatogram produced a fine resolved peak for ethionamide with excellent

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precision. Ethionamide displayed linearity over concentration ranges of 0.1-10 μg/ml. The lower limit of detection of ethionamide in the plasma was 0.01 μg/ml, while the lower limit of quantification was found to be 0.15 μg/ml. No interfering peaks were observed in the plasma constituents of ethionamide peak, and were identified at 2.23 minutes retention time. The average recovery of ethionamide ranged from $104 \pm 2.3\%$ to $128 \pm 11.2\%$. Standard deviation for all analytes was greater than 12%.

The study involved 42 patients (23 males and 19 females), consisting of 17 who were HIV-positive and 25 HIV-negative. The pharmacokinetic parameter results obtained were presented as median and range. In HIV (-) patients, Ka was approximately 0.71 hr^{-1}, Ke was 0.30 hr^{-1}, V_d was 4.24 L/kg, C_{max} was about 1.53 mcg/ml, T_{max} was 2.23 hrs, AUC_{0-24} was 3.55 µg.h/ml, $AUC_{0-\infty}$ was 3.91 µg.h/ml, $T_{1/2}$ was 2.31 hrs and MRT was 3.69 hrs. Likewise, in HIV (+) patients, Ka was just about 1.03 hr^{-1}, Ke was 0.24 hr^{-1}, V_d was approximately 1.99 L/kg, C_{max} was 3.71 mcg/ml, T_{max} was 1.70 hrs, AUC_{0-24} was around 7.74 µg.h/ml, $AUC_{0-\infty}$ was 7.86 µg.h/ml, $T_{1/2}$ was roughly 2.88 hrs and MRT was 3.62 hrs respectively in this study.

No statistically significant differences are observed in all the pharmacokinetic parameters between the HIV (+) and HIV (-) patients. Liver function was excellent in HIV (+) and HIV (-) patients. More than half of the study population (60%) had normal renal function. Forty percent of the population had impaired renal function, consisting of 15 mildly impaired and 2 moderately impaired patients. Renal impairment did not influence ethionamide elimination. ETH steady state concentrations were not attained. Differences in age and sex did not affect the PK of ETH in the present study.

The study involved an imbalanced patients' distribution in HIV (+) group. There was a great variability in HIV (+) patients with regard to viral load, CD4 counts and ART. The findings of this study on the effect of HIV disease and ARVs on ethionamide pharmacokinetics are limited. Thus, further research with a larger number of patients

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should be conducted in order to address the un equal distribution and inconsistency in HIV (+) patients regarding the viral load, ART and CD4 counts.

April, 2017.

DECLARATION

I declare that the thesis *Ethionamide pharmacokinetics in Multi-drug resistant tuberculosis patients with and without HIV infection* is my work, that it has not been submitted before for any degree or examination at any other university, and that all the sources I have used or quoted have been indicated and acknowledged.

Ezeukwu Ifeoma Patricia   April, 2017.

Signed …………….   UWC, Bellville

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ABBREVIATIONS AND ACRONYMS

AIDS	Acquired immune deficiency syndrome
ALP	Alkaline phosphatase
ALT	Alanine aminotransaminase
ART	Antiretroviral treatment
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
cART	Combination antiretroviral therapy
CD4	Cluster difference 4
CKF	Chronic kidney failure
Cl	Clearance
Cl_{tot}	Total body clearance
C_{max}	Maximum concentration
CYP450	*cytochrome P450*
CrCl	Creatinine clearance
CSF	Cerebrospinal fluid
D4T	Stavudine
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic Acid
DOT	Directly observed treatment
DST	Drug susceptible tuberculosis
ELISA	Enzyme-multiplied-immuno assay technique
EMB	Ethambutol
ESRD	End-stage renal disease
GIT	Gastrointestinal Tract

HAART	Highly active antiretroviral therapy
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HIV-TB	Human immunodeficiency virus-tuberculosis
HIVAN	Human immunodeficiency virus-associated nephropathy
HPLC	High-pressure liquid chromatography
GGT	Gamma glutamic transpeptidase
IBW	Ideal body weight
INH	Isoniazid
KDOQI	Kidney disease outcome quality initiation
Ke	Elimination rate constant
LBW	Lean body weight
LC-MS	Liquid chromatography-mass spectrometry
MBC	Minimum bactericidal concentrations
MIC	Minimum inhibitory concentration
MDRD	Modification of diet in renal disease
MDR-TB	Multidrug-resistant tuberculosis
MRT	Mean residence time
MTB	*Mycobacterium tuberculosis*
NAD	Nicotinamide adenine dinucleotide
NRTI	Nucleoside reverse transcriptase inhibitor
NNRTI	Non-nucleoside reverse transcriptase inhibitor
PD	Pharmacodynamics
PI	Protease inhibitor
PK	Pharmacokinetic(s)
PZA	Pyrazinamide
RIF	Rifampicin
RNA	ribonucleic acid
RR-TB	Rifampicin-resistant tuberculosis
3TC	Lamivudine

TB	Tuberculosis
TBW	Total body weight
TDM	Therapeutic drug monitoring
T_{max}	Time to reach maximum concentration
$T_{1/2}$	Half-life
ULN	Upper limit of the normal range
UNAIDS	United Nations Programme on Acquired immune deficiency syndrome
UV	Ultraviolet
Vd	Volume of distribution
WHO	World Health Organization
XDR-TB	Extensively drug-resistant tuberculosis

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CHAPTER ONE
INTRODUCTION

1.1. Background information

Tuberculosis (TB) remains the main health challenge worldwide and causes illness in millions of individuals yearly. In 2015, TB was among the peak 10 causes of mortality globally, ranking beyond HIV/AIDS as among the sources of mortality from a transmissible illness (WHO, 2016). In 2015, there are estimated 10.4 million cases of new TB globally (with 1.2 million amongst HIV (+) persons), of which 5.9 million were men, 3.5 million were women and 1.0 million were children (WHO, 2016). Overall, ninety percent of the cases constitute adults and ten percent children, and the female-male ratio represents 1:6.1 (WHO, 2016). Globally, the thirty high tuberculosis-burdened countries were 87% of the approximated cases. Six countries that made-up the sixty percent of the worldwide TB total include: China, Indonesia, Pakistan, India, South Africa and Nigeria. Of these, in 2015, Indonesia, China and India alone accounted for forty-five percent of worldwide cases (WHO, 2016).

Extensive universal abuse of rifampicin and isoniazid has led to the surfacing of multidrug-resistant tuberculosis (MDR-TB) globally. MDR-TB, which is defined as being TB resistant caused by unresponsiveness of bacteria to isoniazid and rifampicin, the two main potent anti-TB medicines, is of increasing concern (WHO, 2016). An estimation of 3.9% new and 21% of previously treated TB had rifampicin- or multidrug-resistant tuberculosis (MDR/RR-TB) in 2015, according to drug resistance surveillance data (WHO, 2016). In 2015, there were an estimated 580 000 MDR/RR-TB globally, with MDR-TB accounting for eighty-three percent of the whole, and about 250,000 deaths from MDR/RR-TB (WHO, 2016). Thus, the high MDR-TB rates amongst high-incident tuberculosis countries are hindering the improvement concerning care and control of tuberculosis (WHO, 2016).

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TB causes illness in millions of individuals yearly and it is a primary basis of global mortality together with the human immunodeficiency virus (HIV) infection (WHO, 2016). According to WHO, HIV (+) individuals are more prone to developing tuberculosis illness than HIV (-) persons worldwide (WHO, 2016). Globally, in 2015, new HIV infections were estimated to be approximately 2.1 million (1.8 million–2.4 million), adding up to a total of 36.7 million (34.0 million–39.8 million) HIV (+) (including 1.8 million children) (UNAIDS, 2016). According to a UNAIDS report, there is a universal HIV occurrence of 0.8% where most of them reside in low- and middle-income nations, although 40% of the population do not know their status (UNAIDS, 2016). In 2015, 1.1 million individuals died as a result of AIDS-related diseases (UNAIDS, 2016). In 2015, an estimated 11% of tuberculosis incidences were among HIV (+) persons (WHO, 2016). Globally, there were about 1.4 million mortalities due to tuberculosis amongst HIV (-) persons, with 0.4 million deaths from TB patients co-infected with HIV (WHO, 2016). In 2016, WHO reported that individuals' death associated with HIV-TB that was peaked at 570 000 people in 2004, has reduced to 390, 000 people in 2016(a reduction of 32%) (WHO, 2016). Nevertheless, HIV-associated TB is still characterized as a huge problem of avoidable ill-health and deaths. Tuberculosis mortality amongst HIV (+) persons is approximately 25% of all deaths resulting from TB (between HIV (+) and HIV (-) individuals) and one third of the accounted 1.2 million mortalities were from HIV/AIDS in 2014 (WHO, 2016). In addition, there were an estimated 1.5 million deaths resulting from TB (0.4 million amongst HIV (+) persons and 1.1 million amongst HIV (-) persons), of which approximately 480 000 were women, 890 000 were men and 140 000 were children (WHO, 2016).

The tuberculosis increase in sub-Saharan Africa is not associated with unsuccessful control programmes; but as a result of the HIV-TB connection, as each one of the diseases speeds up the progress of the other. The worldwide incidence of TB is about 10,400,000 with 2,720,000 of these in sub-Saharan Africa (WHO, 2016). Sub-Saharan Africa accounts for about of 25.5 million HIV (+) persons, and most of the

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people (approximately 19 million) reside in Southern and Eastern Africa, which in 2015, accounted for 46% of global new HIV diseases (UNAIDS 2016). The percentage cases of HIV-TB were highest in the World Health Organisation African Region (31%). Approximately 834 000 people with HIV-positive TB incidence live in Africa (WHO, 2016). Beginning in the 1980s, HIV endemic caused a severe rise in tuberculosis incidences and tuberculosis death in numerous nations, particularly in Eastern and Southern Africa. Sub-Saharan Africa bears the twofold endemic burden, accounting for about 75% of all associated HIV-TB deaths in 2015 (WHO, 2016). In addition, WHO estimated that the cases of MDR/RR-TB amongst informed cases of pulmonary tuberculosis consist of 42 000 people in Africa in 2015 (WHO, 2016). Likewise, in Africa, the estimated proportion of previous cases of treated tuberculosis with MDR/RR-TB was 15% (7.5 – 22), and new cases with MDR/RR-TB were 3% (1.2 – 4.9) (WHO, 2016). According to WHO, there were an estimated 450 000 TB deaths and approximately 300 000 TB/HIV deaths in Africa (WHO, 2016).

South Africa is among the top 30 TB-burdened countries worldwide and has the second highest tuberculosis incidence rate globally (WHO 2016). In South Africa, the estimation of tuberculosis incidence has risen to 454 000, comprising 263 000 males and 191 000 females (WHO 2016). In 2015, data relating to HIV prevalence in South Africa showed that estimation of 7 million citizens are HIV (+), which is the largest and high-profile global HIV outbreak (WHO, 2016). In 2016, there were 380,000 new infections and 180,000 people dying as a result of AIDS-related illnesses (UNAIDS 2016). Although South Africa is among the nations with the highest antiretroviral treatment (ART) programme internationally, incidence of HIV still remains high (19.2%) amongst the entire population, and differs between regions (SANAC, 2015). For instance, HIV incidence is about forty percent in KwaZulu-Natal, in comparison with eighteen percent in Northern Cape and the Western Cape (UNAIDS 2016). The percentage cases of HIV-TB exceeded 50% in parts of Southern Africa. Thus, South Africa is among the top 30 TB/HIV burdened countries, with approximately 258,000 people with tuberculosis co-infected with HIV. In the

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WHO Global TB Report (2015), South Africa had the second highest absolute number of notified rifampicin-resistant (RR)/MDR cases globally (18 734) (WHO, 2016), with India ranking number one (25 748), though it has a population 20 times that of South Africa. Hence, South Africa is among the top 30 MDR-TB burdened countries, with an estimation of MDR/RR-TB cases of informed pulmonary tuberculosis cases of 10 000 in total. Moreover, an estimated proportion of previous cases of treated tuberculosis with MDR/RR-TB as 7.1% (5.3 – 8.9) and new MDR/RR-TB cases as 3.5% (2.8 – 4.2) (WHO, 2016). The TB treatment success rate in South Africa for cases of RR-/MDR-TB beginning with 2nd-line therapy in 2012 was 49% (WHO, 2014). In addition, there was an estimation of 73 000 TB/HIV mortality in South Africa (WHO, 2016).

Survey conducted in Cape Town, Western Province of South Africa demonstrated that approximately 4.8% new cases of tuberculosis and 10.5% cases of retreatment were due to MDR strains in 2008 (Cox et al., 2010). Also in Khayelitsha, a peri-urban town in the Western Cape, South Africa, MDR-TB burden was estimated to be exceptionally high, at 51/100,000/year based on cases of notified tuberculosis. The prevalence is prone to be significantly elevated considering the discovery of partial tuberculosis case among the community (Cox et al., 2010). MDR-TB is being treated in different selected hospitals in South Africa. In the Western Cape Province, there are four MDR-TB hospitals, namely, DP Marais Hospital in the Cape Metropole region, Brewelskloof Hospital in Worcester, Brooklyn Chest Hospital (BCH) and the Harry Comay Hospital in George.

1.2. Overview of co-infection with Human immunodeficiency virus and Multidrug-resistant tuberculosis

More than half of the tuberculosis patients in South Africa are co-infected with human immunodeficiency virus (HIV) (Meintjes, 2014). Increased incidence of drug-resistant tuberculosis is one of the consequences of HIV-TB co-epidemics. The occurrence of multidrug-resistant tuberculosis (MDR-TB) is increasingly high in

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HIV-prevalence settings. The HIV infection impact on MDR-TB is of immense health concern to the community. HIV co-infection promotes MDR-TB, not only in less developed regions. Infection caused by HIV was demonstrated to be connected with MDR-TB outbreaks in institutional settings, for instance prisons and hospitals (Small et al., 1993; Ritacco et al., 1997; Wells et al., 2007). In the United States, frequently nosocomially developed within closed settings for example, hospitals, shelters, prisons, and is associated with HIV-infection (Alexander and De, 2007). Such extremely institutional-type drug-resistant epidemic usually leads to death except if the patients are accurately and rapidly treated.

HIV and MDR-TB plagues are fuelling each other. Epidemiological factors such as the burden of HIV or TB infection are important aspects that affect the relationship between MDR-TB and HIV-infection (Eldholm et al., 2016). Infection caused by HIV makes individuals vulnerable to developing active tuberculosis by immune system suppression; nevertheless, drugs used for tuberculosis treatment can also interfere with antiretroviral treatment directly. Eldholm and colleagues reported that rifampicin has been demonstrated to considerably lessen plasma levels of HIV reverse transcriptase and protease inhibitors. Likewise, co-infection of HIV-TB is linked with anti-TB drugs malabsorption, such as rifampicin (Eldholm et al. 2016). Multidrug-resistant TB spread is hastened by numerous issues, including ineffective and interrupted therapy, inadequate infection control and co-infection with HIV.

HIV co-infection causes additional problems to the management of drug-resistant tuberculosis. These include immune reconstitution inflammatory syndrome (IRIS) with extrapulmonary sites symptoms, PK drug interactions, increased drug toxicity possibility owing to underlying HIV-related organ disease such as nephropathy, and joint drug toxicities among HIV and TB drugs, (Meintjes Graeme, 2014). The MDR-TB burden surged simultaneously with endemic HIV and is associated with high mortality (Isaakidis et al., 2015). Mortality caused by MDR-TB is high in HIV (+) patients. Current studies revealed higher death rates between HIV (+) XDR and MDR

-TB patients, compared to those with non-HIV infection (Scano et al., 2008). Although ARV therapy for HIV-infection has helped to reduce death rates in patients co-infected with TB/HIV, MDR-TB and drug-resistant TB strains have increased death rates in persons that are co-infected. Thus, the success of ARV treatment programs against HIV-infection, in addition to TB control programs, is threatened.

The standard six months course of 1st-line anti-TB drugs which is effective in tuberculosis patients cannot be used for treatment of MDR-TB. Patients with rifampicin-resistant or MDR-TB are treated with different 2nd-line drug combination. The national guidelines on treatment regimens for MDR-TB recommend a 5-drug regimen (kanamycin, moxifloxacin, ethionamide, terizidone and pyrazinamide) during an intensive phase of 6-months, followed by a 4-drug regimen (pyrazinamide, terizidone, ethionamide and moxifloxacin) in a continuation phase of not less than 18 months (DOH, 2012). Attempts to reduce the length of conventional MDR-TB regimens and to use a combination of drugs which is tolerable have been ongoing for several years through various studies (WHO, 2016). In May 2016, the World Health organization recommended a new short MDR-TB regimen comprising a 4-6 months' initial phase administration of 7-drugs (kanamycin, clofazimine, prothionamide, moxifloxacin, isoniazid, ethambutol and pyrazinamide). This is to be followed by a 5 months' continuation phase administration of 4-drugs (clofazimine, moxifloxacin, ethambutol and pyrazinamide). ETH is one of the second-line orally administered drugs, in combination with other anti-TB drugs for multidrug resistant organisms' treatment. ETH is a thioamide, frequently given to multidrug resistant tuberculosis patients in South Africa, according to the Department of Health National guidelines (DOH, 2012).

1.3. Motivation of the study

Earlier studies have used bioassay for the determination of ETH levels in the serum (Gronroos and Toivanen, 1964; Jenner and Smith, 1987). More recent investigations in the literature have used HPLC, LS-MS/MS, and HPLC–MS–MS among others for the determination of ethionamide concentrations in the serum (Conte et al., 2001; Zhu et al., 2002; Deshpande et al., 2011). Zhu et al. (2002) validated the HPLC assay and concluded that ethionamide (ETH) pharmacokinetic (PK) parameters are different among healthy subjects and TB patients, probably because of dissimilarities in the completeness of their absorption. Conte et al. (2001) were able to develop and validate a sensitive HPLC–MS–MS assay for ethionamide determination in the plasma. They concluded that orally-administered ETH absorption was unaffected by the presence of AIDS or gender. Likewise, Deshpande et al. (2011) validated LC-MS/MS which was applied to a pharmacokinetic study in humans and is useful for therapeutic drug-monitoring of ETH in TB patients. No recent studies in any literature have determined ETH plasma concentrations using LC-MS assay in MDR-TB patients. Hence, it will be interesting to determine ETH plasma concentrations in MDR-TB patients using the LC-MS analytical method.

Previous study involving ETH focused on ETH metabolism for the type of metabolites formed (Jenner et al., 1984; Jenner and Ellard et al. 1981) and ETH sulphoxide was the major metabolite identified. So far, some reports have examined the PK profiles of first-line drugs in patients with tuberculosis and patients with tuberculosis co-infected with HIV (Gurumurthy et al., 2004). In recent years, investigations have focused on ethionamide PKs (Auclair et al., 2001; Zhu et al., 2002; Ahmad et al., 2009; Thee et al., 2011). Few studies have evaluated and compared the PKs of ETH in TB patients and healthy volunteers (Auclair et al., 2001; Zhu et al., 2002; Ahmad et al., 2009). A study done by Auclair et al. (2001) reported that ethionamide PK behaviour was not considerably altered by the different conditions such as fasting, orange juice, food and antacid studied. Zhu et al. (2002) concluded that PK parameters of ETH are different among healthy subjects and TB

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patients, probably because of the dissimilarity in their completeness of absorption. Ahmad et al. (2009) assessed the PKs of ETH in healthy human subjects, and reported that bioavailability and pharmacokinetic parameters assessed showed similarities to the previously reported parameters. Ahmad et al. (2009) concluded that the study would be valuable and useful in dosage regimen design for patients on ethionamide treatment and can be used as a guideline for PK parameters and bioavailability determination in clinical settings. Very limited studies have described the PKs of second line anti-TB drugs. So far, there are apparently, no studies in South Africa that show the PKs of ETH in MDR-TB patients with and without HIV-infection. Hence, it will be essential to evaluate and compare the PKs of ETH in MDR-TB patients with and without HIV infection. The findings of this study may aid upcoming improvement of therapeutic drug monitoring to optimise drug therapy in MDR-TB patients and enhance patients' outcome.

Studies have looked at the effect of efavirenz (EFV) on pharmacokinetics (PKs) of some anti-TB drugs (López-Cortés et al., 2002). López-Cortés and his team investigated PK interaction among rifampicin and efavirenz in HIV (+) patients with TB and recommended increasing efavirenz dosage to 800 mg daily when co-administered with rifampicin, although the minimum effective plasma concentration of efavirenz which guarantees virology success is unknown at present. Patients with slow *CYP2B6* metabolizer genotype experience an increase in efavirenz concentrations during TB treatment, probably because of isoniazid (INH) inhibition of the pathways metabolizing efavirenz (Lawn et al., 2013). PK interaction such as hepatotoxicity was predicted when efavirenz (non-nucleoside reverse transcriptase inhibitors) was used in conjunction with ETH, but no studies on this have been performed (Coyne et al., 2009). Coyne and his colleagues recommended that efavirenz should be avoided if possible or, if used, the concentrations of both EFV and ETH should be measured appropriately and the psychiatric morbidity with efavirenz should be closely monitored. They showed that no studies have been performed for lamivudine and stavudine (nucleoside reverse transcriptase inhibitors),

and their interactions with ETH are difficult to predict (Coyne et al., 2009). However, they recommended the use of standard ETH doses. Similarly, limited information is available on the metabolic pathway of few 2nd-line anti-TB drugs (e.g. cycloserine, para-aminosalicylate and ethionamide), since these anti-TB drugs were licensed and developed decade ago (CDC, 2013). Depending on what is known about the metabolic path ways knowledge, it is assumed that the majority of these anti-TB drugs do not have important interactions with ARVs (CDC, 2013). Considering available information in literature, there are very limited studies that have assessed the effects of lamivudine, stavudine and efavirenz on ETH PKs. Hence, it will be important to investigate the effects of ARVs on ETH PKs.

Some studies have investigated whether age and sex do have any influence on ETH PKs (Conte et al., 2000; Auclair et al., 2001; Zhu et al., 2002). Zhu et al. (2002) reported that analysis of the PK evaluations in healthy subjects and TB patients showed that the values of ETH pharmacokinetic parameters were not linked to gender, weight or age. Research done by Conte et al. (2000) on the consequences of gender and AIDS on plasma and intrapulmonary concentrations of ethionamide, confirmed that plasma concentrations of ethionamide were not influenced by the presence of AIDS or gender. Hence, more studies are required for a comprehensive understanding of the effects of age and sex on the PKs of ETH.

The associations between renal function and ETH elimination have been studied (Malone et al., 1999; Zhu et al., 2002; Coyne et al., 2009). ETH is extensively metabolised in the liver and less than 1% is excreted by the kidneys unchanged (Coyne et al. 2009). ETH PK parameter values with respect to terminal clearance and elimination half-life were independent of creatinine clearance (Zhu et al., 2002). Malone et al. (1999) have determined the haemodialysis clearances of the 2nd-line anti-TB drugs, and reported that ETH was not significantly dialyzed. Therefore, for drugs given to renal impaired patients, pharmacokinetics characterization must be evaluated in renal impaired patients for adequate rational dosing recommendations.

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Hence, supplementary studies are required to investigate the effects of renal function on ETH elimination.

1.4. Hypotheses

1.4.1. Experimental hypothesis

- There is a difference in the pharmacokinetic parameters of ethionamide among patients with MDR-TB and in MDR-TB patients co-infected with HIV.

- HIV infection influences the PK of ethionamide.

- Renal dysfunction influence ethionamide elimination.

- Age and sex influence ethionamide PK.

1.4.2. Null hypothesis

- There are no differences in the pharmacokinetic parameters of ethionamide among patients with MDR-TB and in MDR-TB patients co-infected with HIV.

- MDR-TB patients and MDR-TB patients co-infected with HIV.

- HIV infection does not influence the ethionamide PK.

- Renal dysfunction does not influence ethionamide elimination.

- Age and sex do not influence ethionamide PK.

1.5. Research questions
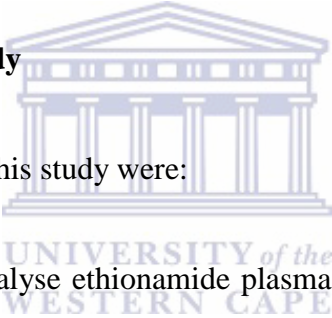
It is pertinent to note that, to date, no research study has been carried out on ethionamide PK parameters in patients with MDR-TB and in MDR-TB patients co-infected with HIV using the LC-MS analytical method. Thus, it would be fascinating to investigate if there are any associations between our PK data and other findings from the literature. In addition, it is significant to investigate if HIV-infection influences PK of ETH in MDR-TB patients with HIV.

Moreover, it will be interesting to investigate if any changes in renal function affect ethionamide elimination in both patients' groups.

1.6. Objectives of the study

1.6.1. General objectives

The general objectives of this study were:

- To develop and analyse ethionamide plasma concentration using the LC-MS method.

- To evaluate and compare the pharmacokinetics of ethionamide in MDR-TB South African patients infected with and without HIV infection.

- To assess the effect of lamivudine, stavudine and efavirenz on ethionamide pharmacokinetics.

- To find out whether age and sex do have any influence on ethionamide PK.

- To find out if there is any association between renal impairment and ethionamide elimination.

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CHAPTER TWO
LITERATURE REVIEW

2.1. Overview of multidrug resistant tuberculosis

Multidrug-resistant tuberculosis (MDR-TB) is caused by bacteria that are unresponsive to rifampicin and isoniazid, the two most potent, first-line anti-tuberculosis drugs (WHO, 2016). Recently, World Health Organization expressed the worldwide effects of multidrug-resistant tuberculosis as a "public health crisis" (WHO, 2014). The mechanism of drug resistance to tuberculosis has advanced rapidly. It was reported by Ramaswamy and Muser (1998) that resistant to drug is caused by a random and spontaneous mutation in the bacterial chromosome MTB. According to Ramaswamy and co-researchers, the mutation modifies the succession of a 27-amino-acid area of the beta subunit of ribonucleic acid (RNA) polymerase in rifampicin. The authors further reported that the katG gene encoding catalase-peroxidase mutations resulted in a changed enzyme arrangement, yielding to reduced INH conversion to a biologically active shape. Hence, MDR-TB strains occur by the chronological accumulation of resistance mutations to individual medications (Ramaswamy and Muser, 1998).

Generally, MDR-TB can be classified as primary, acquired or initial drug resistance (Drobniewski et al., 2002), based on the previous TB treatment history of the patient. Primary drug resistance (resistance of drug among new cases) to anti-tuberculosis drugs happens when a patient with no history of previous TB treatment develops a disease from an organism that is already resistant and becomes infected with a drug-resistant strain of *Mycobacterium tuberculosis*. Drug resistance amongst new tuberculosis cases is categorized by the presence of MTB resistant strains in persons that had previously not received treatment for TB or had received medications for TB treatment below one month (Crofton et al., 1997). In contrast, acquired drug resistance (or drug resistance among previously-treated cases) develops in patients with previous TB treatment or in patients currently receiving treatment (Drobniewski

et al., 2004). This resistance could be as a result of inadequate anti-mycobacterial drugs that were administered to the patient (Friedland, 2007). Other factors reported are the inappropriate treatment regimens administration, poor quality medicines and/or poor patient compliance, particularly in regions with poor control programs for tuberculosis (WHO, 2013a). It is known that acquired resistance can only occur in patients that had received a minimum of four weeks' chemotherapy for tuberculosis (Loddenkamper et al., 2002). Likewise, initial drug resistance (or resistant of drug amongst new TB cases) to anti-tuberculosis drugs refers to patients with a primary resistance, in addition to undisclosed acquired resistance persons (Loddenkamper et al. 2002). According to a report by Crofton and his team, in circumstances where preceding treatment of tuberculosis in individuals is not known, this resistance type is called initial resistance (Crofton et al., 1997).

A study by Pablos et al. (2002) reported that the reasons why MDR-TB continues to increase and emerge are person-to-person transmission and tuberculosis treatment mismanagement. This happened because of inconsistent or incomplete treatment of "ordinary" tuberculosis, either by administering single drugs for "ordinary" tuberculosis or by administering drug combinations that are inappropriate, patients not taking their treatment correctly because of insufficient patients counselling, patients not returning for treatment (defaulting treatment), and clinics running out of drug stocks (Pablos et al., 2002). Other causes include the incorrect use of anti-microbial drugs, especially when the course of antibiotics is interrupted (premature treatment interruption) and the levels of drug in the body are insufficient to kill 100% of bacteria, or administering of ineffective drugs formulations (e.g. bad storage conditions, poor quality medicines), poor infection control, non-adherence by patients, incorrect prescription of drug regimens or erratic drug supply (Pablos et al., 2002).

The clinical presentation, signs and symptoms of drug-resistant TB patients do not significantly differ from those of TB patients; because of pan-susceptible

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Mycobacterium tuberculosis strains (Gandhi et al., 2006; O'Donnell et al., 2009; Schaaf et al., 2009). The clinical picture is further complicated as a result of co-infection with HIV due to other associated opportunistic diseases (Dubrovina et al., 2008; Schaaf et al., 2009; Gandhi et al., 2010; Grobusch, 2010). Commonly presented symptoms of drug resistant-TB are failure to gain weight or loss of weight, coughing for over two weeks, fever, night sweat, convulsions, lymphadenopathy or abscess (Gandhi et al., 2010; Grobusch, 2010). MDR-TB patients co-infected with HIV have considerably more constitutional and pulmonary symptoms, and more extra-pulmonary disease (Dubrovina et al., 2008).

Diagnosis of MDR-TB, which depends on laboratory findings requiring in vitro confirmation of bacterial resistance, ought to be suspected in persons with continuous positive acid-fast bacilli (AFB) cultures or smears, regardless of satisfactory treatment adherence (Weyer, 2005). The diagnostic steps in the treatment of HIV negative and HIV positive persons with drug-resistant TB are the same as those of drug-sensitive TB using conventional diagnostic algorithms (Migliori1 et al., 2010). MDR-TB patients should have their sputum investigated by drug susceptibility testing (DST) and culture of the organism as soon as possible, particularly if TB signs and symptoms are present. Inadequate clinical response to TB treatment is often supported by positive acid-fast bacilli smear in patients at two or three months of treatment, which necessitates doing a culture, as well as a DST against isoniazid (INH) and rifampicin (RIF) (Weyer, 2005). This frequently involves an indirect or a direct drug susceptibility testing in selective media, by means of solid or liquid culture, and it takes a number of weeks before the results are obtainable (Miglioril et al., 2010). When one percent or more of the bacterial population develop resistance to the critical concentration of a drug, isolate of *Mycobacterium tuberculosis* is considered as resistant to that drug (Chaulet and Maher, 1997). Laboratory confirmation of MDR-TB should always be awaited, because the MDR-TB diagnosis critically depends on the quality of laboratory methodology (Weyer, 2005).

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2.2. Chemotherapy of multi-drug resistant tuberculosis

MDR-TB treatment consists of the administration of second-line anti-tuberculosis medications. MDR-TB treatment is difficult and complicated. It must be given under direct observation (DOTS) 7 days a week for 18 to 24 months (based on culture conversion) (DOH, 2012). All MDR-TB patients are hospitalised in designated MDR-TB hospital for the initial six months of intensive treatment, if possible until sputum conversion has occurred, and cultures and sputum smear microscopy turns negative (DOH, 2011). After discharge from hospital, patients are closely monitored with DOTS and followed up at designated clinics until their treatment is completed. All MDR-TB patients with HIV co-infection, irrespective of their CD4 count, are given antiretroviral treatment.

The national guideline treatment regimens for MDR-TB recommend a 5-drug regimen during an intensive phase of 6-months which is guided by TB culture conversion; and is then followed by a 4-drug regimen during a continuation phase of at least 18 months after TB culture conversion (DOH, 2012). The treatment of MDR-TB infection includes an aminoglycoside (kanamycin), a fluoroquinolone (moxifloxacin), a thioamide (ethionamide), terizidone, pyrazinamide, ethambutol (EMB) and glycopeptides (capreomycin) (DOH, 2012). Capreomycin is considered in patients with peripheral neuropathy, renal insufficiency or hearing loss. The dose of the drug is adjusted as patients gain (or lose) weight. The recommended MDR-TB regimen and its dosages are given in Tables 2.1 and 2.2.

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Table 2.1: Standardized Regimen for MDR-TB Treatment in South Africa (for intensive phase: at least 6 months, guided by TB culture conversion) (DOH, 2012)

Drug	< 50 kg	50-65 kg	> 65 kg
Kanamycin	500-750 mg	1000 mg	1000 mg
Moxifloxacin	400 mg	400 mg	400 mg
Ethionamide	500 mg	750 mg	750 – 1000 mg
Terizidone	750 mg	750 mg	750 – 1000 mg
Pyrazinamide	1000-1750 mg	1750-2000 mg	2000-2500 mg

Key: mg =milligram; kg =kilogram.

Ethambutol (15-20 mg/kg) is given as an average daily dosage, where the minimum daily dosage is 1000 mg and the maximum daily dosage is 1200 mg (DOH, 2011).

Table 2.2: Standardized Regimen for MDR-TB Treatment in South Africa (for continuation phase: at least 18 months after TB culture conversion) (DOH. 2012)

Drug	< 50 kg	50-65 kg	> 65 kg
Moxifloxacin	400 mg	400 mg	400 mg
Ethionamide	500 mg	750 mg	750 – 1000 mg
Terizidone	750 mg	750 mg	750 – 1000 mg
Pyrazinamide	1000-1750 mg	1750-2000 mg	2000-2500 mg

Key: mg =milligram; kg =kilogram.

2.3. Pharmacology of drugs used for multidrug-resistant tuberculosis treatment in South Africa

In South Africa, drugs used for multidrug-resistant tuberculosis treatment include an aminoglycoside (kanamycin), a fluoroquinolone (moxifloxacin), a thioamide (ethionamide), terizidone and glycopeptides (capreomycin) (DOH, 2012). Some of the patients, whose mycobacterial tuberculosis is not resistant to pyrazinamide,

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ethambutol and isoniazid, receive these first-line TB drugs, together with second line drugs, used for the treatment of MDR-TB. Hence, pyrazinamide, ethambutol and Isoniazid are also used for MDR-TB treatment where resistance to these drugs is not confirmed. The description of each drug used for MDR-TB treatment is as shown below

2.3.1. Isoniazid

Isoniazid (INH) is regularly administered in combination with other anti-tuberculosis drugs for TB treatment, either as monotherapy or in conjunction for the treatment of latent tuberculosis infection. The minimum inhibitory concentration (MIC) of INH for *Mycobacterium tuberculosis* is approximately 0.02 to 0.20 μg/mL (Arbex et al., 2010). Its mechanism of action involves the inhibition of mycolic acid biosynthesis (Arbex et al., 2010). However, INH also disrupts lipid, carbohydrate, deoxyribonucleic acid (DNA) and nicotinamide adenine dinucleotide (NAD) synthesis or metabolism (DOH, 2012). Isoniazid is completely absorbed and the peak plasma concentrations are reached in about 1 to 2 hours. Concomitant administration of isoniazid with food reduces its bioavailability (Conte et al., 2009). It freely diffuses into all body fluids, organs, tissues and excreta (faeces, sputum and saliva). INH is less than 10% bound to plasma proteins. Its metabolism is via the liver, mainly by acetylation and dehydrazination. Elimination is mostly not dependent on kidney function; but the half-life was prolonged in hepatic disease (Arbex et al., 2010).

Isoniazid is metabolized by arylamine N-acetyltransferase2 (NAT2), glutathione S-transferase (GST) and cytochrome P450 2E1 (CYP2E1) (Sotsuka et al. 2011). It is hepatically metabolized via acetylation by N-acetyltransferase that yields isonicotinic acid and acetylisoniazid. The rate of acetylation is genetically characterised and thus, differs from patient to patient. Rapid acetylator phenotype is observed in certain patients, while others display the slow acetylator phenotype. INH is excreted by the kidneys as inactive metabolites. In rapid acetylator phenotype patients, approximately 7% of excreted INH in urine appears as free INH, while for the slow acetylator

phenotype patients; about 37% appears as conjugated INH. A little quantity is excreted in faeces (Sotsuka et al. 2011). INH half-life is about 1 hour in rapid acetylator phenotype patients and approximately 2 to 5 hours in slow acetylator phenotype persons (Arbex et al., 2010). In patients with renal failure and hepatic disease, INH half-life was longer (Arbex et al., 2010). It is currently postulated that INH is active after transformation by peroxidase into radicals that can either react with vital targets in mycobacterial cells, or ultimately produce isonicotinic acid (Bardou et al., 1998). The only clearly defined target of INH is the biosynthesis of mycolic acids, which are the specific and major compounds of all mycobacterial cell walls.

Isoniazid, as an enzyme inhibitor, inhibits the microsomal enzymes (cytochrome P450 isoenzymes), decreasing the rate of metabolism and excretion of other drugs that are metabolised by the same enzymes. TB drug malabsorption is more likely to occur when concurrent GIT infection/ diarrhoea, or advanced immunodeficiency with or without diarrhoea, is present. There is evidence of poor absorption of isoniazid and rifampicin in persons with HIV and diarrhoea, and also with TB and HIV (Gurumurthy et al., 2004a). There are no observable interactions between isoniazid (INH) with ARV drugs and other anti-TB drugs.

Adverse effects of INH include effects on the liver (fatal and severe hepatitis) and the nervous system. When symptoms of liver impairment are detected, isoniazid should be promptly discontinued, as continual administration of the drug in these cases was reported to cause severe liver damage (Arbex et al., 2010). Other side effects of INH are neurotoxic effects such as toxic psychosis, memory impairment, optic neuritis, atrophy, toxic encephalopathy and convulsions (Coyne et al., 2009).

In adults, INH is given as a single oral dose at 5 mg/kg daily and in paediatrics between 5 and 10 mg/kg/day. In miliary TB, meningitis and TB, 20 mg/kg/day is

administered. At 10 mg/kg daily (maximum 300 mg/day), isoniazid is used as a prophylactic treatment (SAMF, 2016).

2.3.2. Ethambutol

Ethambutol (EMB) is a bacteriostatic agent and is given in conjunction with other antibiotics to treat TB (both drug susceptible and drug resistant) with low toxicity and good tolerance (DOH, 2011). It acts on extracellular and intracellular bacilli, predominantly on fast growing bacilli. Ethambutol MIC for *Mycobacterium tuberculosis* is approximately 1 to 5 µg/mL (Arbex et al., 2010). EMB is given orally, well absorbed from GIT and reaches therapeutic plasma concentrations within 4 hours. It is well distributed and partly metabolized, up to 25% is hepatically metabolized and it is mostly eliminated by excretion in the urine as unchanged drug (Arbex, 2010).

Ethambutol interferes with the arabinogalactan biosynthesis, the main polysaccharide on the mycobacterial cell wall. EMB slows down the arabinosyltransferase enzyme encoded by the *embB* gene, which mediates the polymerization of arabinose into arabinogalactan (Zhang and Yew, 2009). In vitro resistance to EMB slowly develops because of the *embB* gene mutations (Zhang and Yew, 2009).

Concomitant administration of EMB and Ethionamide (ETH) exacerbates the toxic effects of ethambutol by ETH (Arbex et al., 2010; Miglioril et al., 2009). EMB, together with pyrazinamide and thiazide diuretics, has the potential to elevate serum urate levels. Antacids can decrease ethambutol peak plasma concentration by approximately 28% (Caminero, 2003). The drugs should therefore, be administered at longer intervals. In Botswana, a study reported low serum levels for isoniazid, rifampicin and ethambutol in TB patients co-infected with HIV, with delayed absorption for rifampicin and ethambutol (Tappero et al., 2005). A study has shown that food has negligible effect on EMB bioavailability (Caminero, 2003).

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EMB is usually well tolerated in patients. Its adverse effects are time- and dose-dependent, and are common at doses greater than 15 mg/kg. Unwanted effects are uncommon, the most important being optic neuritis, which is dose-related and is possible to happen if the kidney function is decreased (Arbex et al., 2010). It results in visual disturbances, manifesting initially as red-green colour blindness and progressing to decreased visual acuity. Colour vision should be monitored during prolonged treatment with EMB (Arbex et al., 2010).

In paediatrics and adults, EMB is administered as a once daily dose of 15 mg/kg orally, with about 25 mg/kg dose given for TB meningitis (Katzung et al., 2004). In renal impaired patients, the dosage intervals must be increased, whereas the least dosing range should be administered in geriatric patients (maximum 15 mg/kg daily) (SAMF, 2010). The standard EMB dose is 15 to 25 mg/kg of body weight, given as a single daily dose (SAMF, 2010).

2.3.3. Pyrazinamide (PZA)

PZA is a mycobactericidal anti-TB agent (Arbex et al., 2010). Pyrazinamide is absorbed orally from the GIT and is distributed extensively, and CSF concentrations are equivalent to those in the plasma (Coyne et al., 2009). It is among the best cerebrospinal fluid penetrating anti-TB drugs. It is hepatically metabolized and the products of metabolism are renally excreted. PZA elimination half-life is about 9 to 10 hours, and approximately 4% to 14% is eliminated unchanged in the urine, with the remains as metabolites in the liver (SAMF, 2010).

It works by killing certain mycobacteria that cause tuberculosis. It is anticipated that PZA drug will be deaminated by PZA-susceptible strains of *Mycobacterium tuberculosis* into the active metabolite, pyrazinoic acid. This metabolite interrupts membrane energetics and prevents membrane transport function in *Mycobacterium tuberculosis* (Zhang and Mitchison, 2003). It hinders *Mycobacterium tuberculosis* at about 20 µg/ml concentration (Katzung, et al., 2004). Pyrazinamide activity is quite

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poor at acid pH of 5.5, with MICs in the range of approximately 6.25 to 50 μg/ml (Zhang and Mitchison, 2003). Hence, *Mycobacterium tuberculosis* MIC at pH of 5.5 is estimated to be 50 μg/ml, while at pH of 5.95, the MIC is just about 400 μg/ml (Zhang and Mitchison, 2003).

Drug interaction occurs when PZA is taken simultaneously with other drugs. The concurrent administration of zidovudine and pyrazinamide can reduce pyrazinamide effect (Arbex et al., 2010). ETH, probenecid and isoniazid may potentiate the toxic effects of pyrazinamide. Pyrazinamide antagonizes probenecid effects and reduces the plasma level of cyclosporine. It was reported that pyrazinamide can increase the uric acid plasma concentrations, and is essential to adjust regulate the doses of allopurinol and colchicine in patients receiving gout treatment (WHO, 2010; Yew, 2001). Pyrazinamide may interact with and reduce the effects of allopurinol and probenecid, as pyrazinamide inhibits urate clearance (Coyne et al., 2009).

The most severe pyrazinamide side effect is hepatotoxicity that is dose- related. Other adverse effects include joint pain, anaemia, dysuria, interstitial nephritis, malaise, fever, skin rash, nausea, vomiting and so on. It is contraindicated in patients with porphyria or severe hepatic damage. It should be carefully administered in patients with diabetes and gout, and in persons that are hypersensitive to niacin, ethionamide, isoniazid, or PZA (Coyne et al., 2009).

The dosage for adults is 30 – 40 mg/kg daily (maximum dose of 2500 mg), adults with hepatic damage and creatinine clearance (< 30 ml/min) 25 – 35 mg/kg three times per week and for children 30 – 40 mg/kg daily (maximum dose of 1500) (SAMF, 2016).

2.3.4. Kanamycin

Kanamycin is an injectable bactericidal agent and belongs to the aminoglycoside antibiotic group. It is used to treat several bacterial infections, including MDR-TB.

The mechanism of kanamycin is by interaction with the 30S subunit of prokaryotic ribosomes and also damaging the cell membrane of the mycobacterium (Coyne et al., 2009). When administered intramuscularly, kanamycin absorption is complete and the peak serum concentration is reached in about 30 to 90 minutes (Budha et al., 2008; Coyne et al., 2009). In addition, aminoglycosides display lesser protein binding at about 10% and its distribution is limited to extracellular spaces (Coyne et al., 2009). They do not penetrate many mammalian cells, hence, they do not undergo significant metabolism. Aminoglycosides elimination is predominantly by the kidneys, and about 80% to 98% is renally excreted unchanged over a 24-hour period, with a half-life of approximately 4 hours (Coyne et al., 2009). Thus, kanamycin drug excretion is extended in patients with renal impairment (Budha et al., 2008).

Kanamycin demonstrates a non-saturable concentration-dependent effectiveness; hence, it is effective even when its plasma concentrations are lower than the MIC (Arbex et al., 2010). Its bacterial activity against *Mycobacterium tuberculosis* is evident at a concentration of almost 6 μg/ml in vitro and it displays a quick inhibitory effect at levels exceeding its MIC (Nuermberger and Grosset, 2004). Its mechanism of action depends on a high C_{max}: MIC ratio (Douglas and Mcleod, 1999).

Aminoglycoside anti-TB drugs (kanamycin, amikacin and capreomycin) are principally excreted renally as unchanged drugs and are unlikely to have metabolic drug interactions with ARVs (CDC, 2013). There have been no observable interactions between kanamycin and ARV drugs or other anti-TB drugs (CDC, 2013).

The most severe side effect caused by aminoglycosides is tinnitus or loss of hearing (ototoxicity), in which there is a higher risk in patients who are elderly, undergoing prolong treatment periods or receiving high kanamycin doses (Arbex et al., 2010). Other serious adverse effects include allergic reactions and toxicity to kidneys (Arbex et al. 2010). This kidney toxic effect is due to kanamycin accumulation in the kidney

tubules, because aminoglycosides are mostly eliminated by the kidneys (Arbex et al., 2010).

Kanamycin is administered intramuscularly or intravenously in two or three divided doses of 15 mg/kg per min, with a reduction of dose in kidney impaired patients (SAMF, 2016).

2.3.5. Moxifloxacin

Moxifloxacin is a bactericidal agent and belongs to the fluoroquinolone group. Their effect is exerted by deoxyribonucleic acid (DNA) gyrase inhibition, thus making the synthesis of bacterial DNA (Katzung et al., 2004). This fourth-generation fluoroquinolone acts like DNA gyrase and topoisomerase IV, hence its dual action in slowing down the development of resistance (Arbex et al., 2010). Consequently, moxifloxacin is an ideal drug for XDR and MDR-TB treatment. Moxifloxacin absorbed well subsequent to its administration orally, is extensively distributed in the tissues and undergoes kidney excretion. The half-life of moxifloxacin is approximately 9 to 10 hours (Katzung et al., 2004). Approximately, 52% of moxifloxacin is intravenous or oral dose is metabolized through glucuronidation and sulphate conjugations. About 14% of the dose is converted to a glucuronide conjugate in the urine, while approximately, 38% is accounted for as sulphate conjugate, and is excreted primarily in faeces (Stass and Kubitza, 1999). The remainder of the drug is excreted unchanged in faeces (25%) and urine (20%) (Stass and Kubitza, 1999).

Moxifloxacin is unlikely to have significant drug interactions with ARVs. It was reported that concurrent rifampicin administration accounted for 27% decrease in moxifloxacin's area under curve and a noticeable increase in the inactive sulphate metabolite concentration, possibly by the sulphate conjugation induction (Weiner et al., 2007). Fluoroquinolones should not be administered with antacids or minerals such as magnesium, iron or calcium, as they can interact with and decrease the absorption of the drugs (Katzung et al., 2004).

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Generally, fluoroquinolones are well-tolerated after administration, and their usual adverse effects being gastrointestinal disturbances, including diarrhoea and nausea. Care must be applied while using moxifloxacin in renal or hepatic impaired patients or in patients below 18 years old, as the drug can harm growing cartilage. It is contraindicated in known allergic or sensitive patients, and also in lactating or pregnant women (SAMF, 2016).

Moxifloxacin adult dose is 400 mg daily (orally or IV), and 7.5 mg to 10 mg/kg daily dose is for children (DOH, 2011). Moxifloxacin doses do not require any modification in patients with renal failure (DOH, 2011).

2.3.6. Terizidone

Terizidone causes an inhibition of cell-wall synthesis by competitively inhibiting two enzymes, D-alanine ligase and L-alanine racemase, thus, damaging formation of peptidoglycan required for bacterial cell wall synthesis (Arbex et al., 2010). It is quickly and completely absorbed subsequent to its administration orally, and the drug bioavailability is approximately 70% to 90% (Arbex et al., 2010). Hence, terizidone demonstrates enhanced absorption and distribution into the tissues and fluids, as well as the CSF (SAMF, 2016). After the drug ingestion, the peak plasma concentration is reached in approximately, 3 to 4 hours, and its half-life is about 10 hours. Terizidone is not protein bound, and an insignificant terizidone amount is metabolized in the liver. Approximately, 70% of terizidone dose is renally excreted unaltered within 72 hours and the majority of the drug dose is excreted in the urine in its active form. Only a small proportion of the drug is excreted in faeces (WHO, 2008; Blumberg et al., 2003).

Co-administration of terizidone with isoniazid and ETH can intensify neurotoxic effects (Coyne et al., 2009). Likewise, concurrent administration of fluoroquinolone

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and terizidone can deteriorate the neurotoxic effects on the central nervous system. Thus, patients ought to be monitored carefully for the adverse effects of this drug combination (Arbex et al., 2010). Terizidone increases oral anticoagulant and phenytoin serum concentrations, in addition to reducing that of pyridoxine. It was reported that in patients taking neuroleptics and anticonvulsants, terizidone dose ought to be adjusted (Blumberg et al. 2003). Likewise, simultaneous administration of alcohol and terizidone increases the risk of convulsions (Blumberg et al., 2003; WHO, 2008).

Terizidone usually causes a variety of psychiatric and neurological disturbances, in addition to other side effects including dizziness, tremor, drowsiness, anxiety, depression, seizures, and psychosis (Arbex et al., 2010). These adverse effects are common in persons taking higher terizidone doses or in renal impaired patients (Katzung et al., 2004).

Terizidone is contraindicated in patients with severe renal impairment, epilepsy, depression, psychosis and porphyria (SAMF, 2016). Thus, the dosing interval of terizidone ought to be prolonged in renal impaired patients (SAMF, 2016). Similarly, it can cause vomiting, skin allergies and nausea (DOH, 2011). Hence, pyridoxine, 150 mg, should be administered concurrently to reduce any neurological side effects (DOH, 2011).

Terizidone adult daily dose is 15 to 20 mg/kg, with a maximum dose of 1000 mg/day; whereas children daily dose is 10 to 20 mg/kg, with a maximum dose of 1000 mg/day (SAMF, 2016). Every patient taking terizidone should be receiving pyridoxine, 50 mg for each 250 mg of terizidone (SAMF, 2016).

2.3.7. Ethionamide

Ethionamide (ETH) is a bacteriostatic anti-TB agent used together with other anti-TB drugs for MDR-TB treatment. The usual adult ETH dose for MDR-TB treatment is

500 mg to 1000 mg, in one or two divided doses per day (DOH, 2012). The maximum dose is 1000 mg orally per day, but the daily dose is limited by gastrointestinal toxicity (DOH, 2012). Therapy ought to proceed until bacteriological conversion is permanent and utmost clinical improvement has happened, usually 18 to 24 months (DOH, 2012).

2.3.7.1. Ethionamide Pharmacodynamics

ETH is a pro-drug and its mechanism of action is structurally similar to that of isoniazid. Thee et al. (2016) reported that ETH causes energetically growing bacilli to lose its acid-fastness and inhibits mycolic acid synthesis subsequent to its activation. According to Thee and co-researchers, activation of ETH is by the monooxygenase EthA enzyme, resulting in the formation of sulfoxide metabolite which possesses the same activity as the parent drug (Thee et al., 2016). The authors further proposed that ethionamide and its sulfoxide are transformed by EthA to a metabolite which intracellularly accumulates and acts as the final toxic compound. They postulated that the activated ETH forms covalent adducts with nicotinamide adenine dinucleotide (NAD), an InhA enzyme inhibitor in *M. tuberculosis*. Thus, inhibition of InhA results in mycolic acid biosynthesis inhibition, an essential component of the bacterial cell wall, and cell lysis (Thee et al., 2016). ETH may be bactericidal or bacteriostatic in action, depending on the drug level attained at the infection site and the vulnerability of the infecting organism.

According to a report by Thee et al. (2016), for anti-tuberculosis treatment, a targeted ethionamide peak plasma concentration (C_{max}) was proposed at approximately 2.5 mg/ml for susceptible strains of *Mycobacterium tuberculosis*. In therapeutic drug monitoring, subsequent to the administration of 250 to 500 mg of ETH, Cmax of 1 to 5 mg/ml range are recommended targets (Peloquin, 2002). ETH minimum inhibitory concentrations for drug-susceptible *Mycobacterium tuberculosis* strains were approximately 0.60 to 2.5 mg/ml using Broth's method (Heifets, 1988). Strains of

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Mycobacterium tuberculosis susceptible to minimum inhibitory concentration of less than 1.25 mg/ml are categorized as extremely susceptible and so C_{max} of ETH of about 1.0 mg/ml is possibly effective against these strains (Thee et al., 2016). A study done by Heifets and co-researchers on determination of ETH minimal bactericidal and inhibitory concentrations (MBC and MIC), using 7H12 broth with 14 drug-susceptible strains of *Mycobacterium tuberculosis (MTB),* showed that ETH MICs for MTB was approximately 0.3 to 1.25 µg/ml (Heifets et al., 1991). Therefore, ethionamide, at therapeutic concentrations, is bacteriostatic against *Mycobacterium tuberculosis*, but at higher concentrations of about 2 to 5 mg/ml in vitro, it has shown extremely bactericidal activity against *Mycobacterium tuberculosis* (Heifets, 1991).

2.3.7.2. Ethionamide Pharmacokinetics

Pharmacokinetics' properties of ethionamide can be explained in terms of its absorption, distribution, metabolism and excretion after its administration.

2.3.7.2.1. Absorption

Ethionamide is rapidly and completely absorbed from the gastrointestinal tract upon oral administration. ETH peak plasma concentration occurs about an hour after its administration (Arbex et al., 2010). Following a single 250 mg oral ETH dose given as film-coated tablets in fasting adults, ETH peak serum levels, average concentration 2.16 µg/ml, are attained within one hour (McEvoy, 2006). Likewise, when a single 250 mg oral dose of ethionamide is given as sugar-coated tablets in healthy adults, peak serum levels, average concentration 1.48 µg/ml, are attained within 1.5 hours (McEvoy, 2006). After a single oral 500 mg dose, the peak serum concentration is about 2.2 µg/ml (McEvoy, 2006).

ETH does not undergo any appreciable first-pass metabolism and bioavailability is approximately 80% (Arbex et. al., 2010). A study done by Conte et al. (2000) reported that absorption of orally administered ethionamide, together with the ethionamide plasma concentrations, was unaffected by the presence of AIDS or sex.

Also, Auclair et al. (2001) concluded that ETH absorption from the GIT was approximately complete and antacid and food had little effect on this process.

2.3.7.2.2. Distribution

Ethionamide is distributed extensively all over the body fluids and tissues. The ETH is about 30% plasma protein bound. The mean (SD) oral volume of distribution perceived in forty healthy subjects subsequent to oral administration of 250 mg dose of film-coated tablets, was approximately 93.5 litres (McEvoy, 2006). A study that used ETH sugar-coated tablets showed that ETH is distributed widely and rapidly into body fluids and tissues after a sugar-coated tablet administration, with other organs and serum concentrations being roughly equally distributed (McEvoy, 2006). Subsequent to sugar-coated tablet administration, significant concentrations are present in cerebrospinal fluid (CSF). ETH crosses the placenta and enters the meninges, appearing in the CSF after the sugar-coated tablet administration, in concentrations equal to concurrent serum concentrations of the drug. A study in children with TB meningitis demonstrated that ETH peak plasma levels in CSF normally happened 1.5 to 2.5 hours after oral doses of 15 or 20 mg/kg, but established significant interindividual and intra-individual variation (McEvoy, 2006). The in vivo ETH penetration into the epithelial lining fluid (ELF) and pulmonary macrophages in humans has not been reported (Conte et al., 2000).

2.3.7.2.3. Metabolism

ETH is metabolized extensively to active and inactive metabolites. It undergoes extensive hepatic (liver) metabolism into several different metabolites, some of which are biologically active. ETH metabolites include 2-ethylisonicotinamide, 2-ethylisonicotinic acid and ethionamide sulfoxide (Arbex et al., 2010). However, ethionamide sulfoxide is the major active metabolite; and it has been demonstrated to have an antibacterial activity against mycobacterium tuberculosis (DeBarber et al., 2000). The metabolism happens by the process of sulphoxidation, followed by desulphuration and deamination, finally by methylation (Coyne et al., 2009).

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Conversion to the active sulfoxide metabolites by monooxygenase is the first step in ethionamide metabolism in the liver. The monooxygenase possesses several properties that are common with the cytochrome P450 system (CYP) and frequently, have overlying substrate specificities (Henderson et al., 2008).

2.3.7.2.4. Excretion

After extensive hepatic metabolism of ethionamide into several metabolites, only approximately, less than 1% of a given dose is eliminated unchanged in the urine by the kidneys (Coyne et al., 2009). About 1% to 5% is eliminated as unaltered (active) drug and the remains are excreted as metabolites (Arbex et al., 2010). The plasma half-life of ethionamide is about 2 to 3 hours in humans (Conte et al., 2000; Auclair et al., 2001).

2.3.7.3. Clinical uses

ETH, a thioamide drug, is used with other anti-tuberculosis drugs in combination for treatment of all resistant TB caused by *Mycobacteria tuberculosis.* It is only designated as a 2nd-line anti-TB drug, when toxicity from or resistance to 1st-line ant-TB drugs has developed. Specifically, ETH has demonstrated effectiveness in clinical studies and contributes significant constituents for tuberculosis meningitis treatment in children and adults and for MDR-TB treatment regimens (Thee et al., 2016).

Ethionamide is orally administered, with or without food. Intake with food may improve gastrointestinal tolerability (Auclair et al., 2001). The optimal adult daily dose is 15-20 mg/kg, and the standard daily dose is about 500 mg to 1000 mg, depending on tolerance and body weight (DOH, 2013). This daily dose can be taken either as a single daily dose or daily divided (two) doses to improve tolerability.

2.3.7.4. Special warnings, precautions and adverse effects

Rapid resistance development is experienced once ETH is administered alone in tuberculosis treatment as a monotherapy. Thus, it is important to give other appropriate anti-TB drugs; the selection will be based on the outcomes of susceptibility testing. Nevertheless, treatment can commence before the results of susceptibility tests is received, as considered suitable by the doctor.

ETH exhibits intense GIT effects, as well as loss of appetite, a metallic taste in the mouth, excessive salivation, abdominal pain, vomiting (commonly severe) and nausea (Arbex et al., 2010). These symptoms subside if the drug is administered at bedtime or at meal time. In a number of cases, it may be essential to use anti-emetics or to gradually increase the doses until the total dose is achieved (Arbex et al., 2010). Neurological effects, including psychotic disturbances, encephalopathy, hallucinations, depression, diplopia, irritability, convulsions, anxiety, peripheral and optic neuritis, with a pellagra-like syndrome, have been reported with ethionamide (Coyne et al., 2009). Psychosis has been found to happen in about 1% to 2% of the patients (Coyne et al., 2009). Likewise, in patients with mental instability history, ETH must be given with care. In some cases, these symptoms have improved with nicotinamide and pyridoxine administration. Therefore, the neurological effects can be reduced by concurrent administration of 50 to100 mg/day pyridoxine dose (Arbex et al., 2010). Simultaneous commencement of efavirenz and ethionamide is not advisable, because adverse effects are experienced frequently in the first few weeks of efavirenz treatment (Arendt et al., 2007).

Skin reactions such as photosensitivity, acne, and exanthema are seen in people cured with ETH. Purpura and thrombocytopenia were reported to happen intermittently during ETH administration (Arbex et al., 2010).

Hepatic toxicity is one of the severe adverse effects of ETH. Toxic hepatitis, obstructive jaundice, acute hepatic necrosis, with modest elevations of hepatic

transaminase levels, bilirubin and alkaline phosphatase, with or without jaundice, has been described during ethionamide treatment (Fajardo et al., 2006). Toxic hepatitis (hepatotoxicity) happens in about 4.3% of the patients, particularly in patients with liver disease or alcoholism history. Prior to treatment, base line liver function tests should be done, and serum transaminases ought to be examined every two to four weeks during treatment. If transaminase levels exceed three times the upper limit of the normal range (ULN) with hepatitis symptoms and/or jaundice, or five times the ULN, with or without symptoms, ETH along with other potentially hepatotoxic co-administered drugs, should be temporarily discontinued while waiting for the laboratory anomalies to resolve (Fajardo et al., 2006). These drugs may then be reintroduced, in sequence, to resolve the drugs responsible for the liver toxicity. Changes in liver can happen about five months subsequent to the commencement of therapy with the drug, and it remains uncertain whether these alterations are as a result of hypersensitivity or direct toxicity (Arbex et al., 2010). Toxic hepatitis usually resolves once the drug is stopped. An increased possibility of toxic hepatitis was experienced in patients with diabetes mellitus (Arbex et al., 2010).
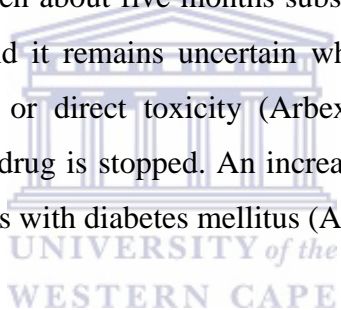
Endocrine effects are among ethionamide adverse effects. Patients receiving ETH may develop menorrhagia, impotence, alopecia, gynecomastia or hypothyroidism. ETH makes control of glycaemia more problematic in patients suffering from diabetes mellitus (Arbex et al., 2010).

Cardiovascular effects, such as postural hypotension, may happen in persons treated with ETH (Arbex et al., 2010).

Hypoglycemia has been experienced during ETH therapy, and thus glucose levels in the blood ought to be determined earlier to the commencement of and intermittently all through the treatment. Blood glucose control in diabetes mellitus may be more difficult during ethionamide treatment, including an increased risk of hypoglycemia (Arbex et al., 2010).

Likewise, hypothyroidism is one of ETH's adverse effects. Thus, thyroid function is recommended to be monitored periodically, as hypothyroidism, with or without goiter, was experienced with ETH therapy (Arbex et al., 2010).

2.3.7.5. Ethionamide interaction
2.3.7.5.1. Drug interactions between Ethionamide and anti-tuberculosis drugs

Co-administration of ethionamide and isoniazid increases the serum concentration of the latter in both rapid and slow acetylators (Arbex et. al., 2010). If co-administration is deemed necessary, supplemental pyridoxine should be given, as well as monitoring for adverse effects of isoniazid such as peripheral neuritis, hepatotoxicity and encephalopathy (Arbex et. al., 2010).

Adverse hepatic effects have been associated with capreomycin, particularly, with the concomitant administration of other anti-tuberculosis drugs identified to modify hepatic function. Theoretically, co-administration of capreomycin and ethionamide increases the danger of hepatotoxicity. Close monitoring of patients is required for changes in liver function during treatment if these drugs are co-administered (Coyne et al., 2009).

There is a bigger risk of neurotoxic effects of ethambutol during concurrent administration of ethambutol and ethionamide. In addition, ETH, cycloserine and terizidone cause increased adverse effects on the central nervous system, especially seizures. A reversible pellagra-like encephalopathy has occurred when ethionamide and cycloserine were co-administered (Coyne et al., 2009). This may have been caused by disturbances in the metabolism of pyridoxine. During ETH co-administration with hepatotoxic drugs including rifampicin, a high hepatotoxicity incidence was reported (Coyne et al., 2009).

Adverse effects of ETH can increase during concomitant administration of pyrazinamide and ETH. Cautious monitoring of hepatic function is recommended

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during the concomitant administration of ETH and pyrazinamide. Each drug has the ability to cause hepatotoxicity, and the hepatotoxicity hazard may be increased with the concurrent use of both drugs. Also, patients must be assessed for other risk factors for hepatotoxicity, such as alcohol use, other hepatotoxic drugs and underlying hepatic disease (Arbex et al., 2010).

In addition, there is an increased possibility of hypothyroidism and hepatotoxicity with the co-administration of para-aminosalicylic acid and ETH.

2.3.7.5.2. Drug interaction between Ethionamide and anti-retroviral drugs

Based on limited existing information on ethionamide metabolism, ETH drug might possess harmful adverse interactions with antiretroviral drugs. ETH is considered to undergo CYP450 system metabolism, although the CYP enzyme responsible for the metabolism is unknown. Whether doses of certain ARV drugs and/or ETH doses ought to be altered during concurrent administration in HIV and DR-TB treatment is totally unknown. At present, no adjustment is recommended (Coyne et al., 2009).

Ethionamide drug interactions with antiretroviral drugs are unknown, but their potential combined toxicities are well known (Coyne et al., 2009). Therefore, caution should be exercised in the co-administration of ARVs that are powerfully connected with hepatic function abnormality, such as the protease inhibitors (e.g. tipranavir and darunavir) and the NNRTIs (e.g. nevirapine and efavirenz) (Coyne et al., 2009). Administration of ethionamide with efavirenz (a NNRTI) causes hepatitis and psychiatric symptoms. Hence, it was recommended that efavirenz should be avoided if possible, and if used, ethionamide and NNRTI concentrations must be measured, with psychiatric morbidity in patients receiving efavirenz being monitored. Also, NRTIs go through intracellular phosphorylation in relation to the active drug and contain a low possibility for considerable drug–drug interactions (Barry et al., 1999). Administration of ethionamide with stavudine (a NRTI) causes peripheral

neuropathy. Hence, it was recommended that standard doses be used (Barry et al., 1999).

2.3.7.5.3. Ethionamide-associated diseases

There are four diseases associated with ethionamide, which include peripheral neuropathy, diabetes mellitus, hepatotoxicity and liver disease.

Peripheral neuropathy has been associated with ETH administration, but less often than with isoniazid, a structurally-related agent. Thus, treatment with ETH should be administered carefully in individuals with pre-existing peripheral neuropathy or with risk factors for developing the condition, including diabetes, alcoholism and malnutrition. The use of pyridoxine (vitamin B6) at 25 to 50 mg/day has been recommended to stop or attenuate isoniazid-related peripheral neuropathy and could be considered for patients receiving ETH (Arbex et al., 2010).

ETH has occasionally, been associated with poor diabetes control. Patients with diabetes mellitus should be monitored closely during ETH treatment, and their anti-diabetic regimen adjusted consequently (Coyne et al., 2009).

ETH is hepatotoxic and its administration is contraindicated in patients with severe liver damage. Five percent of patients treated with ethionamide are reported to have transient elevations in bilirubin, serum SGOT (AST) and SGPT (ALT). Hepatitis, with or without jaundice, was observed especially, in diabetes mellitus patients. Therefore, liver function tests should be measured at baseline and monthly during treatment, and ETH withdrawn at the first signs or symptoms indicative of liver damage (Peloquin, 2008).

Liver disease is associated with ethionamide interactions. Ethionamide is primarily metabolized by the liver. Patients suffering from liver damage may possibly be at

bigger risk of ethionamide adverse effects because of decreased drug clearance. Dosage reductions are recommended in these patients.

2.3.7.5.4. Ethionamide alcohol interactions

There is a possibility of alcohol interaction with certain medications, thereby causing reduction in the efficiency of administered drugs, increased danger of illness, harm or death by modification of the bioavailability of these drugs. Psychotic reactions have been reported in patients with excess alcohol and ETH combination (Coyne et al., 2009). Excessive use of ethanol during ethionamide therapy has been reported to precipitate a psychotic reaction and should thus, be avoided. Therefore, patients ought to be counselled to avoid consumption of excessive alcohol. Also, ETH appears to be metabolized by the liver. Chronic alcohol ingestion may increase enzymatic activity of the liver, whereas acute alcohol ingestion can inhibit liver enzymatic activity (Arbex et al., 2010). Patients suffering from alcoholism may have hepatic impairment and injury. Further hepatic injury can result from administration of ETH, when added to the disease state of TB itself. Thus, pharmacokinetic and pharmacodynamic interactions could occur between the ETH and alcohol. It is essential to avoid intake of alcohol or other things that cause drowsiness while taking ETH. Liver function tests have to be done before commencement of treatment and should be monitored monthly in all patients treated with ethionamide (Coyne et al., 2009).

2.3.7.6. Ethionamide pharmacokinetic parameters

Few studies have reported ethionamide PK parameters in healthy volunteers and in patients with tuberculosis (Peloquin et al., 1991; Conte et al., 2000; Auclair et al., 2001; Zhu et al., 2002; Ahmad et al., 2009). No information is available in the literature on the plasma pharmacokinetics of ethionamide in patients infected with MDR-TB and HIV. Therefore, this study is of great importance.

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2.3.7.6.1. Pharmacokinetic data in healthy volunteers

Ethionamide pharmacokinetics studies in healthy volunteers have been described in the literature, using different routes of administration and different doses (Peloquin et al., 1991; Conte et al., 2000; Auclair et al., 2001; Ahmad et al., 2009; Deshpande et al., 2011).

Ahmad et al. (2009) assessed the pharmacokinetics in healthy male subjects where 250 mg ETH tablets were administered orally. The PK parameters and bioavailability evaluated in their study were equivalent to the earlier stated parameters. ETH given orally, displayed an improved absorption profile, as more than 90% of ETH was absorbed, with exceptional distribution and elimination profiles. Ahmad and his team concluded that their ETH findings were in agreement with previous values reported in the literature (Ahmad et al., 2009).

Auclair et al. (2001) evaluated ETH pharmacokinetics administered in healthy subjects under different conditions using 500 mg oral dose of ETH. Auclair and colleague reported that ETH PK characteristics were not extensively changed by the different conditions used in their study. They concluded that ETH can be given with food because antacids and food intake had no significant effects on ETH pharmacokinetics (Auclair et al., 2001).

Deshpande et al. (2011) determined ETH pharmacokinetics in healthy subjects after oral administration of 250 mg tablet of ETH. They concluded that their PK parameter values obtained were in accord with the reported values in the literature (Deshpande et al. 2011).

Peloquin et al. (1991) estimated the pharmacokinetics of ethionamide in 12 healthy subjects using 250 mg ETH tablets and 500 mg ETH suppositories. Peloquin and his research group reported that subsequent to rectal ETH administration, their bioavailability value was about 57.3% compared to that from oral administration.

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Likewise, after rectal ETH administration, their maximum serum concentration value was approximately 33% in contrast to that of oral administration. They recommended that serum concentration monitoring and ETH higher doses should be used while administering suppositories (Peloquin et al., 1991).

Ethionamide pharmacokinetic parameters in healthy volunteers after drug administration are summarized in Table 2.3

Table 2.3: ETH pharmacokinetic parameters in healthy volunteers after different doses

Dose (mg)	Subjects	Vd (L)	$T_{1/2}$ (hrs)	Cl(l/hr)	Cmax (µg/ml)	AUC0-∞ (mg/L/hr)	Ke (hr⁻¹)	Ka (hr⁻¹)	Tmax (hrs)	MRT (hrs)	References
250	12	64.81[a] (0.5359)	2.00[a] (1.16)	32.6[a] (0.30)	1.94[a] (1.39)	8.75[a] (0.54)	0.39[a] (2.63)	0.38[a] (3.19)	1.75[a] (1.49)	2.24[a] (1.20)	Ahmad et al. (2009)
500	12	2.4[a]	1.8[a]	64.5[a]	2.3[b] (0.99–6.10)	10.0[b] (5.4–17)	0.39[a]	0.48[a]	1.7[b] (0.75–3.0)	NA	Auclair et al. (2001)
250	40	NA	1.92[b] (0.27)	NA	216[b] (614)	767[b] (1688)	0.37[b] (0.05)	NA	1.0[b] (0.5)	NA	Korth-Bradley et al. (2014)
250	40	NA	4.06[b] (2.52)	NA	148[b] (636)	660[b] (176)	0.23[b] (0.11)	NA	1.5[b] (0.9)	NA	Korth-Bradley et al. (2014)
500	12	(2.75±1.24)[b]	(2.92±1.05)[b]	(51.16±13.65)[b]	(2.24±0.82)[b]	(10.34±2.29)[b]	NA	NA	(1.75±0.75)[b]	NA	Peloquin et al. (1991)
500	12	NA	NA	NA	(0.74±0.29)[b]	(5.45±1.90)[b]	NA	NA	(4.42±1.78)[b]	NA	Peloquin et al. (1991)

| 500 | 10 | 2.38[a] (1.33-4.42) | 1.94[a] (1.34-3.81) | 0.76[a] (0.53-1.32) | 1.97[a] (0.99-6.10) | 8.00[a] (5.9-13.30) | 0.36[a] (0.18-0.52) | 0.69[a] (0.36-2.99) | 1.50[a] (0.75-3.00) | NA | Zhu et al. (2002) |

V_d= volume of distribution; T½ = half-life; Cl= clearance; C_{max}=maximum concentration; $AUC_{0-\infty}$= area under the plasma concentration-time curve from zero to infinity; K_e= elimination rate; K_a= absorption rate; T_{max}= time to reach peak serum drug concentration (C_{max}); MRT= mean residence time. Median[a] (standard deviation), Mean[b] (range), NA= not available
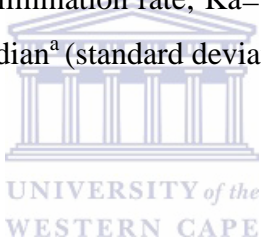
2.3.7.6.2. Ethionamide pharmacokinetic data in patients co-infected with the human immunodeficiency virus (HIV) and multidrug-resistant tuberculosis (MDR-TB)

To date, the pharmacokinetic characteristics of ETH in patients with MDR-TB and patients with MDR-TB co-infected with HIV have not been studied in South Africa.

Nevertheless, a study evaluated ethionamide pharmacokinetic parameters in patients suffering from tuberculosis (Zhu et al., 2002). According to studies by Zhu and his team, the pharmacokinetic parameters of ETH were different when comparing TB patients and healthy volunteers, probably because of variations in the completeness of the absorption. This implies that patients with tuberculosis absorbed ETH more gradually compared to healthy subjects. Hence, a 500 mg dose of ethionamide appeared as the least amount necessary to attain plasma concentrations above the MIC (Zhu et al., 2002).

Lee et al. (2009) presented a study on prothionamide (PTH) pharmacokinetics in multidrug-resistant tuberculosis patients. In their study, 6 ml of blood was taken from 17 patients each, with MDR-TB to determine their steady-state PK parameters. After steady-state administration of PTH, results from HPLC/UV assay revealed that mean $AUC_{0–12}$ was 11.0 ± 3.7 μgh/ml, mean T_{max} and $T_{1/2}$ were respectively 3.6 hrs and 2.7 hrs respectively (Lee et al., 2009). Lee and colleague reported that PTH and ETH showed similar pharmacokinetic properties, with the half-life and plasma concentration being lower for PTH than for ETH. They concluded that the PTH pharmacokinetics did not relate with the degree of emaciation in a MDR-TB patient (Lee et al., 2009).

2.4. Tuberculosis and human immunodeficiency virus co-infection

According to Harries et al (2010), HIV infection is the highest threat factor for TB. It has increased its renaissance. In 2015, 1.2 million of the 10.4 million new cases of tuberculosis globally were infected with HIV (WHO, 2016). In the same year, 1.4

million TB deaths were estimated, with supplementary 0.4 million deaths from HIV-positive tuberculosis cases (WHO, 2016). The *Mycobacteria tuberculosis* (MTB) is the main frequent source of death amongst human immunodeficiency virus patients co-infected with TB. The two infections are strongly related. People with healthy immune systems may not fall sick from TB infection; whilst HIV-positive people with a low CD4 count are at higher risks of developing tuberculosis infection (Abdool et al., 2010; Abdool et al., 2011).

However, in 2014, from the estimation of 9.6 million people that developed TB worldwide, 1.2 million (12%) were HIV-positive and 74% of these HIV-positive TB cases reside in the African region (WHO, 2016). In the same year, mortality due to HIV-associated TB increased by 570 000 in 2004 but the number have reduced to 390 000 in 2014, with a reduction rate of 32% (WHO, 2016). This still represents a huge problem of ill-health and deaths. TB deaths among people living with HIV accounted for about 25% of all tuberculosis deaths (between HIV-negative and HIV-positive persons) and one third of the accounted 1.2 million mortality were from HIV/AIDS worldwide (WHO, 2015). Hence, HIV and TB infections are the two principal causes of infectious disease–associated mortality worldwide. Studies demonstrated that tuberculosis causes risk to the achievement of the worldwide investment in treatment of HIV-infection and that tuberculosis cases in HIV-positive patients are restricting progress in TB control globally (WHO, 2009).

The impact of HIV on TB incidence cases in sub-Saharan Africa is very alarming. Both human immunodeficiency virus (HIV) prevalence rates and TB incidence are very high in sub-Saharan Africa (Amor et al., 2008; WHO, 2010). TB is the main common sickness among HIV-positive people, as well as those on antiretroviral therapy. Of the accounted 1.2 million global HIV (+) new tuberculosis cases in 2014, about 74% of these group reside in sub-Saharan Africa (WHO, 2016). In the early 1980's, outbreaks of HIV resulted in a major increase in cases of tuberculosis and death in numerous countries, particularly in southern and eastern Africa (WHO,

2015). In Africa, though the MDR-TB emergences characterized a severe threat in countries rigorously affected by the HIV endemic, most countries lack information on drug-resistant tuberculosis. Hence, MDR-TB increase in sub- Saharan Africa is not linked to failure of control programmes, but is predominantly as a result of the connection between HIV and TB, as each of the diseases speeds up the progress of the other.

South Africa is the site of the global sixth biggest tuberculosis outbreak. In South Africa, the HIV plague fuels the outbreak of tuberculosis as HIV-positive individuals have a greater possibility of tuberculosis development, owing to their compromised immune system. In South Africa, it was noted that approximately, 60% of HIV-positive patients are also co-infected with tuberculosis (SANAC, 2015). In 2012, about 65% of TB cases tested for HIV were co-infected with HIV (WHO, 2013) and jointly, these pathogens are accountable for about 46% of the disability-adjusted life years (DALY) lost in South Africa (Bradshaw et al., 2003). Tuberculosis remains a principal cause of mortality in HIV positive persons and in South Africa, where life expectancy is only 49 years of age (Bradshaw et al., 2003). Although incidence of HIV alone was a setback for local hospitals and caused an increase in demands for resources on an already strained public health system, the increase of drug-resistant tuberculosis in South Africa creates additional challenge to successful management of the disease (Schaaf et al., 2009a). The occurrence of HIV co-infection was approximately, 26.6% in children with MDR-TB in a study from Cape Town between 2005 and 2007 (Schaaf et al., 2009a).

HIV positive people also suffer the risk of drug-resistant TB. HIV itself does not increase the chance of drug resistance, but it speeds up the development of TB infection into active tuberculosis disease (Valway et al., 1994). Despite the introduction of control programmes, the number of cases of MDR-TB is still increasing. HIV infection was linked to MDR-TB outbursts in institutional settings, including prisons and hospitals (Valway et al., 1994). The rising rate of HIV

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infection outbreak amongst countries with high incidence of tuberculosis presents challenges to tuberculosis management programs and can promote additional increases in anti-tuberculosis drug resistance. Also, HIV co-infection complicates treatment of MDR-TB due to insufficient information concerning drug-drug interactions, numerous possible causes of clinical deterioration for the duration of treatment, and overlapping toxicities of antiretroviral and 2nd-line anti-TB medications (Shenoi et al., 2009). Delay in diagnosis, makes HIV positive patients more vulnerable to high mortality from extensively drug-resistant and multidrug-resistant tuberculosis (WHO, 2016).

2.5. Management of HIV positive TB patients taking ART: South Africa National Guidelines

Active TB treatment in HIV positive patients must adhere to the principles guiding treatment for non-HIV individuals. All individuals suffering from TB/HIV disease ought to be treated with ART. Significant concerns to be considered when using ART in active TB patients are: when to initiate ART; major pharmacokinetic drug-drug interactions among anti-TB agents and ARV drugs; added toxicities connected with concomitant anti-TB and ARV drug usage; and the progress of TB-linked immune reconstitution inflammatory syndrome (IRIS) subsequent to the beginning of ART.

If tuberculosis is diagnosed in individuals on ARV therapy, the antiretroviral regimen ought to be evaluated, with consideration given to possible pharmacokinetic reactions between ARVs and anti-TB drugs. The ARV regimen of the patient needs to be customized to allow for optimal administration of tuberculosis treatment regimen. Nevertheless, if a patient is not yet receiving antiretroviral therapy at the period of tuberculosis diagnosis, postponing ART initiation for a long period may result to supplementary immune suppression, with greater possibility of new opportunistic infections and deaths, particularly in persons with advanced HIV disease. Numerous controlled trials have made an effort to work out the best timing for ART initiation in

active TB disease. The outcome of these trials favoured earlier ART initiation in patients with TB (Abdoolet al., 2010; Abdool et al., 2011).

Huge randomized clinical trials in patients co-infected with HIV/TB, carried out in Asia and Africa, proved that early initiation of antiretroviral therapy in patients with CD4 counts <50 cell/mm^3 considerably decreased events of AIDS and/or death (Abdoolet al., 2010; Abdool et al., 2011; Blanc et al., 2011; Havlir et al., 2011). In all these studies, early antiretroviral therapy was defined as ART initiation within two weeks and not later than four weeks following TB therapy commencement. In these studies, IRIS was most prevalent in persons that were on ART earlier than in persons commencing ART later, but the condition was occasionally linked with death. These trials are mutually in agreement of antiretroviral therapy initiation in the first two weeks of treatment of tuberculosis in patients with CD4 cell counts approximately <50 cells/mm^3 (Abdool et al., 2010; Abdool et al., 2011; Blanc et al., 2011; Havlir et al., 2011).

Furthermore, studie**s** stated above showed no survival advantage for patients with CD4 count ≥50 cells/mm^3 that started antiretroviral therapy at less than two weeks versus later (that is, eight to twelve weeks) after commencement of tuberculosis treatment. Significantly, none of the studies confirmed any damage from earlier commencement of ART. Therefore, antiretroviral therapy must not be postponed until after the completion of tuberculosis treatment, because this approach was associated with higher mortality (Abdool et al., 2010). However, Abdool and his team recommended starting of ART within eight weeks of tuberculosis treatment initiation for individuals with ≥50 cells/mm^3, after consideration of evidence in support of early ART and lack of available information showing injury in tuberculosis co-infected patients (Abdool et al., 2010).

For patients with drug-resistant tuberculosis, the death rates in persons co-infected with HIV and extensively drug-resistant or multidrug-resistant tuberculosis are extremely high (Gandhi et al., 2010). Retrospective case series and case control

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studies offer increasing confirmation of improved results linked with the receiving of antiretroviral therapy in such co-infected patients (Dheda et al., 2010; Pietersen et al., 2014), though the best timing for ART initiation is unknown. Managing HIV (+) patients with drug-resistant tuberculosis is difficult, and necessitates specialist consultation.

2.6. Pharmacology of first-line antiretroviral drugs

2.6.1. Tenofovir

Tenofovir is a prodrug, converted to tenofovir by tissue esterases and plasma which is phosphorylated intracellularly to tenofovir diphosphate which is the active form. A study done in healthy volunteers showed that tenofovir, given at 300 mg once daily, minimally binds to proteins in human plasma or serum (0.7 and 7.2%, respectively). It is not metabolized and is eliminated renally as an unaltered drug by combining active tubular secretion and glomerular filtration (Droste et al., 2004). Gastrointestinal disorders are well-known adverse events of tenofovir.

No information is accessible for every ARV-TB drug interaction (Luetkemeyer, 2013). Tenofovir drug interactions with other anti-TB and ARV drugs were not observed. Limited PK interactions have been observed between tenofovir and other drugs, as well as other drugs which are cleared mostly by CYP450 isoenzymes, including the protease inhibitors such as indinavir and nelfinavir (Chittick et al., 2006). In addition, protease inhibitors (Lopinavir-ritonavir) and tenofovir cause an increase in tenofovir plasma concentrations.

2.6.2. Stavudine

Stavudine (D4T) is nucleoside reverse transcriptase inhibitors (NRTI) which is phosphorylated to stavudine triphosphate, which is active metabolite, by cellular kinases. It can be administered without or with food; and food considerably lowers the absorption rate. Subsequent to oral administration, stavudine is absorbed rapidly, with maximum plasma concentrations reached within one hour after dosing of drug.

Stavudine bioavailability in the adult HIV positive patients is, approximately, 82% to 99% (Lea and Faulds, 1996). The area under the plasma concentration (AUC) and maximum plasma concentration (C_{max}) are concentration dependent. That is, after both single and multiple doses, these parameters increase in proportion to the dose ranging from about 0.03 to 4 mg/kg. Tmax is reached within 0.5–0.75 hours after oral administration. No significant accumulation of stavudine was observed with frequent administration after 6, 8, or 12 hours. The steady state volume of distribution was roughly 47.3–68.9 litres in adults, and stavudine-5-triphosphate elimination half-life ($t_{1/2}$) was 3.5 hours (Lea and Faulds, 1996). Stavudine distributes evenly among plasma and red blood cells. The parent compound's mean renal clearance is about 272 ml/min, accounting for around 67% of the apparent oral clearance.

Some drug interaction has been observed between stavudine and anti-TB drugs or ARVs. Concurrent administration of ethionamide and stavudine (a NRTI) causes peripheral neuropathy. Hence, it is recommended that standard doses be used.

2.6.3. Efavirenz

Efavirenz (EFV) is non-nucleoside reverse transcriptase inhibitors (NNRTI). It is used as part of HAART regimens combined with other drugs for HIV-1 infection treatment. It can be given with or without food; but typically administered at bedtime on an empty stomach to reduce adverse neurological and psychiatric effects. Meals that are high in fat have to be avoided for appropriate drug absorption. In vitro studies and human studies using human liver microsomes, established that efavirenz was metabolized principally to hydroxylases metabolites by the cytochrome P450 system, after glucuronidation of these hydroxylated metabolites. Terminal half-life of efavirenz was approximately 52 to 76 hrs following single doses and about 40 to 55 hrs subsequent to multiple doses, with high protein binding of >99%. Oral clearance was estimated to be about 9.4 L/hr, orally, the volume of distribution was approximately 252 L and constant rate of absorption was roughly 0.3 hr^{-1} (Almond et al., 2005). Efavirenz can cause severe, life-threatening side effects, such as serious

mental problems, liver problems and serious rash similar to that observed with nevirapine. These adverse effects may be decreased by administering the once-daily dose at bedtime.

Depending on the protease inhibitors, efavirenz will increase the plasma levels of protease inhibitors (ritonavir, nelfinavir) or decrease the plasma levels of other protease inhibitors. In addition, co-administration of ethionamide and efavirenz causes hepatitis and psychiatric symptoms with efavirenz.

2.6.4. Lamivudine

Lamivudine (3TC) is a nucleoside reverse transcriptase inhibitor (NRTI). It is regularly used as a major component of HAART regimens, together with other drugs for HIV-1 infection treatment and as monotherapy in hepatitis B viral infection treatment (Perry and Faulds, 1997). It can be given with or without food. Lamivudine is absorbed rapidly following oral administration with maximum serum concentrations (C_{max}) reached at 0.5 to 1.5 hrs subsequent to the dosing (Johnson et al., 1999). Bioavailability was estimated to be approximately 82% in adults and about 68% in children. It is distributed extensively into total body fluid; the mean apparent volume of distribution is almost 1.3 L/kg subsequent to intravenous administration and protein binding is less than 36%. The lamivudine elimination half-life ($t_{1/2}$) is approximately five to seven hours (Johnson et al., 1999). About 5% is recovered as transsulfoxide metabolite in the urine, and almost 70% is eliminated unchanged in the urine. Active tubular secretion seems to perform a role in the clearance. Lamivudine dosage forms including, oral solution, capsules and tablets are bioequivalent.

Lamivudine drug interactions with other ARVs and anti-TB drugs were not observed. Studies indicating drug-drug interactions among most of the 2nd line anti-mycobacterial medications and ARV drugs are still few in number, even though the possibility of adverse drug interactions is considerable (CDC, 2013). Hence, therapeutic drug monitoring (TDM) will be helpful in the measurement of the

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concentration of drug in the plasma, proper management of drug interactions between anti-TB drugs and ARV drugs, and in proper management of TB therapy, mostly in HIV positive patients (Peloquin, 2002).

2.7. Overlapping drug toxicities between anti-tuberculosis and antiretroviral drugs

The use of antiretroviral treatment in TB patients is complicated by overlying toxicity profiles of some anti-tuberculosis and ARV medications. Adverse reactions are common amongst HIV-related TB patients, particularly in concomitant administration with HAART. TB as well as ARV drugs have numerous toxicity profiles in common, and anti-TB drug adverse reactions seem to be common in patients with HIV-infection. Studies conducted in adults showed that the most adverse events happened within two months of initiating concomitant treatment (Dean et al., 2002). Dean and his team concluded that adverse reactions led to interruption and/or cessation of HIV or TB treatment in approximately 63 (34%) of their 183 patients. Overlapping toxicities, such as gastrointestinal (GIT) intolerance, nephrotoxicity, peripheral neuropathy, skin rash and psychiatric side effects, can reduce choices for MDR-TB and HIV co-treatment (CDC, 2013).

ARVs with GIT adverse reactions can increase identical effects in persons receiving treatment for MDR-TB. Diarrhoea, which is among the utmost frequent symptoms of gastrointestinal tract disturbance described in patients with HIV-infection, can result in malabsorption of drug (Gurumurthy et al., 2004). Malabsorption of MDR-TB drugs was reported in HIV-related enteropathology patients (Weyer, 2005). Diarrhoea may increase ETH gastric motility, resulting in resident time decrease in the intestine and reducing drug absorption. The selective poor absorption of one anti-tuberculosis drug used in the treatment regimen can encourage resistance of the drugs. Moreover, diarrhoea which is a major protease inhibitors' side effect can decrease anti-tuberculosis drugs' bioavailability. Likewise, ETH yields extreme GIT side effects including vomiting, nausea and diarrhoea. All antiretrovirals with ethionamide, para-

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aminosalicylic acid and fluoroquinolones cause gastrointestinal intolerance, but it is less common with emtricitabine and lamivudine. Although TB drugs are one of the most likely drugs that cause GIT distress, this is hardly a reason to discontinue treatment, because splitting the dose may reduce GIT disturbances such as nausea and vomiting. GIT distress side effects, such as nausea and vomiting, are experienced from co-administration of anti-TB drugs (rifabutin, pyrazinamide, isoniazid, and rifampin) and ARV drugs (amprenavir, ritonavir, indinavir and zidovudine).

The use of ARV and anti-TB drugs is associated with a number of toxicities, including those affecting the kidneys. Nephrotoxicity, as a consequence of prolonged administration of nephrotoxic drugs, and co-administration with additional drugs that are toxic, particularly aminoglycosides, can lead to accumulation anti-tuberculosis drugs in kidney tubules. In South Africa, tenofovir, used in the first-line ART regimen, induces nephrotoxicity when aminoglycoside is prescribed for between 2-6 months in MDR-TB treatment (Kenyon et al., 2011). Hence, concurrent administration of two nephrotoxic drugs contributed to the development of severe renal failure (Kenyon et al., 2011). Kenyon and colleague were concerned that the risk of nephrotoxicity with tenofovir and aminoglycoside would be greater if co-administered for a longer duration. Hence, they recommended close monitoring of serum creatinine level during concomitant administration of both drugs for MDR-TB/HIV treatment. In 2011, Kalyesubula with his team reported that the main risk factors for acute kidney injury and associated mortality are severe immune-suppression (CD4 count, <200 cells/mm^3) and opportunistic infections (Kalyesubula et al. 2011).

Peripheral neuropathy, which is one of the most common toxicities in adults, happens during concurrent administration of isoniazid and stavudine (Dean et al., 2002). A study done in South Africa reported that patients taking ART together with TB treatment were found to be at increased risk of stavudine discontinuation, primarily due to peripheral neuropathy (Westreich et al., 2009). Peripheral neuropathy is rare in

childhood TB patients on isoniazid, but if administered with HAART, pyridoxine supplementation may be advisable. Also, stavudine and ethionamide cause peripheral neuropathy.

Skin rash is another aspect of toxicity that is common in anti-tuberculosis therapy, but treatment can usually be continued if it is not severe. Additional drugs that could potentially cause severe skin rash are co-trimoxazole, nevirapine and, to a lesser extent, efavirenz and abacavir may also cause rashes. These drugs should all be discontinued and reintroduced individually if the rash is severe. But if a hypersensitivity reaction is suspected (such as fever, respiratory and GIT symptoms, and elevated transaminases), then both abacavir and nevirapine should not be reintroduced. Hence, side effects such as skin rash are seen with concomitant administration of anti-TB drugs (isoniazid, rifampin, pyrazinamide, rifabutin) and ARV drugs (efavirenz, nevirapine, abacavir, delavirdine).

One of the most serious adverse events is hepatitis (hepatotoxicity), defined as aspartate or alanine transaminase levels of more than five times the normal level, while others look for clinical jaundice. All potentially hepatotoxic anti-TB drugs should be discontinued, and if on HAART, probably also the ARV drugs. This is because anti-tuberculosis drugs take precedence over ARVs and should be reintroduced individually, while monitoring liver function tests. Hepatotoxicity prompted by ETH administration can result in an injury of the liver. When main hepatic impairment happens, as shown by jaundice, with lengthy transaminases elevation for the duration of 6 to 8 weeks, administration of ETH ought to be stopped. ETH must be examined cautiously in patients with mental instability, alcoholism, diabetes or liver disease (Weyer, 2007). Consequently, hepatotoxicity side effects found in HIV/TB co-treatment are derived from concurrent administration of anti-TB drugs (isoniazid, rifampin, pyrazinamide and rifabutin), ARV drugs (HIV-1 protease inhibitors, nevirapine) and immune reconstitution, following commencement of ARV treatment amongst patients suffering from chronic viral hepatitis.

Overlapping toxicities threaten adherence, may lead to treatment interruption and are a concern in HIV (+) patients treated with the poorly-tolerated 2nd-line regimens for drug-resistant tuberculosis.

2.8. Laboratory findings on liver and kidney functions

Numerous tests are accessible for the detection of abnormalities. Blood examinations usually designated as kidney and liver function tests, are amongst the most frequently used.

2.8.1. Liver function

Liver enzyme tests detect inflammation and damage to the liver, which are measurements of *enzyme levels,* with the exception of bilirubin. The liver performs various metabolic functions, detoxifies harmful substances, makes blood-clotting proteins, secretes bile and performs many other vital functions. The liver function tests that assess the ability of the liver to eliminate substances that undergo hepatic metabolism, such as the 14C-aminopyrine breath test, are limited by complexity and availability (DiPiro et al., 2014). Routine liver testing includes measuring of alkaline phosphatase (ALP), gamma glutamic transpeptidase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin and albumin levels in the blood.

Severity of liver disease is assessed by means of Child-Pugh score. This scoring system is used to evaluate the possibility of liver disease development, likelihood of recovery or survival and for assessing chronic liver disease prognosis, mainly cirrhosis. The score utilizes biochemical and clinical measures (grading scale) to evaluate liver disease diagnosis in patients. Each biochemical measurement is assigned a score from 1-3, where point 3 represents severe derangement (Durand and Valla, 2005).

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Table 2.4: Child-Pugh score classification system

Biochemical and clinical measurements	Increasing abnormality points scored		
	1	**2**	**3**
Serum albumin (g/Litres)	>35	28-35	<28
Prothrombin* (s)	<4	4-6	>6
Ascites Bilirubin (µmol/Litres)	Absent (no ascites) <34	Mild (medically controlled) 34-51	Refractory (poorly controlled) >51
Hepatic encephalopathy	None (no encephalopathy)	Minimal (medically controlled)	Advanced (poorly controlled)

(Durand and Valla, 2005).

*Prothrombin time values of 4 and 6 s correspond approximately to 50 and 40% of normal, respectively.

Table 2.5: Disease classification

Points	Class	One year survival	Two year survival
5-6	A	100%	85%
7-9	B	81%	57%
10-15	C	45%	35%

(Durand and Valla, 2005).

Chronic liver disease was categorized as A to C Child-Pugh class, using the added score as of above. The score, which is equivalent to the summation of individual points, permits for patients' classification in Child– Pugh grades A to C. Therefore, according to the classification, class A (5–6 points: is for mild liver impairment),

class B (7–9 points: for moderate impairment) and class C (10–15 points: for severe liver impairment) (Durand and Valla, 2005).

2.8.2. Risk factors that might impair kidney function
2.8.2.1. Human immunodeficiency virus-related liver disease

Hepatic disease is currently the most mutual non-AIDS associated cause of mortality amongst patients infected with HIV, estimating to approximately 14% to 18% of all mortality and about half of the deaths amongst hospitalized patients with HIV-infection (Price & Thio, 2010). Hepatitis, which is inflammation of the liver, is relatively common in people with HIV and can also limit HIV treatment options. AIDS-related hepatic disease, such as AIDS cholangiopathy, happens when strictures that is infection-related in biliary tract, results to biliary obstruction. It, characteristically, presents with right upper quadrant pain (RUQ), fever, nausea, vomiting, diarrhoea inter alia, and a markedly increased alkaline phosphatase. It is, typically, observed in low CD4 counts (that is, <100/mm3) (Price and Thio, 2010). Also, calculus cholecystitis (AIDS-related liver disease) was properly documented in HIV-infection and patients usually present with fever with cholestasis and abdominal pain. Therefore, it is important to always monitor the liver function by doing regular blood tests, especially when initially diagnosed with HIV, at all HIV routine clinic appointments and when ill.

2.8.2.2. Antiretroviral-related liver disease

Increased mortality rate amongst persons infected with human immunodeficiency virus using combination antiretroviral therapy (cART), is attributable to the hepatic disease complications (Price and Thio, 2010). Retrospective studies done by Price and Thio (2010) demonstrated that prevalence of ART-related severe hepatotoxicity is about 10%, and events that are life-threatening happen at a rate of approximately 2.6 per 100 person's years (Price and Thio, 2010). This shows that hepatic complications may be adverse effects of numerous anti-HIV drugs. These drugs consist of the non-nucleoside reverse transcriptase inhibitors (NNRTIs) which cause

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direct drug toxicity or hypersensitivity reactions (e.g. nevirapine, most associated with hepatotoxicity), etravirine and efavirenz (also causing hepatotoxicity but less often than nevirapine). Conversely, liver failure and clinical hepatitis were reported and associated with protease inhibitors, tipranavir in combination with ritonavir boosting. Nucleoside reverse transcriptase inhibitors (NRTI's) remain connected with toxicity of mitochondrial, because of their capacity to hinder mitochondrial polymerase γ. Four principal mechanisms through which ART can cause hepatic impairment, including IRIS, mitochondrial toxicity, hypersensitivity reactions and drug metabolism and/or direct drug toxicity (Price and Thio, 2010). Patients living with HIV-infection on ARVs may have an elevation of liver enzymes (Gisolf et al., 2000), and this is a mutual cause of morbidity and mortality in HIV positive patients (Soriano et al., 2008). Therefore, a healthy liver is essential for HIV-infected people, because it plays an important part in the processing of anti-HIV medications. For this reason, consistent hepatic function tests are vital for HIV therapy which is started recently (that is, after one month and three months), and ought to be examined every 2 weeks for the first 2 months if therapy is commenced with the anti-HIV drug, nevirapine (Viramune).

2.8.2.3. Drug-induced liver injury in Human immuno-deficiency virus (HIV)/tuberculosis co-infected patients

Drug-induced liver injury in patients co-infected with tuberculosis/HIV is a mutual challenge in the South African setting, and it is a recognized adverse drug reaction to ART and TB treatment, which complicates tuberculosis therapy in approximately 5% to 33% of patients. Analysis of the previous data conducted, showed that 41 of 296 (13.85%) patients co-infected with HIV/TB that consumed anti-tuberculosis drugs, had symptom of elevated liver enzyme levels (Ali et al., 2013). Also, concurrent administration of antiretroviral and anti-tuberculosis drugs is complicated by serious overlying adverse effects, e.g. hepatotoxicity and drug-drug interaction (Ali et al., 2013). An unpredictable or idiosyncratic hypersensitivity reaction that occurs in DILI is independent of dose and can result in portal tract inflammation with cholestasis

and/or hepatocellular injury. The 1st-line anti-tuberculosis drugs, rifampicin, isoniazid and pyrazinamide were linked with hepatotoxicity and the danger is improved when the drugs are used in combination (Ali et al., 2013). Hepatotoxicity can cause mortality, morbidity, prolong duration of illness and economic problem, and may necessitate dosage regimen modification.

Toxic hepatitis, obstructive jaundice, acute hepatic necrosis, as well as modest elevations of hepatic transaminase levels, bilirubin and alkaline phosphatase with or without jaundice, have been described during ethionamide treatment. Baseline liver function tests should be obtained prior to therapy, and serum transaminases should be monitored every 2-4 weeks during therapy.

2.8.2.4. Drug-induced related liver toxicity

Certain medications are main source of hepatic damage (hepatotoxicity) because most drugs are metabolized by enzymes in the liver called cytochrome P450 (CYP) or the microsomal enzymes. Drug-induced liver impairment is the utmost mutual motive quoted for an approved drug withdrawal. The appearances of hepatotoxicity induced by drugs are extremely variable, extending from asymptomatic raise of hepatic enzymes to fulminate liver failure. Therefore, hepatotoxic drugs such as alcohol, aspirin, phenytoin, methotrexate, statins, isoniazid and acetaminophen should be avoided if possible. Several drugs used for the treatment of other infections which HIV-positive patients are vulnerable to can cause hepatic problems. The drugs include drugs used for treatment of tuberculosis and statins, used for high cholesterol treatment.

2.8.2.5. Co-existing diseases

Apart from HIV-infection, other patient's co-existing diseases might result in elevation of serum hepatic enzymes. A number of these diseases consists of chronic viral hepatitis and alcohol-related hepatic disease. Chronic viral hepatitis condition is characterised by a slight increase in AST and ALT concentrations, that is,

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approximately 2 to 3 times greater than the upper limit. Likewise, alcohol-related hepatic disease is characterised by an increase in AST and ALT concentrations, that is, the AST concentration is about 2 to 3 times greater than the standard level, in conjunction with patients' history of excessive alcohol use. (Aragon and Younossi, 2010).

2.8.2.6. Alcohol-induced related liver toxicity

Alcohol-induced liver disease is among the global causes of chronic liver disease, and the severity of the liver damage associated to alcohol differs between different persons. Heavy drinkers are referred to as women that frequently consume beyond 20 grams of ethanol each day or men who usually drink above 80 grams of ethanol each day. In a number of heavy drinkers, merely fatty liver, which is a clinically benign liver damage, develops, whereas, in other persons that drink the same quantities of alcohol, cirrhosis, a form of advanced liver damage which frequently results in morbidity and death, develops (Diehl, 2002). Fatty liver (steatohepatitis/ hepatic steatosis) is the initial phase of alcoholic hepatic disease (which is asymptomatic in ambulatory patients) and it eventually progresses to cirrhosis (which is the final phase of alcoholic hepatic disease) in certain patients, causing clinically observable cholestasis and portal hypertension (Diehl, 2002). Diehl reported that cirrhosis advances infrequently and gradually in chronic hepatic steatosis patients, while, approximately, 40%−50% chronic alcohol-induced steatohepatitis patients develop cirrhosis within 5 years (Diehl, 2002).

2.8.3. Kidney function

The kidneys play several vital roles in maintaining health by controlling the levels of water and various minerals in the body, filtering waste materials from the blood and expelling them from the body as urine.

In clinical practice, glomerular filtration rate (GFR) cannot be easily estimated; instead, it is measured from equations by using body size, sex, race, age and serum

creatinine level (Levey et al., 2009). Standard GFR estimate is 90 or above, but standard GFR varies depending on age (it decreases in old age). A GFR below 60 is a warning sign of kidney disease, and a reading of less than 15 implies that kidney failure treatment, including dialysis or a kidney transplant is needed.

The creatinine clearance (CrCl) is among the index of renal function in disease and health, that measures the level of creatinine (a chemical waste product of creatine), which gives an indication about the working of the kidneys. The test compares the blood creatinine level with the urine creatinine level. The creatinine clearance test is used to estimate glomerular filtration rate (GFR). The kidney eliminates creatinine from the body completely. Thus, in abnormal renal function, levels of creatinine rise in the blood because a smaller amount of creatinine is released via the urine. Blood creatinine concentrations vary depending on body size, muscle mass and age. A standard CrCl estimate is approximately 0.7 to 1.3 mg/dL for men and about 0.6 to 1.1 mg/dL for women, but standard values may differ between different laboratories. Typically, women have lower creatinine concentration than men, as a result of the lesser muscle mass in women than in men.

Other kidney tests done include: sodium, potassium, urea, and modification of diet in renal disease.

The table below shows illustrations of the stages of chronic kidney failure (CKF), according to estimated creatinine clearance, and also kidney disease outcome quality initiation (KDOQI) stages of kidney disease.

Table 2.6: Stages of chronic kidney failure (CKF) according to creatinine clearance

Stage	Estimated creatinine	Description	Treatment stage

	clearance (millilitres/minutes)		
1	>80 ml/min	"Normal" Normal kidney function but urine findings or structural abnormalities or genetic trait point to kidney disease.	Observation, control of blood pressure. More on management of Stages 1 and 2 CKD.
2	50–80 ml/min	"Mild renal impairment" Mildly reduced kidney function, and other findings (as for stage 1) point to kidney disease.	Observation, control of blood pressure and risk factors. More on management of Stages 1 and 2 CKD.
3	30–49 ml/min	"Moderate renal impairment" Moderately reduced kidney function.	Observation, control of blood pressure and risk factors. More on management of Stage 3 CKD.
4	<30 ml/min	"Severe renal impairment" Severely reduced kidney function.	Planning for end-stage renal failure. More on management of Stages 4 and 5 CKD.
5	Requiring dialysis	"Kidney failure" Very severe, or end-stage renal failure (sometimes called established renal failure).	Treatment choices. More on management of Stages 4 and 5 CKD.

(CDER and CBER, 1998)

The stages of chronic kidney disease shown above are based on estimated or measuredcreatinine clearanceand are a useful aid to planning.

2.8.4. Risk factors that might impair kidney function

2.8.4.1. Human immunodeficiency virus-related kidney dysfunction

Numerous categories of kidney disease appear to be indirectly or directly triggered by HIV, including HIV-linked thrombotic microangiopathy, HIV-linked-immune-mediated glomerular nephritides and classic HIV-associated nephropathy (HIVAN)

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(Roling et al., 2006). In 1984, kidney disease was first described as a complication of the acquired immunodeficiency syndrome (AIDS), but now it is known as HIV-associated nephropathy (HIVAN) (Wyatt et al., 2009). Most HIV (+) individuals has abnormal functioning of the kidneys, leading to end-stage renal disease (ESRD), which is the almost complete or complete failure of the kidneys (Wyatt et al., 2009). Hence, human immunodeficiency virus (HIV)-associated nephropathy has established itself as an important cause of kidney failure and therefore, dialysis or a kidney transplant will be required for those patients. It is now felt that HIVAN is caused by direct viral infection of the renal cells, mainly the tubular epithelial cells and the visceral epithelial cells of the glomerulus (Gerntholtz et al., 2006). This is because naturally, HIVAN happens late in HIV-1 infection, and the risk factors for HIVAN development consist of a high viral load and a 200 cells/mm CD4 cell count (Roling et al., 2006). Patients with chronic kidney disease (CKD) and HIV-infection are at a risk of drug toxicity, acute renal failure, end-stage renal disease and higher risk of cardiovascular disease (Wyatt et al., 2007). Wyatt and colleagues showed that factors associated with CKD include risk factors reported previously, lower CD4 cell count, hepatitis C virus (HCV), black race and older age; compared to studies done earlier, which showed that virological suppression was common amongst patients suffering from CKD.   Therefore, early recognition would aid in the identification and reversible causes treatment, expectation of complications and dialysis preparation or transplantation in progressive disease patients.

2.8.4.2. Antiretroviral-induced nephrotoxicity

HAART (highly active antiretroviral therapy) have a defensive kidney effect. Prior to HAART introduction, different adverse drug reactions on the kidneys were documented, and differ from proteinuria development to acute kidney failure (Roling et al., 2006). Most drugs utilized for the treatment of HIV-infection are linked with nephrotoxicity.

A protease inhibitor such as indinavir, was linked with recurrent urological and kidney adverse reactions, including nephrolithiasis, acute renal failure, dysuria, crystalluria, papillary necrosis and stone formation. Also, a number of studies have associated the use of ritonavir to reversible kidney failure. Furthermore, antiretroviral treatment using a combination of low-dose ritonavir and indinavir increases indinavir toxicity on the kidney (Roling et al., 2006).

Nucleotide reverse-transcriptase inhibitors (NRTI), including adefovir, cidofovir and tenofovir, were linked with damage of renal tubule. Case reports have linked HAART regimens that contain tenofovir to fanconi syndrome and tubular toxicity with variable severity, decreased glomerular filtration rate and elevated serum creatinine level (Roling et al., 2006).

With regard to nucleoside reverse-transcriptase inhibitors (NRTI), toxicity to the kidneys due to the use of nucleoside analogues is usually infrequent. Studies have established that didanosine and lamivudine-stavudine treatments are connected with fanconi-like syndrome or tubular dysfunction (Roling et al. 2006). Likewise, a study associated efavirenz (a NNRTI) toxicity to the kidneys, based on hypersensitivity reaction including interstitial nephritis, hepatitis and pneumonitis. These symptoms reappeared after a re-challenge (Roling et al., 2006).

2.8.4.3. Drug-related renal injury

Drugs are among the common cause of acute kidney injury because patients take multiple medications, and are exposed to therapeutic and diagnostic procedures which may harm the kidney function (Hoste and Kellum, 2006). The toxic effects on kidneys linked to drugs are both expected and common, given the kidney's function in the maintenance of metabolic homeostasis and plasma filtration. Some drugs associated with kidney malfunction include acyclovir, aminoglycoside, fluoxetine,

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amphotericin B, non-steroidal anti-inflammatory drugs, anti-depressants, amitriptyline and antibiotics. According to Choudhury and Ahmed (2006), the occurrence of nephrotoxic injury due to administration of antibiotics (such as, aminoglycosides) was reported to be 36%. The exact occurrence of drug-induced nephrotoxicity is difficult to determine, because detection is delayed until an obvious change in kidney function is measured as an increase in creatinine or serum blood urea nitrogen. Most incidents of drug-induced renal damage are reversible, with functions returning to baseline after discontinuation of the drug. Therefore, preventive strategies should be to identify drugs responsible for drug-induced kidney malfunction, with a further assessment to resolve if some can be stopped, reduced to a lesser dose, or replaced with a safest option. But if the change of drug or discontinuing is inappropriate, then non-medicinal strategies should be implemented to decrease the risk of damage, such as an increase in monitoring efforts or increasing hydration to help reduce the danger of dehydration.

2.8.4.4. Co-existing diseases related to kidney toxicity

Co-existing diseases are among the essential risk factors that cause development of kidney malfunction in patients infected with or without HIV. Hypertension, which is one of the major causes of kidney infection, can harm blood vessels of the kidneys, thereby, making the removal of body fluids and wastes difficult. Also, hepatitis C virus (HCV) co-infection is among the factors triggering kidney dysfunction (Roe and Hall, 2008). The link between kidney disease and HCV infection is documented in a study done by Lee et al. (2010), which shows that the presence of anti-HCV antibodies is connected with renal disease progression, with higher rate of positive anti-HCV in those with more severe stages of CKD. Hence, chronic renal disease is a global health crisis, with unpleasant effects of cardiovascular disease, kidney failure and premature death. Advanced adults are disposed to acute kidney infection (AKI) and kidney insufficiency due to the reduction in blood flow to the kidney that happens at approximately, one percent after the age of 30.4 each year. In addition, these patients frequently have several risk factors for AKI, including the use of

multiple nephrotoxic drugs, increased exposure to diagnostic procedures with radio-contrast dye, hypotension, co-existing diseases (e.g. heart failure, diabetes and cardiovascular disease) and dehydration.

2.8.4.5. Alcohol-induced kidney toxicity

Excessive consumption of alcohol causes distinct harmful effects on kidneys and its functions in maintaining acid-base balance, electrolytes and body fluids. It also causes other kidney-related health problems to alcohol abusers or their dependents. Alcohol consumption has been identified as a potential cause of kidney disorder. Some studies have shown excessive alcohol consumption to be associated with hypertension (HTN), which is among the major risk factors for chronic kidney disease (CKD) (Cheungpasitporn et al., 2014). This is because when alcohol dehydrates (dries out) the body, the drying effect can affect the normal function of organs and cells, including the kidneys.
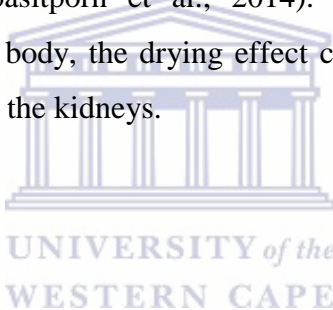
2.9. Pharmacokinetics

2.9.1. Overview

Pharmacokinetics (PKs) illustrates what the body does to the drug. PK is the study of the association between the drug plasma concentrations and a dosage regimen. Mechanisms for the determination of this plasma-concentration profile are characterized as PK parameters. The concentration-time profile of a drug is studied by means of several PK parameters.

2.9.2. Factors affecting pharmacokinetics of a drug

PK parameters of a drug can be influenced by inter-individual variations, resulting in different plasma concentration-time profiles and, consequently, a change in PKs profile after administration of equivalent drug dose to different patients. Considerable variations in drugs response exist between individuals. Hence, a drug therapeutic standard dose, on clinical trials in patients and healthy volunteers, is inappropriate for all patients. Differences are present in both pharmacodynamics and pharmacokinetics

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parameters. The main significant features in the variability of PK parameters include use of concomitant drugs, body weight, sex, age, disease and genetic factors (Balasubramanian and Tamil, 2013).

2.9.2.1. Genetic factors

Considerable drug variability factors include drug transport and metabolism. Inter-individual dissimilarity metabolism of drug is as a result of genetic polymorphism. This is defined by the presence of monogenic traits which exist in a minimum of two phenotypes, neither of which is rare. Genetic polymorphism of metabolic enzymes might be as a result of gene duplication, gene suppression, modification in gene inducibility and allelic variants which result to dissimilar catalytic activities of enzymes. Genetic variability was previously illustrated by isoniazid metabolism, largely hepatically acetylated to N-acetylisoniazid, a hepatotoxic compound precursor. Huge ethnic differences that are genetically-controlled are present in the rapid and slow acetylators' status respectively. Adverse reactions occur prevalently in slow acetylators. Alternatively, rapid acetylators are most susceptible to side effects (e.g. liver damage that is isoniazid-induced) (Balasubramanian and Tamil, 2013).

Genetic effects on metabolism of drug interact among other extrinsic (that is, environmental, behavioural and cultural) and intrinsic (that is, physiologic) characteristics of an individual for the determination of treatment outcome with any pharmacological agent. For example, the cytochrome P450 2C9 (CYP2C9) is the major enzyme accountable for *S*-warfarin drug metabolism. Individuals with poor CYP2C9 metabolizing enzymes have decreased clearance of *S*-warfarin. Clinical studies demonstrated that these individuals need warfarin in low dosages and are at a higher risk of excess anticoagulation (Belle and Singh, 2008). Specialists reported that genetic factors accounted for approximately 20 to 95 percent of patients' variation to individual drug responses. This provides useful clinical information for individualizing drug therapy and improving clinical outcomes (Belle & Singh, 2008).

2.9.2.2. Disease states

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Drug responses can be modified by the existence of coexisting diseases in the patient, as well as the disease for which the drug is used. Diseases of organs diseases responsible for elimination of drugs (e.g. the kidneys and liver) and circulatory disorders are accountable for great differences in pharmacokinetics of drug. For example, a patient with compromised renal function experiences diminished urinary excretion of drugs; as a result, clearance of many drugs is also reduced, thereby, inducing toxicity. Renal diseases influence the pharmacokinetics of drugs eliminated via excretion such as inadequate elimination of metabolites which can induce toxicity.

Furthermore, absorption is influenced by hepatic disorders (via the first-pass effect), distribution process (via the protein binding), metabolism and excretion of drugs. In patients with impaired liver, there is a decrease in synthesis of plasma protein by the liver, thereby, affecting the volume of distribution of drugs which are widely bound to these proteins. Also, liver conditions such as cirrhosis, influence drugs metabolized mainly in the liver, and obstructive jaundice (a liver condition), altering biliary excretion.

In addition, dissimilar pharmacokinetic mechanisms can be influenced by reduced vascular perfusion of body parts encountered in cardiac failure. For example, drug absorption is influenced by perfusion of the absorption sites, and dissimilarity of body perfusion can change the drug distribution to certain organs. Similarly, excretion and metabolism of drugs are affected by the liver and kidney perfusion. It has been reported that absorption of anti-mycobacterial agents as well as ETH, is impaired in patients suffering from AIDS (Thee et al., 2011).

Hence, special attention is needed in the evaluation of patients' hepatic and renal function, and the dosage regimen of drugs should be customized if organ damage is observed. If repeated drug administration is required, attention is needed while adjusting the dosage regimen in patients suffering from conditions that decrease

drug clearance. In such circumstances, toxic accumulation may happen. In addition, there would be an elongated drug half-life; hence, the time needed to attain steady state concentration would be longer (Balasubramanian and Tamil, 2013).

2.9.2.3. Age

Pharmacokinetics (PK) of many drugs changes as a function of age, between children and adults. This is characterized by physiological differences, clearance mechanisms, immaturity of enzyme systems and a progressive decrease in the functional reserve of multiple organs and systems that may affect drug disposition (Ginsberg et al., 2002). Generally, the absorption of a drug does not change significantly with age, but changes are found in the rate of absorption, than in the extent of absorption. Visible differences in absorption are observed in the elderly and neonatal period. For both, there is a reduction in hepatic metabolism and first-pass effect which might result in increased oral bioavailability of certain drugs. Also, the volume of distribution of a drug is often directly proportional to the weight of the body and is modulated by age. For instance, changes related to age in the binding of drug may affect the volume of distribution (e.g. reduction in the extracellular fluid of the elderly). Moreover, metabolism of drug is obviously affected by age, whereby, the enzymes responsible for phase I and phase II metabolism gradually mature, in the first 2 to 4 weeks after postpartum. But complete development shows in the second decade of life, following a slow decrease in function connected with aging. The apparent volume of distribution and half-life of a drug differ with different age groups (new born, infant, children, adults and elderly). Therefore, full-term and premature neonates are most likely to have a 3-9 times longer half-life for certain drugs compared to adults. The dissimilarity vanishes by two to six months of age, but half-life can be shorter beyond this age, compared with adults for particular pathways and drugs (Ginsberg et al., 2002). Furthermore, in the excretion of drugs, the kidney clearance standardized according to bodyweight is low in neonates, but increases rapidly to a maximum at 6 months. During maturity, there is association of age with a standard reduction in kidney function of one percent each year and with a rise in the kidney clearance

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variability between individuals. Also, some important pharmacokinetic changes that happen with aging include a reduction in hepatic and renal clearance and first-pass metabolism, and an increase in the volume of distribution of lipid soluble drugs (therefore, elimination half-life prolongation) but the bioavailability of some drugs can be increased. Subsequently, lipophilic drugs have larger volume of distribution with a lengthy half-life and hydrophilic drugs have a lesser volume of distribution (Balasubramanian and Tamil, 2013).

2.9.2.4. Sex

Biological dissimilarities exist among women and men which can result to differences in responses of drug. Sex variation in pharmacokinetics can be because of dissimilarities in physical constitution, such as organ function, organ blood flow, body water space, muscle mass and body size (Huang et al., 2014). Although total drug absorption is not considerably influenced by sex, rates of absorption can be a little slow in women and bioavailability subsequent to oral administration of drug, for CYP3A substrates particularly, may be fairly high in women in contrast to men. Typically, men are mostly larger than women; hence dissimilarities in body size result in larger volume of distribution and quicker total clearance of several drugs in men, in contrast to women. Also, larger body fat in women can enhance volume of distribution for lipophilic drugs in women. Likewise, in women metabolism of drug is affected by female–specific issues, including menopause, pregnancy, menstruation and oral contraceptive use, as well as alcohol consumption, drug ingestion and cigarette smoking which are more commonly observed factors in men (Tanaka, 1999). Elimination of anti-epileptic drugs may be increased by pregnancy thereby, reducing the effectiveness of the drug. In addition, metabolism of most drugs can be impaired with oral contraceptive use and, equally, some drugs may interfere with contraceptive efficiency. Other studies confirm that the difference in pharmacokinetics (drug metabolism and elimination) between men and women was mainly attributable to the difference in metabolic enzymes and levels of steroid hormone (Huang et al., 2014). Recently, clinical and *in vitro* evidence showed that

the expression of cytochrome P450 (CYP) 3A4 of women was subtly higher than that of men, and total clearance of substrates for CYP3A are mildly faster (milligrams per kilograms) in women, in contrast with men (Huang et al., 2014). Therefore, close attention ought to be paid to the toxicity and adverse effects occurring from sex dissimilarities from drug metabolism in clinical situations.

2.9.2.5. Body weight

Pharmacokinetics of lean patients differs from that of obese patients in many conditions. Overall, studies indicate no significant difference in absorption between obese and lean subjects. Different body weights lead to different volumes of distribution, which result in different drug concentrations in the plasma. Also, administration of drug in obese patients is problematic, as recommendations of dose depend on PK information acquired from individuals with normal weight; thus, errors in the determination of the appropriate dose are frequently made. As a consequence of co-morbidity in obese patients, functioning of organs responsible for elimination of drug (e.g. liver and kidney) may be affected, making PK more complex and difficult (De Baerdemaekern et al., 2004). Predominantly, dosage recommendations in package inserts are based on total body weight (TBW), not on ideal body weight (IBW) or lean body mass (LBM) and it is assumed that PK is proportional to weight. Also, body weight is subject to many factors, such as age, height and degree of "fatness", hence the need for a scientific way to administer drugs according to the surface area, which counteracts the imbalance between body weight and physique.

2.9.2.6. Concomitant drug therapy

Drug-drug interaction is a condition where a drug interacts and influences the action of other drugs the person is taking, i.e. the drug effects are deceased or increased, or they yield a different outcome that wouldn't have been caused by one drug. Numerous drugs are given concurrently, in order to treat infections happening simultaneously or to increase the efficiency of treatment, thereby, causing pharmacokinetic interactions among the drugs and also affecting the therapeutic efficacy or toxicity of the drugs. During absorption, interactions of drug may possibly take place whereby a drug influences the extent or rate of absorption of other drugs. For instance, insoluble complexes are formed by calcium in the intestinal lumen with tetracycline, thereby, decreasing their bioavailability (Balasubramanian and Tamil, 2013). Drug distribution can be affected by drug interactions, where a drug which is highly bound to tissue or plasma proteins may be displaced by a different drug from its binding sites, leading to toxicity. Also, metabolism can be inhibited or induced by concomitant drug administration, thereby, leading to toxicity or ineffective therapy. Likewise, drug interactions can influence drug elimination, where a drug competes with the kidney tubular transport of a different drug, thereby, inhibiting the excretion of that particular drug by the kidney. Clinically, the half-life of the above drug can be elongated, and subsequently, with frequent drug administration, the phase to attain steady state concentration and to experience changes in plasma level such as toxicity are intensified.

2.10. Methods used to determine plasma concentrations of anti-tuberculosis drugs

Numerous efficient techniques were recommended in the literature for the analysis of anti-TB drugs in biological fluids. These analytical methods may be either non-chromatographic methods like bioassay and fluorimetric assay, or chromatographic methods, such as liquid or gas chromatography.

Microbiological assays, also known as bioassay, were widely utilized in the literature for the determination of the potency of anti-bacterial agents (Stead, 2000). Although this method is reproducible, simple and does not necessitate the use of highly technical equipment, it is time- consuming, and it lacks sensitivity and specificity (Immanuel and Kumar, 2001). Additional influence that contributed to the decrease in the use of agar diffusion method (microbiological assay), is due to inaccuracy in the analysis and the longer time taken before reading the results (Stead, 2000). Fluorimetric analytical technique was validated for ethionamide determination in biological fluids, such as plasma and urine. This recommended technique is highly sensitive and is based on the measureable quenching outcome of ETH on the native fluorescence of eosin in acidic medium because of formation of complex (Walash et al., 2004). Although microbiological assay and fluorimetric analysis are easy to use, they are not specific enough.

Chromatographic methods are based on a separation in which the drug and/or the material under analysis is distributed between two phases: a stationary and a mobile phase, which move in a definite direction. Different chromatographic methods, including gas and liquid chromatography for anti-tuberculosis drugs analysis, were reported in previous studies (Hemanth Kumar et al., 2014). A larger number of high-performance liquid chromatographic techniques for determining plasma levels of anti-TB drugs were reported in the literature (Jenner et al., 1981; Peloquin et al., 1991; Auclair et al., 2001; Thee et al., 2011). Other studies utilized LC-MS/MS, HPLC–MS–MS and HPLC with UV detection for determination of the plasma concentrations of anti-TB drugs (Conte et al., 2001; Lee et al., 2009; Deshpande et al., 2011).

2.11. Determination of Ethionamide plasma concentrations

A number of assay methods for the measurement of ethionamide levels in serum have been developed. Both non-chromatographic and chromatographic techniques were used in the literature for ETH plasma level determination in biological fluids (Jenner

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et al., 1981; Peloquin et al., 1991; Stead, 2000; Auclair et al., 2001; Conte et al., 2001; Walash et al., 2004; Hemanth Kumar et al., 2014).

Earlier studies used a bioassay (Gronroos et al., 1964; Jenner et al., 1984), while more recent investigations in the literature, have used high performance liquid chromatography for evaluation of ETH plasma concentrations (; Auclair et al., 2001; Zhu et al., 2002; Ahmad et al., 2009). The HPLC analytical method has been sensitive and specific, and detects 0.5 mg/ml (Peloquin et al., 1991) or 0.05 mg/ml (Jenner et al., 1981) of ethionamide in plasma. Jenner et al. (1984) used HPLC to analyse the urinary excretion and blood levels of ETH in human, and observed a lower limit of detection of 0.01 mg/ml. Auclair et al. (2001) reported a linear range of standard curves for serum ETH in 0.2 to 10 μg/ml ranges, using HPLC assay. Auclair and colleagues concluded that the lack of detectable concentrations of ethionamide in urine correspond with extensive metabolism of ethionamide, taking place in the liver.

Zhu et al. (2002) developed a validated HPLC technique for the determination of ethionamide concentrations, following multiple oral doses in patients suffering from tuberculosis. They found that the PK model developed could be used for the determination of individual pharmacokinetic parameter and afterwards, for the adjustment of doses when required. Zhu and his co-researchers concluded that at least the dose of ethionamide, 500 mg, is necessary for the purpose of attaining inhibitory serum concentrations.

A study done by Jenner et al. (1986) also used HPLC to analyse the blood levels of ETH and prothionamide (PTH), following intravenous and oral dosages. Jenner and colleague analyzed their extracted sample by HPLC, using the method previously reported for the thioamides determination in the plasma (Jenner et al., 1984). Their estimates of the concentrations of thioamides in plasma samples were made by reference to a series of calibration curves, prepared as described previously in the literature (Jenner et al., 1981, 1984).

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Recently, a HPLC–MS–MS procedure was developed using an electrospray mode that provides reliable, rapid, and specific evaluations for ETH plasma concentrations. Compared with other techniques, this method has the advantage of an increased sensitivity of 0.05 mg/ml in the plasma and the ability to analyse volumes of small sample. Thus, the limit of quantitation was attained at 0.05 mg/mL (Conte et al., 2001; Jenner and Ellard, 1981; Peloquin et al., 1991). Specificity of HPLC–MS–MS technique significantly reduces the risk of interference from other sample constituents (Conte et al., 2001). They found that ETH was accessible in the plasma at levels which approximated the MICs stated for *mycobacterium tuberculosis* where the concentration of the drug was in the epithelial lining fluid. Thus, it can be practically applied during the analysis of specimens from AIDS patients that are taking many drugs concomitantly.

Deshpande et al. (2011) developed and validated LC-MS/MS analytical technique that is extremely reproducible, specific, sensitive and gives high-throughput, for plasma levels of ethionamide determination using prothionamide as an internal standard. Retention time of ETH was approximately 2.50 minutes, with overall chromatography run time of 3.5 minutes respectively. Deshpande and colleagues reported a linear response function ranging from 25.7 to 61.20 µg/mL concentration of ETH (Deshpande et al., 2011). They concluded that the technique would be practically helpful for therapeutic drug monitoring of ethionamide in TB patients with regard to LC-MS/MS high sensitivity (LLOQ 25.7 µg/mL).

Limited studies are available in the literature for ethionamide plasma level determination in human plasma using LC-MS method of analysis.

2.1.2. Ethionamide plasma concentration determination using liquid chromatography–mass spectrometry (LC-MS) assay

Liquid chromatography joined with mass spectrometry is an analytical method in which the abilities of physical separation of liquid chromatography were combined

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with the mass analytical abilities of mass spectrometry. This analytical technique provides higher specificity, selectivity and sensitivity detection, which makes it capable of accurate quantitative analysis of drugs and their metabolites (Want et al., 2003; Kostiainen et al., 2003). It has become the basis for bio-analytical measurements of anti-TB drugs in human plasma. Part of its application is oriented towards the quantitative measurement of the drug, but it also offers qualitative differentiation between the parent drug and its metabolites, as well as unknown endogenous components in the analysis samples.

Liquid chromatography, coupled with mass spectrometry is one of the methods for the determination and assay of ETH. Limited studies used LC-MS, for ETH determination in biological samples. Therefore, the key advantages of utilizing LC-MS analytical techniques over HPLC techniques include its selectivity, specificity, rapidness, and peak assignment - that is, generating chemical fingerprint for the compound of interest and guarantee accurate peak assignment in the presence of multifaceted matrices. In addition, qualitative and quantitative data can easily be acquired, with limited instrumental optimization. Also, compared to conventional HPLC or immunoassays, it provides enhanced analytical specificity for analyte with low molecular weight and has an advanced throughput than gas chromatography coupled with mass spectrometry (GC-MS).

LC-MS is utilized owing to its short analysis time, excellent specificity when compared to ultra-violet (on condition that the analyte can be ionised accurately), and high sensitivity. The method shows high sensitivity, specificity, and recovery similar to that described by Jenner and Ellard (1981), and by Shepard et al. (1985), while replacing the liquid-liquid extraction with a simple, reproducible solid-phase extraction method. HPLC, coupled with mass spectrometric analysis of anti-tuberculosis drugs, is specific, sensitive and rapid, and can be applied successfully to therapeutic drug monitoring in patients with tuberculosis (La and Feng, 2007). Besides, Conte and his team used the new column chromatographic mass

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spectrometric method to determine ethionamide levels in alveolar cells and plasma epithelial lining fluid (ELF).

In this study, LC-MS was utilized for the analysis of ETH plasma concentrations in patients, because of its high sensitivity and specificity in comparison to other described methods. Chapter three has more in-depth information about the methods, chemicals used and the LC/MS settings.

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CHAPTER THREE
METHOD

3.1. Study site

This study was performed at the Brewelskloof Hospital (BKH) in Worcester, Western Cape Province, South Africa. Brewelskloof Hospital is one of the South African hospitals specializing in management of multidrug resistance tuberculosis (MDR-TB).

3.2. Study design

The study was designed as a prospective, non-randomized, two-group pharmacokinetic study that involves female and male HIV-negative patients and HIV-positive patients admitted for treatment of MDR-TB.

3.3. Study population

Demographic characteristics were recorded for every patient on a data collection form: age, weight, and gender. The study comprises HIV-negative and HIV-positive patients, and patients suffering from multidrug resistance tuberculosis. Most of the HIV (+) patients were taking antiretroviral drugs.

3.4. Inclusion criteria

A patient was incorporated in this study only if he/she fulfilled all of the following criteria:

1. Signed informed consent form after explaining to the patient, clearly using their first language, about the study procedures, objectives, advantages and disadvantages.

2. MDR-TB must be responsive to 2nd-line anti-tuberculosis drugs.

3. MDR-TB must be responsive to 2nd-line anti-tuberculosis drugs and co-infected with HIV.

4. Patients on ethionamide therapy at least two weeks before the study.

5. Matured patients between 18 to 54 years of age

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3.5. Exclusion criteria

Patients were barred in this study due to one of the following reasons:

1. Patient's request.

2. Patients on other drugs apart from anti-retroviral drugs, known to interfere or interact with the pharmacokinetics of ETH.

3. Pregnancy or breast-feeding.

4. Patients that are below 18 years and above 54 years old.

5. Case of XDR-TB.

3.6. Clinical and therapeutic characteristics

Each patient's body weight and vital signs were checked.

3.7. Laboratory tests:

Renal function (electrolytes, sodium, potassium, creatinine and urea) and liver function (bilirubin, gamma-glutamyl transferase, aspartate amino transferase, alkaline phosphatase, albumin and alanine aminotransferase) tests were conducted at Path Care Laboratory (Worcester). Virological and immunological tests (CD4 count and viral load) were performed at the National Health Laboratory Services (NHLS) at Tygerberg Hospital.

3.7.1 Liver function tests (LFT)

Liver function was assessed by means of Child-Pugh score. The scoring system utilizes biochemical and clinical factors to evaluate the diagnosis of chronic liver disease in patients. When assessing this score, biochemical and clinical measurements such as prothrombin-time, serum albumin, total bilirubin, hepatic ascites and hepatic encephalopathy were graded, based on standardised points. Three of the factors evaluated the synthetic function of the liver (that is, prothrombin, serum albumin, and total bilirubin level) and two of the factors are based on clinical evaluation (that is, degree of hepatic encephalopathy and degree of ascites) (Durand and Valla, 2005).

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Table 3.1: Child-Pugh score classification system

Biochemical and clinical measurements	Increasing abnormality points scored		
	1	2	3
Serum albumin(g/Litres)	>35	28-35	<28
Prothrombin* (s)	<4	4-6	>6
Ascites Bilirubin (µmol/Litres)	Absent (no ascites) <34	Mild (medically controlled) 34-51	Refractory (poorly controlled) >51
Hepatic encephalopathy	None (no encephalopathy)	Minimal (medically controlled)	Advanced (poorly controlled)

(Durand and Valla, 2005)

*Prothrombin time values of 4 and 6 correspond approximately to 50 and 40% of normal values, respectively.

Each biochemical measurement is assigned a score from 1-3, where point 3 represents severe derangement. The individuals' scores were summed together and ranked in accordance to the severity of the liver disease. Chronic disease of the liver was categorized as A to C Child-Pugh class, using the added score as of above. The score, which is equivalent to the summation of individual points, permits for patients' classification in Child– Pugh grades A to C. Therefore, according to the classification, class A (5–6 points: is for mild liver impairment), class B (7–9 points:

for moderate impairment) and class C (10–15 points: for severe liver impairment) (Durand and Valla, 2005).

3.7.2. Renal function tests

Renal function was estimated by creatinine clearance calculated by the application of the following formula;

CrCl (ml/minutes) = [(140 − age) x weight (in kg)] / [0.82 x serum creatinine (μmol/litres)]

CrCl = creatinine clearance.

In women, creatinine is multiplied by 0.85 instead of 0.82 (DOH, 2012).

3.7.3. Immunological and virological tests

Viral load level and CD4 count tests (indicate the immune-competence level) were performed on patients who were HIV-positive by the National Health Laboratory Services (NHLS) at Tygerberg Hospital.
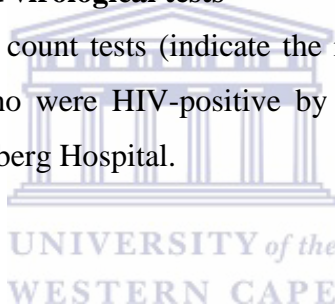
3.8. Study procedures
3.8.1. Blood sampling and Ethionamide dose

Patients were informed to do an overnight fast (8 hours) before the day of the study. On the morning of the day of the study, vital signs were assessed, while baseline blood samples (5 ml) were collected for virological, renal function and liver function tests, before drug administration. Another 5 ml blood sample was collected in a heparinized tube, for the determination of ethionamide baseline plasma concentrations. Subsequently, each patient received his/her standard dose of anti-TB drug, as well as ETH tablet orally, and the dosing time was recorded. Each patient received single dosage form anti-TB drugs, at a dose of 25 mg/kg. They were then allowed to have breakfast, four hours after the drug administration.

A blood sample (5 ml) was collected after drug administration, in a heparinized tube at 0.5, 1, 2, 4, 8, 16, and 24 hours for the measurement of ethionamide plasma

concentrations. Blood samples of the patients were obtained using an intravenous catheter fixed at each patient's forearm vein. After collection, blood centrifugation was done for 5 minutes at 5,250 rpm. The plasma was then separated from the sediment with the use of a micropipette and stored at -80^{0}C until the day of analysis.

3.9. Determination of ethionamide plasma concentrations

Plasma concentrations of ETH were determined using liquid chromatography coupled with mass spectrometry (LC-MS) assay, at the Central Analytical Facility, Department of Biochemistry, University of Stellenbosch, Cape Town, South Africa.

3.9.1. Chemicals

The chemicals utilized in this study were acquired from Sigma-Aldrich, in Western Cape. These include trifluroacetic acid (TFA), phosphoric acid, trichloroacetic acid, acetonitrile, dimethyl sulfoxide (DMSO), formic acid, ethionamide tablets and propranolol.
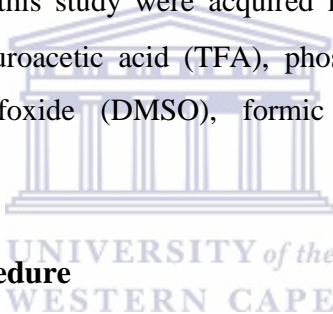
3.9.2. Experimental procedure

The LC-MS assay was conducted according to the method used by Abaniwonda, (2012) in her study. The steps are as follows: ethionamide stock solution, preparation of ethionamide standards, preparation of patients' plasma samples, setting up of mass spectrometry and liquid chromatography.

3.10. Determination of ethionamide pharmacokinetic parameters

Patients' plasma concentration-time profile was manually plotted. PK parameters of ETH were determined using a non-compartmental analysis (NCA) and the results were expressed as median (range).

3.10.1. The area under the plasma concentration-time curve (AUC$_{0-24}$)

The area under the concentration from 0 to 24 hours (AUC$_{0-24}$) was manually determined using trapezoidal method.

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3.10.2. The area under the plasma concentration-time curve ($AUC_{0-\infty}$)

Area under the concentration from zero to infinity ($AUC_{0-\infty}$) was determined using the formula below:

$AUC_{0-\infty} = AUC_{0-24} + CP_{last} / Ke$

[Where CP_{last} = the last measurable plasma concentration].

3.10.3. The mean residence time (MRT)

Mean residence time (MRT) of a drug was determined as follows:

$MRT = AUMC\infty / AUC\infty$

[$AUMC\infty$ = the area under the momentum curve from zero to infinity]

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

[AUMC= the area under the momentum curve, t_{last} = time of the last measurable concentration and C_{last} = last plasma concentration].

$AUMC = (t_i c_i + t_{i-1} \times c_{i-1}) / 2 \times (t_i - t_{i-1})$
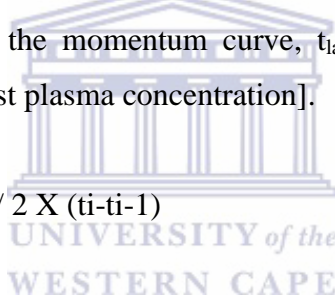
3.10.4. The elimination rate constant (K_e)

Elimination rate constant (Ke) was determined from terminal linear phase of the plasma concentration-time profile.

Since natural log was used, the slope was calculated as follows

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

Ke was obtained as – (slope)

3.10.5. The elimination half-life ($T_{1/2}$)

Elimination half-life ($T_{1/2}$) was determined using the following formula:

$T_{1/2} = 0.693 / K_e$

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3.10.6. The absorption rate constant (K_a)

Absorption rate constant (K_a) was determined by utilizing the residuals method to plot the residual concentrations graph and finding the slope graphically. Absorption rate constant was obtained from slope of the straight line, which represents the absorption phase.

Ka is equal to – (slope) = – (ln y2- ln y1/x2-x1)

3.10.7. The time to reach maximum concentration (T_{max})

Time to reach the maximum concentration (T_{max}) was calculated using the formula below:

T_{max}= (Ln ka – Ln ke) / (ka – ke)

[Where: ke = elimination rate constant and ka = absorption rate constant]

3.10.8. The maximum concentration (C_{max})

The maximum concentration (C_{max}) was calculated using the formula below:

$C_{max} = B \times (e^{-bt} – e^{-at})$

[Where: B is rate of y-interception, b is slope, t is time, a is absorption]

[But y-intercept B: Ln ct = Ln B – bt]

3.10.9. The volume of distribution (V_d)

Volume of distribution (V_d) was determined using the formula below:

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

[Where: V_d is volume of distribution, Ke is elimination rate constant].

3.10.10. The total body clearance (Cl_{tot})

Total body clearance (Cl_{tot}) was determined by the formula below:

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

3.11. Statistical data analysis

Data obtained from the study was coded, captured and stored in the computer system using Excel (Microsoft Office 2010) for easy management of the data. ETH pharmacokinetic parameters and its concentrations in the plasma following ETH oral administration were calculated and statistically analysed by non-compartmental model for all variables. The distributions of all variables were tested for normality using the Shapiro Wilk Normality Test, visual inspection of q-q plots and histograms. Variables that were normally distributed were analysed using a parametric test such as independent T-Test, while variables that were skewed were analysed using a non-parametric test such as Mann-Whitney U- Test (Wilcoxon Rank Sum Test).

The comparisons of interest were between patients with MDR-TB co-infected with HIV and MDR-TB patients without HIV-infection. Median termed "as measure of location" and range termed "as a measure of spread" were utilized in analyzing the quantitative variables. ETH pharmacokinetic parameters and concentrations were evaluated by means of median (range) statistically. Results were expressed as medians and ranges, because of the small sample size. Nonparametric Wilcoxon Rank Sum test was used for comparisons of results and statistical differences were believed to be significant at $p<0.01$ level for avoidance of a false positive.

3.12. Ethics considerations

This research study was approved and registered by UWC (Ref nb 07/6/2014) and UCT (ref nb 777/2014) ethics committees. The Provincial Department of Health in conjunction with the Medical Superintendent of Brewelskloof Hospital granted the permission to carry out the study at the Brewelskloof Hospital. HIV test-informed consents were utilized and information acquired at the course of the study was handled with strict confidentiality.

3.13. Dissemination of research results

This study results will be disseminated through the following:

a. Presentation at department, School of Pharmacy, University of the Western Cape.

b. Conference presentations at the national and international level.

c. Publication in scientific journal.

d. Thesis for master's degree at the University of the Western Cape.

CHAPTER FOUR
RESULTS

4.1. Validation of the liquid chromatography-mass spectrometry analysis

4.1.1. Liquid chromatography-mass spectrometry

Liquid chromatography coupled with mass spectrometry (LC/MS), was utilized for the determination of ethionamide levels in human plasma. The LC/MS analytical method was validated by determining the linearity, specificity, low limit of quantification, low limit of detection and recovery.

4.1.2. Calibration curve and linearity

Calibration curve of ethionamide was formed by plotting responses on y-axis against the equivalent concentrations (in μg/ml) on the x-axis, as indicated in figure 4.1 below. Linearity of the method was determined by analysing a set of calibration standards. The calibration curve displayed linearity over the concentration ranges of 0.1-10 μg/ml. The correlation coefficient was 0.99 or greater.

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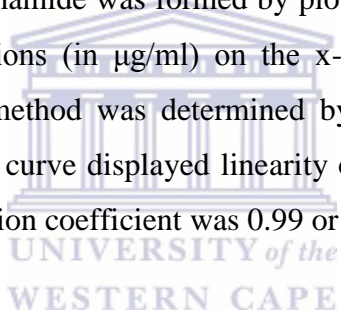
Compound name: ethionamide
Correlation coefficient: r = 0.998921, r^2 = 0.997844
Calibration curve: 0.0111144 * x + -0.000119122
Response type: Internal Std (Ref 3), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Include, Weighting: 1/x, Axis trans: None

Ethionamide concentration (μg/ml)
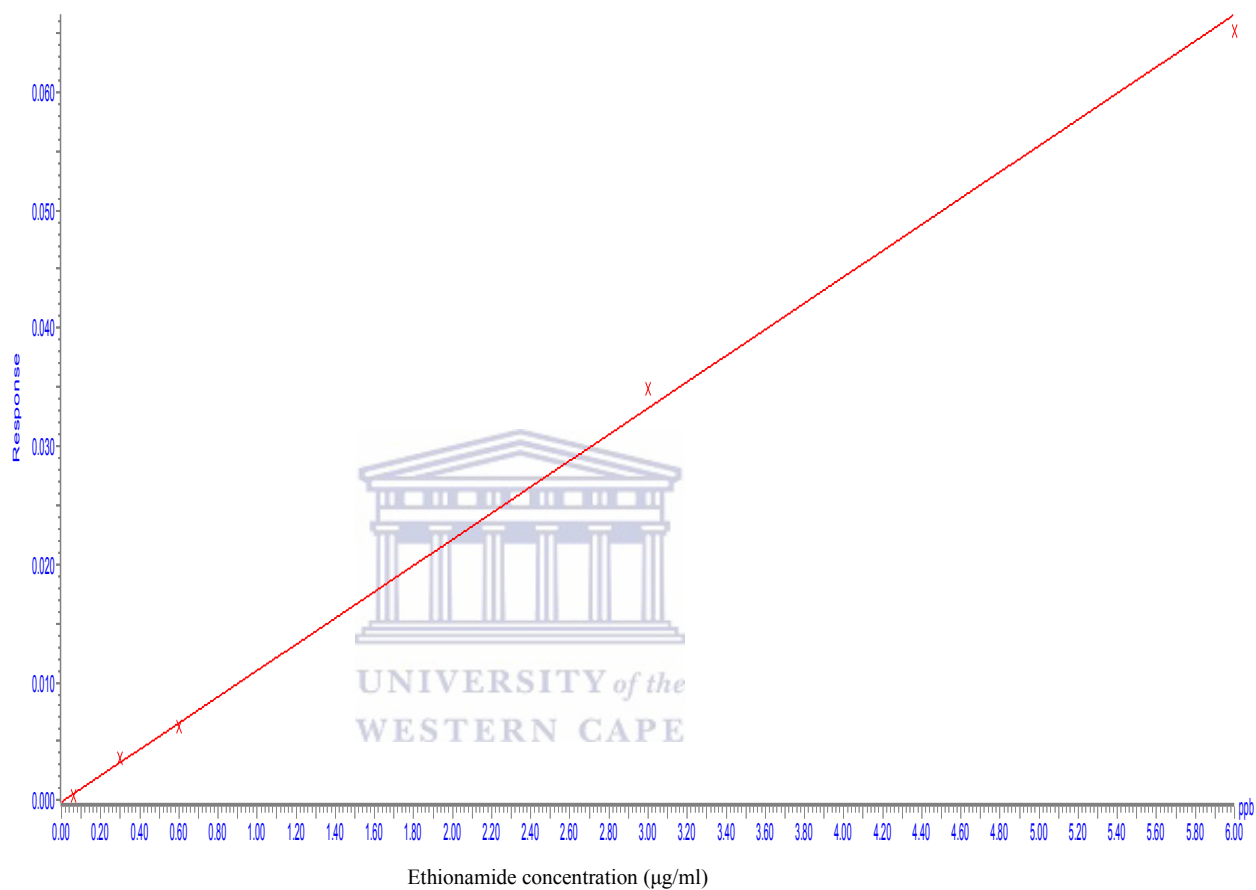
Fig 4.1: Ethionamide calibration curve

4.1.3. Specificity

No interfering peaks were observed from the components of serum with ethionamide peak, detected at 2.23 minutes retention time. Chromatograms demonstrating the separation of plasma extract of ethionamide are revealed in Figure 4.2.

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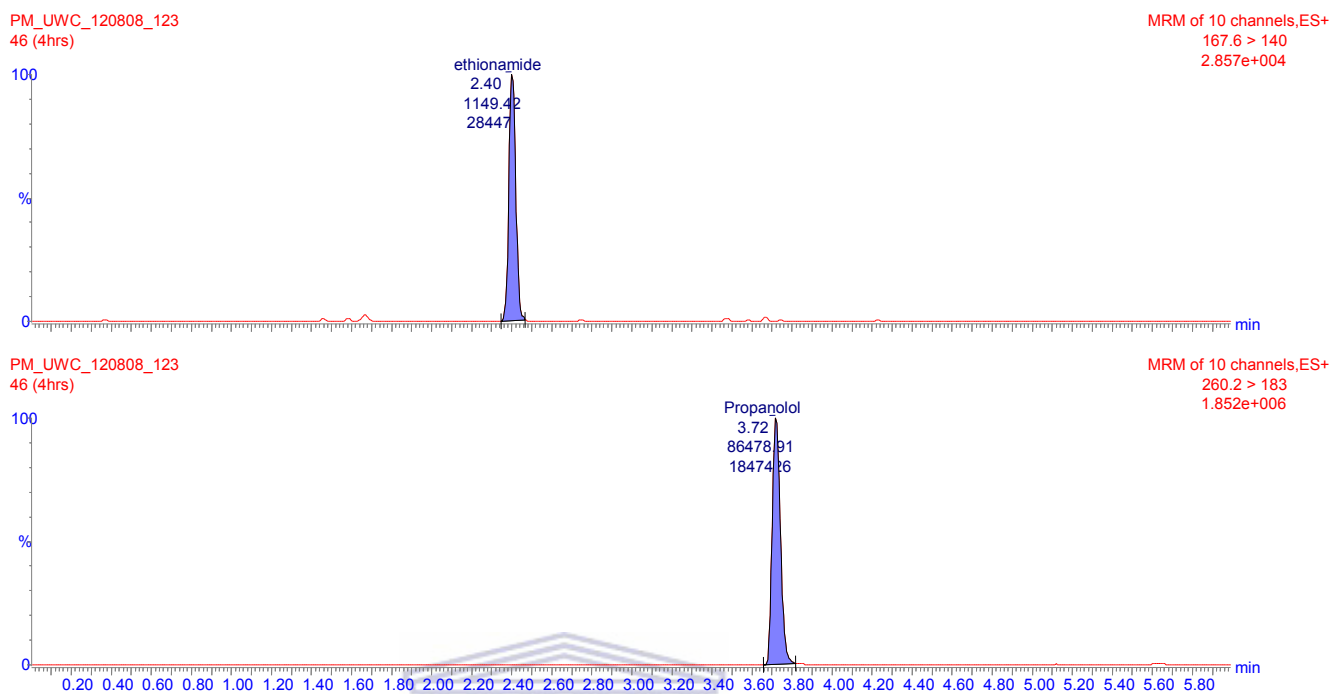
Figure 4.2: Chromatogram of ethionamide plasma extract.

4.1.4. Limits of Detection (LOD) and Limits of Quantification (LOQ)

Ethionamide lower limit of detection in plasma was 0.01μg/ml. Likewise, ethionamide lower limit of quantification in plasma was 0.15 μg/ml.

4.1.5. Recovery

Determination of ethionamide recovery involves peak areas in comparison with samples of plasma that were spiked with ethionamide subsequent to extraction with the subsequent standard ethionamide solutions. Blank plasma was spiked in triplicate with 1 and 5 ppm of the drugs for repeatability and determination of the recoveries. The average recovery of ethionamide ranged from 104%±2.3% to 128%±11.2%. The standard deviation was greater than 12% for all the analytes. The results of the spiking experiments, detection limits and quantification limits of ethionamide are shown in table 4.1 below.

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Table 4.1: Results of the spiking experiments, LOD and LOQ

Specimen	1ppm spike: % Recovery std dev	1ppm spike: % Recovery std dev	LOD (ppm)	LOQ (ppm)
Ethionamide	128±11.2%	104±2.3%	0.01	0.15

4.2. Patients' demographics

A sample size of 42 patients (23 males and 19 females) comprising black and mixed raced patients participated in this study. These patients included 32 MDR-TB patients and 10 rifampicin mono-resistant patients. Of the 42 patients, 17 (11 males and 6 females) were HIV-positive. The patients' demographic data, which are age in years, body weight in kilograms and gender, are listed in Table 4.2 below.

Table 4.2: Demographic characteristics

Age intervals(years)	Females No	Females Weight(kg): median(range)	Males No	Males Weight(kg): median(range)	Total (F + M)
18-24	4	49.28 (42.7-55)	1	60.28 (60.28)	5
25-34	8	60.11 (44-98.02)	9	56.91 (46.58-77)	17
35-44	6	52.69 (40.96-64.54)	8	53.81 (43.4-72)	14
45-54	1	45 (45)	5	53.24 (39.82-61)	6
Total	19		23		42

Key: F= Females; M= Males.

In table 4.2 above, the study population was sub-divided into different groups, according to their age, sex and weight. Seventy-four percent of the study population was between 25-44 years of age, consisting of 31 patients (17 males and 14 females). These 31 patients

had the highest incidence of MDR-TB, and were categorized as 8 new cases, 16 relapsed cases and 7 defaulted cases. None of the patients was above 54 years of age.

4.3. Renal function profile

Renal function findings in HIV-negative and HIV-positive patients who enrolled in this study are represented in table 4.3 below.

Table 4.3: Renal function in HIV (-) and HIV (+) patients

Group	Description	Estimated creatinine clearance (millilitres/minutes)	HIV positive	HIV negative	Total
1	Normal renal function	> 80 ml/min	7 (2F + 5M)	18 (13F + 5M)	25
2	Mild renal impairment	50 – 80 ml/min	8 (3F + 5M)	7 (0F + 7M)	15
3	Moderate renal impairment	30 – 49 ml/min	2 (1F + 1M)	0	2
4	Severe renal impairment	< 30 ml/min	0	0	0
5	End stage renal disease	Requiring dialysis	0	0	0

Key: M=male, F=female.

The above table represents kidney function in HIV-negative and HIV-positive patients of the study population. Fifteen patients (7 HIV-negative and 8 HIV-positive) had mild renal impairment, with a creatinine clearance (CrCl) ranging from 50 to 80 ml/min inclusive. Likewise, two patients (2 HIV-positive) had moderate renal impairment, with a CrCl ranging from 30 to 49 ml/min, inclusive. More than half of the study population (60%) had

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normal renal function, consisting of 18 HIV-negative and 7 HIV-positive patients. None of the patients had compromised renal function.
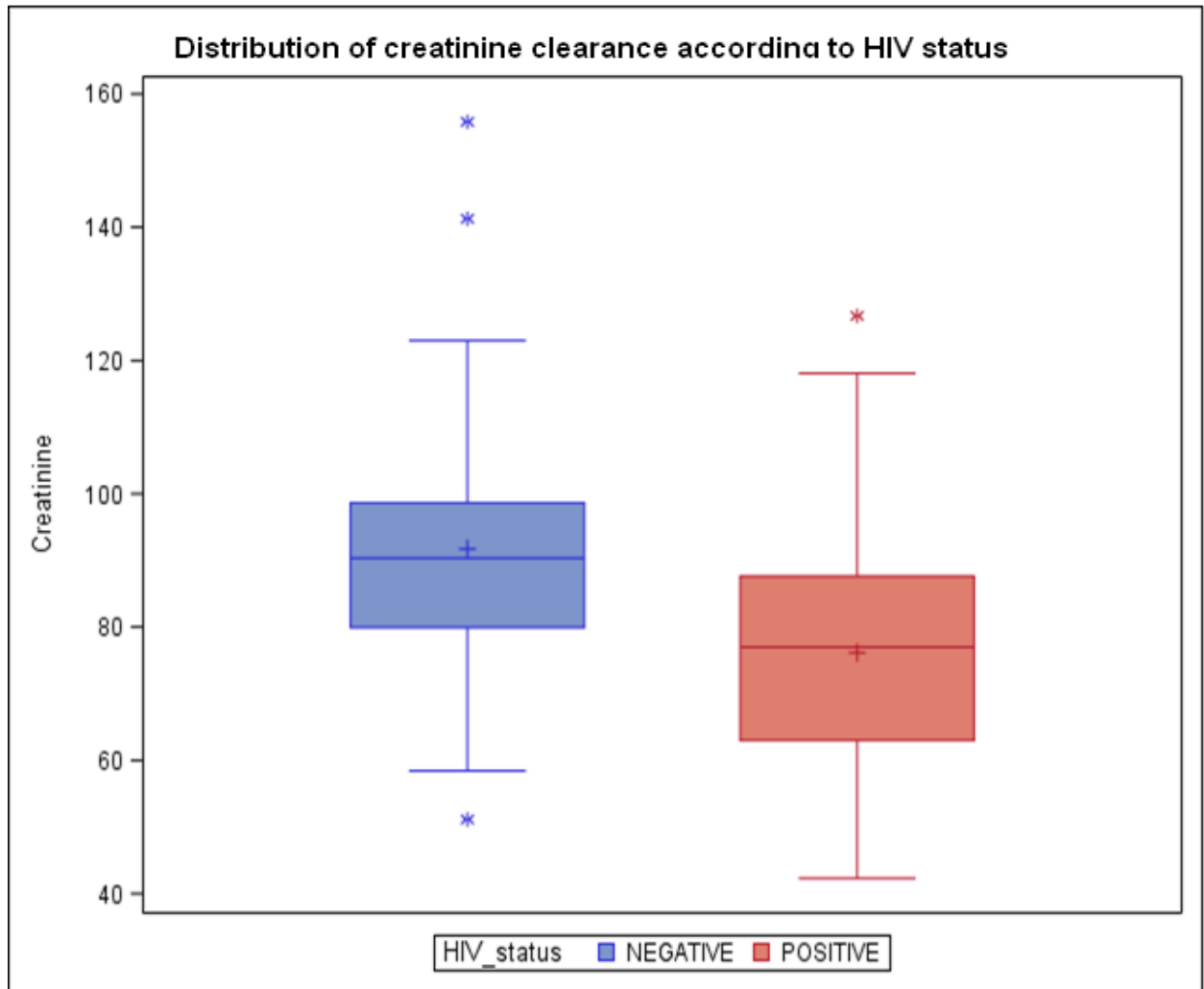
Figure 4.3: Creatinine clearance distribution of HIV-negative and HIV-positive patients

The median CrCl in HIV-negative patients is within the normal range. But the median CrCl in HIV positive patients is moderately decreased.

4.4. HIV status of study population and antiretroviral treatment

Out of the 17 patients that were HIV-infected, 16 were on antiretroviral treatment. These patients were receiving the following ARVs: stavudine (D4T): 30 mg/po/bd, efavirenz

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(EFV): 600 mg/po/nocte and lamivudine (3TC), 150 mg/po/bd. The CD4 counts of the HIV-infected patients are indicated in the table below.

Table 4.4: Immunological profile in HIV-infected patients

CD4 count (range) cells/mm3	Total (F + M)	Number of females	Number of males
<200	8	2	6
201-400	8	3	5
593	1	1	0

Key: F= Females; M= Males.

Table 4.4 above shows that the number of patients with severe immune depression is equivalent to the number of patients with moderate immune depression. Only one patient had CD4 >500 cells/mm3.

Table 4.5: Virological profile in HIV-infected patients

Viral load (ranges) (copies/ml)	Total (F + M)	Number of females	Number of males
Unknown	1	1	0
<600	12	5	7
600-1000	1	0	1
>1000-5000	3	0	3

Key: F= Females; M= Males.

From table 4.5 above, 12 patients from the study population had viral load <600 copies/ml. Also, 4 patients had viral load ranging from 600-5000 copies/ml.

4.5. Anti-TB drugs, other medications and co-existing diseases of study population

The patients were given anti-TB drugs to which mycobacterium tuberculosis had been found to be sensitive. Ethionamide (500-750 mg daily) was given in conjunction with

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pyrazinamide (1.0-2.0 g), kanamycin (1.0 g), ethambutol (1.0-1.6 g), terizidone (750 mg), ofloxacin (800 mg) and isoniazid (550-800 mg). All the drugs were orally administered, except kanamycin, which was administered intramuscularly. Most of the patients were also taking co-trimoxazole (150 mg daily) as a prophylactic treatment for pneumocystis (carinii) jiroveci pneumonia in individuals who are immuno suppressed. Co-existing diseases recorded include: hepatitis B virus (1 patient), substance abuse (5 patients), epilepsy (1 patient), diabetes mellitus (3 patients), hypertension (2 patients) and STI (1 patient). The table below represents the co-morbidity and co-treatments of the study population.

Table 4.6: Co-morbidity and co-treatments of the study population

Patients' Numbers	Co-morbidity	Co-treatments
1	hepatitis	Vitamin B complex (2 tablets), cimetidine (400 mg), metoclopramide (10 mg), tramadol (400 mg).
2	Epilepsy	Epanutin (300 mg), phenytoin (300mg), codeine phosphate (10ml), panado (500 mg), phenergan (25mg), amikacin (1000mg), sorbitol (30 ml).
3,6	substance abuse	Pyridoxine (150mg), vitamin B complex (2tablets), codeine phosphate (10 ml), sorbitol (30ml), panado (500 mg), phenergan (25mg), maxalon (10 mg), brufen (400mg), metoclopramide (10mg).
12	STI	Valium (5mg), amitriptyline (25mg), ranitidine (300 mg).

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23	diabetes, hypertension	Metformin (500 mg), enalapril (10 mg), hydrochlorothiazide (25 mg).
26,28,29,30,31,33,34,35,36, 42	HIV positive	Stavudine (D4T) (30mg), efavirenz (EFV) (600 mg), lamivudine (3TC) (150mg), bactrim (150 mg), prednisone (150 mg), phenergan (25mg), losec (20 mg).
27	HIV positive, Substance abuse (dead)	Stavudine (D4T) (30 mg), efavirenz (EFV) (600mg), lamivudine (3TC) (150mg), bactrim (150 mg), pyridoxine (150mg), vitamin C (1000 mg), paracetamol (500 mg), amphotericin B (50 mg), metoclopramide (10 mg), fluconazole (150 mg), nystatin drop (100 000 units/ml), phenergan (25 mg), sorbitol (30 ml).
32	HIV positive, Renal insufficiency, substance abuse	Stavudine (D4T) (30 mg), efavirenz (EFV) (600mg), lamivudine (3TC) (150mg), bactrim (150 mg), pyridoxine (150mg), vitamin C (1000 mg), paracetamol (500 mg), amphotericin B (50 mg), metoclopramide (10 mg), fluconazole (150 mg), nystatin drop (100 000 units/ml), phenergan (25 mg), sorbitol (30 ml), isoniazid (550 mg).

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| 4,5,7,8,9,10,11,13,14,15,16, 17,18,19,20,21,22,24,25,37, 38,39,40,41 | None | Pyridoxine (150 mg), doxycycline (100 mg), metoclopramide (10 mg), amitriptyline (25 mg), bactrim (150 mg), tramadol (400 mg). |

Table 4.7: Ethionamide interactions with other patients' drugs

Drugs	Interactions
Ethionamide + Efavirenz	Administration of ethionamide causes hepatitis and psychiatric symptoms with efavirenz. Ethionamide may increase the risk of liver problem.
Ethionamide + Ethambutol	Concomitant administration of ethambutol and Ethionamide exacerbates the toxic effects of ethambutol by ETH. Combing these medications may increase the risk of nerve damage.
Ethionamide + Isoniazid	Co-administration of ethionamide and isoniazid increased the serum concentration of isoniazid in both rapid and slow acetylators. Combing these medications may increase the risk of nerve damage.

4.6. Ethionamide plasma concentrations and pharmacokinetic parameters

4.6.1. Ethionamide plasma concentrations

Plasma levels of ethionamide at various time points after ethionamide administration over a period of 24 hours for both HIV (-) and HIV (+) patients' groups are illustrated in the figures 4.4 and 4.5 below.

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4.6.1.1. The ethionamide logarithm plasma concentration-time profile in HIV-negative and HIV-positive patients

Plot of log ethionamide plasma concentrations (µg/mL) against its corresponding time (hrs) for both HIV (-) and HIV (+) patient groups of this study are demonstrated in figure 4.3 below.
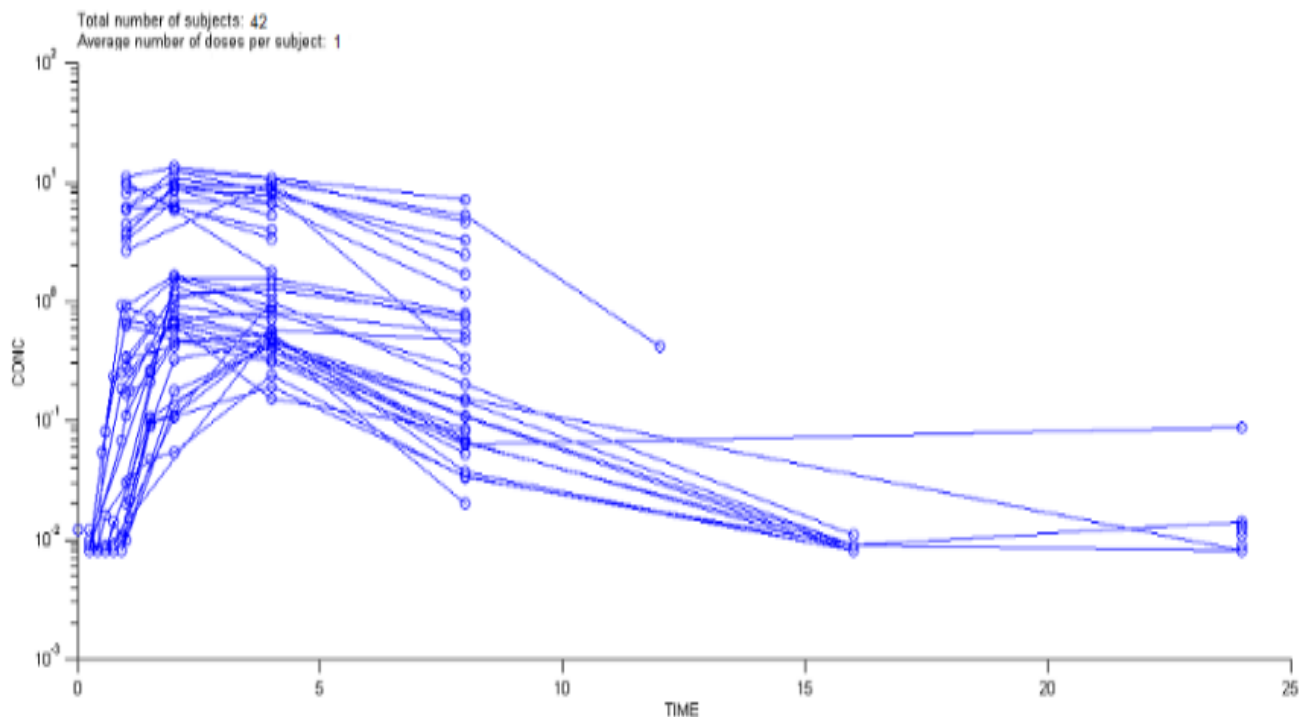
Fig 4.4: Plot of ethionamide logarithm plasma concentrations (µg/mL) vs. time (hrs).

Most of the patients do not have value for baseline (before ethionamide administration), because previous ethionamide concentrations had been eliminated completely from the body. This implies that the plasma concentrations were below detectable levels of 0.05 µg/ml. The peak plasma concentration was observed at 2 hrs after ethionamide administration. Ethionamide plasma levels were below detectable levels after 8 hours in the majority of the patients. Similarly, ethionamide plasma levels were detected at 16 hrs and 24 hrs in 8 patients infected with HIV. This could be due to HIV-infection and ARVs that the patients are taking.

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4.6.1.2. The ethionamide median plasma concentration-time profile in HIV-negative and HIV-positive patients

Figure 4.4 below shows a plot of ethionamide median plasma concentration-time profile in HIV (-) and HIV (+) patients group of this study.
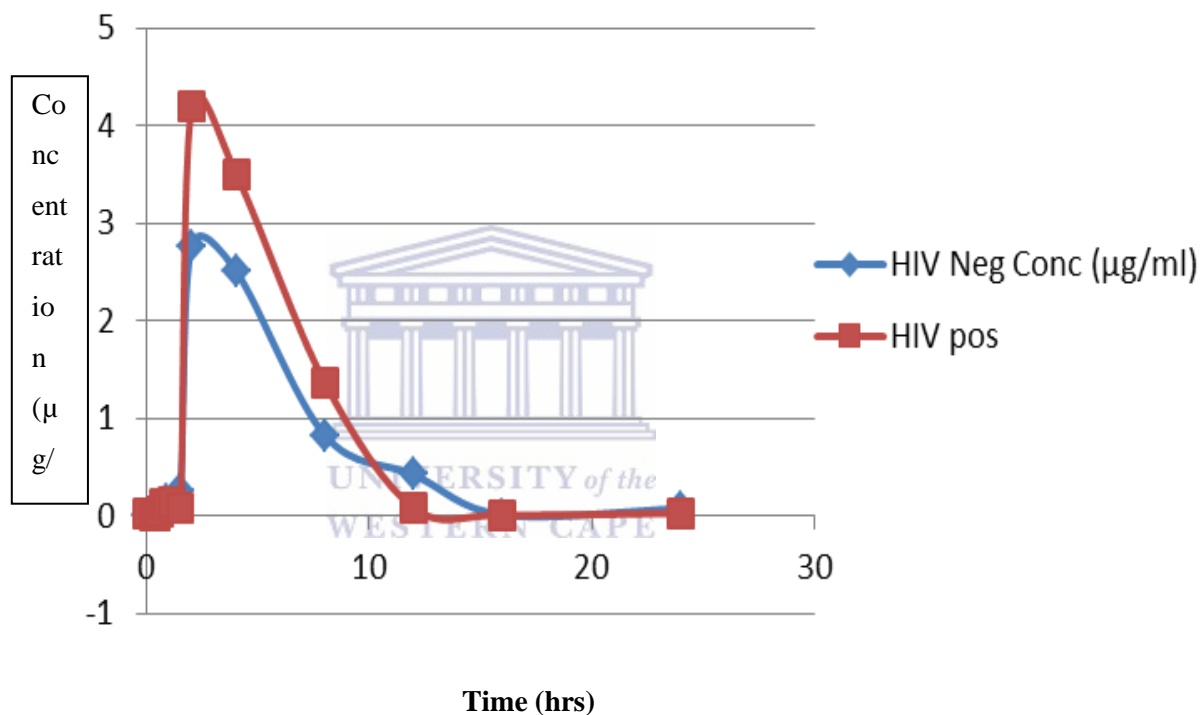
Fig 4.5: Plot of ethionamide median plasma concentrations vs. time

Figure 4.5 depicts a graph of ethionamide concentration (μg/ml) versus time (hours). Two concentration curves were observed, representing ETH concentrations in HIV-positive patients (red curve) and ETH concentrations in HIV-negative patients (blue curve) respectively. The concentration-time plot was linear from time zero until at 2 hours, where a decline was observed in both curves. The median ETH plasma concentration is relatively higher in HIV (+) patients at a concentration of 4.3 μg/ml.

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4.6.2 Ethionamide pharmacokinetic parameters

Table 4.8 below shows ethionamide pharmacokinetic parameters in both HIV (-) and HIV (+) patients who participated in this study. Patients were receiving MDR-TB therapy for at least two weeks. Similarly, related data from other previous studies in-addition to this present data are included in Table 4.9.

Table 4.8: Results of ethionamide pharmacokinetic parameters of this study

Variables	Median (range)		P. values 99% confidence interval estimates for difference in location (Positive-Negative)			
	HIV-negative (Nb:25)	HIV-positive (Nb:17)	P-value	Shift	Lower CI	Upper CI
AUC0-24 (µg/ml)	3.550 (0.780-63.120)	7.740 (0.370-79.350)	0.3486	1.2400	-1.6700	11.8400
AUC0-∞ (µg/ml)	3.910 (1.200-64.050)	7.860 (0.470-80.680)	0.3486	1.1800	-1.7000	13.2600
MRT (hrs)	3.690 (2.370-14.350)	3.620 (1.560-8.240)	0.5846	-0.2600	-1.2500	0.6800
Ke	0.300 (0.100-0.780)	0.240 (0.130-0.600)	0.4236	-0.0400	-0.1300	0.0600
T½ (hr^{-1})	2.310 (0.890-6.790)	2.880 (1.150-5.330)	0.4310	0.0290	-0.5800	1.1900
Ka (hr^{-1})	0.710 (0.290-2.190)	1.030 (0.240-2.100)	0.3817	0.1600	-0.1600	0.5800
Tmax (hrs)	2.230 (1.010-4.220)	1.700 (0.880-5.060)	0.8585	-0.0800	-0.7100	0.7300
Cmax (µg/ml)	1.530 (0.180-12.800)	3.710 (0.055-17.110)	0.5504	0.3500	-0.8300	4.1300

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Vd (L)	242.950 (36.150-1169.000)	119.150 (25.530-4137.930)	0.5588	-20.3200	-182.8000	180.9500
Cl (L/hr)	63.630 (8.00-281.250)	34.220 (5.330-731.250)	0.7795	-6.0300	-50.7600	69.3000
Vd/Wt (L/kg)	4.243 (0.762-27.377)	1.996 (0.451-98.946)	0.5588	-0.4514	-3.2103	2.9319
CrCl (ml/min)	90.350 (51.120-155.820)	77.000 (42.260-126.690)	0.0313	-15.8000	-29.2400	-2.4200

Key: Vd = volume of distribution; T½ = elimination half-life; Tmax = time to attain Cmax; Cmax = plasma concentration, AUC0-24 = area under the concentration-time curve from zero to 24 hrs; AUC0-∞= area under the plasma concentration-time curve from zero to infinity; Ka= absorption rate constant; Cltot = total plasma clearance; Ke = elimination rate constant; MRT = mean residence time.
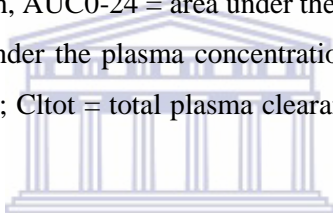
As indicated in table 4.8 above, there were no differences statistically in ethionamide pharmacokinetics parameters in HIV negative and HIV positive patients

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Table 4.9: Ethionamide pharmacokinetics parameters in HIV-negative patients

Study conditions	Vd (L)	Tmax (hrs)	Cmax(µg/ml)	T½ (hr^{-1})	AUC0-24 (µg/m)	AUC0-∞(µg/m)	Cltot (L/hr)	Ke	Ka (hr-1)	MRT (hrs)	Reference
TB patients	5.07 (2.16-9.06)	2.00 (1.25-2.22)	1.35 (0.48-5.63)	1.63 (0.90-2.97)	NA	2.80 (1.00-8.96)	2.11 (0.86-5.89)	0.43 (0.23-0.77)	0.40 (0.25-0.78)	NA	Zhu et al.; 2002
MDR-TB Patients	2.5 ± 1.3	3.6 ± 1.3	2.2 ± 1.1	2.7 ±0.7	NA	NA	0.6 ± 0.2	0.3 ± 0.1	NA	NA	Lee et al.; 2009
Healthy volunteers	18.154 ± 0.2958	1.75 ± 1.487	1.941 ± 1.3857	1.8 ± 1.362	NA	8.745 ± 0.566	32.591 ± 0.298	0.39 ± 2.864	0.384 ± 3.185	NA	Ahmad et al., 2009

from previous studies

Key: Vd = volume of distribution; T½ = elimination half-life; Tmax = time to attain Cmax; Cmax = maximum concentration, AUC0-24 = area under the concentration-time curve from zero to 24 hrs; AUC0-∞= area under the plasma concentration-time curve from zero to infinity; Ka= absorption rate constant; Cltot = total plasma clearance; Ke = elimination rate constant; MRT = mean residence time; NA = not available

CHAPTER FIVE
DISCUSSION

Liquid-Chromatography coupled with mass spectrometry method (LC-MS)

This study aimed to determine ethionamide (ETH) plasma concentration using the LC-MS method. In this study, a highly specific liquid chromatography coupled with mass spectrometric method for the determination of ETH concentrations in plasma samples of the patients was developed and validated.

A number of assay methods for the measurement of ethionamide level in serum have been developed. Both non-chromatographic and chromatographic analytical methods have been utilized in the literature for the ETH plasma level determination in the biological fluids (Jenner et al., 1981; Peloquin et al., 1991; Auclair et al., 2001; Conte et al., 2001; Walash et al., 2004; Hemanth Kumar et al., 2014). Published analytical methods used for the determination of ETH plasma concentrations included titrimetric method, fluorimetry, spectrophotometry, electro-analysis (Walash et al., 2004) and mass spectrometry (Conte et al., 2000). Several high performance liquid chromatography (HPLC) assay methods for the determination of ETH plasma concentrations have been reported (Auclair et al., 2001; Zhu et al., 2002; Ahmad et al., 2009; Hemanth Kumar et al., 2014).

A fluorimetric analytical method for the determination of ethionamide in biological fluids was developed and validated. This method utilizes the use of native fluorescence of eosin in acidic medium because of formation of complexes (Walash et al., 2004). In this method, pH study is extremely essential because pH affects ionic bonding ionization and eosin is a pH sensitive fluorophore. Fluorimetric assay is linear over concentration range of 1 to 8, ug/mL, with 0.08, ug/mL the lower limit of detection. The percentage recoveries for spiked plasma were 0.824, 1.58 and 1.15%, respectively (Walash et al., 2004).

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In 2013, a study done by Bhanushali and his team employed a reversed-phase ion-pair chromatographic method for the determination of ETH level in the plasma. They reported that the calibration curve was linear from the concentration range of 0.1 to 3.0, ug/ml, through a correlation coefficient of $r^2>0.999$. The accuracy for ETH ranged from 94 to 106% for all the quality control (QC). The method was precise with relative standard deviation of less than 2% at all QC levels. The lower limits of detection and lower limits of quantitation were 0.015 and 0.05, ug/ml correspondingly (Bhanushali et al., 2013).

Hemanth Kumar et al. (2014) utilized HPLC analytical method for the determination of ETH level in human plasma for four patients. The HPLC assay was linear from 0.25 to 10.0, µg/ml range, and was specific for the determination of ETH level. Lower limits of detection (LOD) and lower limits of quantitation (LOQ) were 0.05 and 0.16, ug/ml correspondingly. The standard deviation for inter-day and intra-day assays was lesser than 10%. The retention time of 4.3 minutes reduces the total chromatographic run time, thus allowing the processing of larger number of samples. This analytical method consistently removed interfering components from serum, producing ETH recovery which ranged from 86 to 96 %. The total ethionamide recovery from plasma was 91% (Kumar et al., 2014).

Moreover, Ahmad and colleagues reported that the concentration of ETH and their ratios of peak height were linear from 0.1 to 4, ug/ml concentration range using HPLC in 12 healthy volunteers. The within-day precision was 0.42 to 5.26% coefficient of variation, whilst the overall precision was 0.65 to 3.89% during sample validation process. The lower limit of detection (LOD) was 0.01 ug/ml of ETH per ml of serum. The HPLC assay specificity for ETH was evaluated by testing spiked samples. The run time was 6 minutes and absolute recovery of ETH was 86% (Ahmad et al., 2009).

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Zhu et al. (2002) developed a validated HPLC method of analysis for the determination of ethionamide concentrations following multiple oral dosing in 55 patients suffering from tuberculosis. They observed that the ETH linear plasma curves for ETH were from 0.5 to 20 µg/mL range. Overall validation precision was from 0.8 to 4.7% range, while the within-day precision was 0.4 to 6.8% coefficient of variation (% CV) for the validation of the samples' quality control. The lower limit of detection was 0.2 µg/mL, assessed by ratio of peak height to noise ($\geq$ 5:1). The total ethionamide recovery from plasma was 90.7% (Zhu et al., 2002).

In addition, Auclair et al. (2001) reported linear curves for plasma ETH from 0.2 to 10 µg/ml range using HPLC assay in 12 healthy adults. The complete ethionamide recovery from plasma was 91%. No interferences with the measurement of ETH were experienced. Overall precision during validation was 0.81 to 4.66%, whereas the within-day precision (CV) for samples quality control (QC) validation process was 0.36 to 6.39% (Auclair et al., 2001).

Validation of LC-MS/MS analytical method for simultaneous quantitation of ETH in plasma was developed by Deshpande and colleagues for four healthy human volunteers (Deshpande et al., 2011). They reported that a linear response was produced at concentration range of 0.0257 to 6.12 µg/mL, with the precision (% coefficient of variation) values of 0.87–2.75. The retention time was 2.50 minutes, total chromatographic run time was observed at 3.5 minutes and calibration curve had correlation coefficient (*r*) of 0.99 or better (Deshpande et al., 2011).

Conte et al. (2000) were able to develop and validate a sensitive new column chromatographic mass spectrometric technique (HPLC–MS–MS) for ethionamide determination in plasma in 40 adult volunteers. Conte and his team observed the limit of detection (LOD) at 0.05 µg/ml, a 3.2 minutes for run time and accuracy range for the determination of ETH plasma level was 28.0 to 5.0%. The mean coefficients of

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variation were 9.66 to 62.67% and ranges of the assay for inter-day and intra-day determinations as 5.0 to 12.47% (Conte et al., 2000).

In this study, LC-MS method was employed to determine ETH plasma levels in the samples obtained from forty-two MDR-TB patients, following oral administration of 500-750 mg of ETH tablet. Ethionamide calibration curve constructed by plotting the responses against the corresponding concentrations, displayed linearity over the range of 0.1-10 μg/ml. These values were in accordance with the report of Auclair et al. (2001), Zhu et al. (2002) and Kumar et al. (2014). Auclair et al. (2001) found linear range of the standard curves for serum ETH from 0.2 to 10 μg/ml using HPLC assay. The limit of detection (LOD) of 0.01 μg/ml and limits of quantitation (LOQ) of 0.15 μg/ml found in our study, were similar to the work done by Ahmad et al. (2009), Jenner et al. (1984) and Hemanth Kumar et al. (2014). No interfering peaks were observed from the components of plasma with ethionamide peak, detected at 2.23 minutes retention time. The retention time generated in our study was relatively low compared to values reported by Deshpande et al. (2011) and Hemanth Kumar et al. (2014). The average recovery of ethionamide ranged from 104%±2.3% to 128%±11.2%. This is moderately high when compared with the results cited by Ahmad et al. (2009), whose absolute recovery of ETH was 86%, and Zhu et al. (2002) whose absolute recovery of ETH from serum was 90.7%.

The LC-MS analytical method has numerous advantages: this instrumentation is highly sophisticated; it uses gradient mobile phases or multifaceted mobile phase and solid phase extraction cartridges. It is a specific, rapid, sensitive and simple method of extraction for the determination of the plasma ETH levels. No interfering peaks were observed from the blood samples, and peak of ethionamide was detected at 2.23 minutes retention time. This reduces run time, so they can be processes for larger number of samples and pre-treatment of samples is simple without loss of any analyte. Therefore, the specificity and sensitivity of the LC-MS analytical method

was sufficient for the precise determination of the pharmacokinetics of the plasma levels of ETH in humans.

The sample size, forty-two (23 males and 19 females) patients, was adequate to explain ethionamide pharmacokinetic parameters in MDR-TB patients. The number of patients that participated in this study was higher than the sample size of some of the previous studies. Twelve males participated in the study performed by Ahmad et al. (2009) titled "In-vitro release and pharmacokinetics of ETH drug in healthy male volunteers". Likewise, 12 patients (6 males and 6 females), participated in a research study on ETH pharmacokinetics administered with antacids, food or orange juice, or under fasting conditions (Auclair et al., 2001). A sample size of 31 patients was observed in pharmacokinetics studies of ethionamide in children (Thee et al., 2011).

The age and sex were well balanced across all the study subgroups. All the patients that enrolled in this study were within the active age group. Hence, the median age [33 (18-52) years] and median weight [53.4 (41-98) kg] were in comparison to the previous published studies. A previous study done by Ahmad et al. (2009) showed that the volunteers' ages ranged from 21- 30 years and their body weights ranged from 56-70kg. Also, Zhu et al. (2002) had median age of [36.2 (12.2-57.6) years] and median weight of [65 (42-71) kg].

Ethionamide pharmacokinetics

Table 4.9 above shows ethionamide (ETH) pharmacokinetic parameters in patients who had been on treatment and the pharmacokinetic (PK) parameters were determined using the non-compartmental analytical method. The PK parameters for all patients were determined and expressed as median and range. ETH area under the concentration (AUC) vs. time curve was linearly increased, with each increasing oral

doses from 500-750 mg signifying that patients' ETH dose was proportional to its exposure in the blood.

The absorption rate constant (Ka) is an essential pharmacokinetic parameter. The rate and extent of gastrointestinal (GIT) ETH absorption is complete and rapid following oral administration and greater than 90% of the dose given was absorbed from GIT tract (Ahmad et al., 2009). Although, some studies cited pharmacokinetic parameters of ETH in healthy subjects and TB patients, studies on the ethionamide PK in adult patients with multi-drug resistant tuberculosis (MDR-TB) have not been done, particularly in South Africa. In this study, the absorption rate constant (Ka) was approximately 0.71 hr^{-1}, which is similar to the 0.40 hr^{-1} reported in TB patients and 0.69 hr^{-1} in healthy volunteers (Zhu et al., 2002). Our patients had a rapid rate of absorption with serum levels of the drug peaking approximately at 2.23 hrs after ETH administration. None of our patients had severe liver disease, or were taking other medications that could have altered ETH absorption. They were subjected to fasting prior to taking their medications. Thus, the timing of the food intake in connection to the dosing of ETH was controlled in our patients. ETH may be given on an empty stomach, or with antacids, orange juice, or food, since no statistical differences were found in the AUC and C_{max} between the four treatments (Auclair et al., 2001). Also, in comparison to our population pattern, reduced absorption was observed in 4.76% of our patients. This appears to be more recurrent in HIV (+) patients than HIV (-) patients (Peloquin, 1993). Likewise, the presence of other medical problems such as diabetes mellitus and severe liver disease (which causes increase risk of side effects) may affect the rate of absorption of anti-tuberculosis drugs.

The absorption rate constant (Ka) influenced the AUC, T_{max} and C_{max}. In practice, the shorter the T_{max}, the faster the rate of absorption, and C_{max} increases with increase in Ka value. Subsequent to oral ethionamide administration to humans, the time to reach maximum serum concentrations, T_{max} was 2.23 hrs, indicating rapid and complete absorption by our subjects. This was similar to the value reported by Auclair et al.

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(2001), who studied the effect of a dose of 500-mg of ETH in twelve healthy subjects under different conditions. The study revealed that T_{max} was somewhat prolonged subsequent to food or antacid administration (2.3 to 2.6 hrs), in comparison to administration on an empty stomach or with juice (1.7 to 1.9 hrs). There is a possibility that food intake might have influenced absorption of ethionamide and subsequently, affect its T_{max}. But the influence of foods on the ethionamide bioavailability is insignificant (Arbex et al., 2010). Nevertheless, pharmacokinetics study by Zhu et al. (2002), who studied multiple oral doses (from 250 to 1000 mg) of ETH in fifty-five patients with tuberculosis, failed to detect T_{max} value for patients in group B that had blood taken at 0.5 and 12 hours post-dosing. It was concluded that sparse data could not permit for clear evaluation of T_{max} in that study (Zhu et al., 2002). Although, T_{max} estimated was about 1.50 hrs for healthy volunteers and approximately 2 hrs for TB patients in their study. This was in line with the T_{max} value obtained in our study.

Moreover, C_{max} must be higher than the minimum inhibitory concentration (MIC) to have 100% therapeutic response. Therefore, the standard C_{max} range is 1– 5 µg/ml, that is at or over the standard minimum inhibitory concentration (MIC) (Auclair et al., 2001). In clinical setting, there was unavailability of individual MIC values for *M. tuberculosis*, so the reported median broth MIC of ETH against susceptible *M. tuberculosis* (1.0 mg/ml) was used (Berning and Peloquin, 1999). Sensitive strains of *M. Tuberculosis* MICs, tested with the BACTEC method was reported to be between 0.25-0.50 mg/ml (Rastogi et al., 1996) and 0.3-1.2 mg/ml (Heifets et al., 1991). For medical purposes, suggested breakpoints for susceptible, moderately susceptible, moderately resistant, and resistant strains of TB are, <1.25, 2.5, 5.0, and >5.0 mg/ml, respectively (Salfinger and Heifets, 1988). In this study, the maximum plasma concentration (C_{max}) was 1.53 µg/ml at 2.23 hours. Hence, the C_{max} obtained in our study possesses a good therapeutic outcome because it was above the MIC for susceptible *M. Tuberculosis*. Surprisingly, no information is available in literature about the C_{max} of a drug after administration to MDR-TB patients, thereby, making it

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impossible for comparison with previous data. Although, a study done by McEvoy (2009) reported that after oral administration of 250 mg of ETH as a film-coated or sugar-coated tablet, both were readily absorbed from the GIT. The sugar-coated tablets generated a peak plasma level of approximately 1.5 µg/ml after 1.5 hrs, whereas the film-coated tablets produced a peak plasma level of 2.16 µg/ml after 1 hour. A study showed 2.2 µg/ml peak plasma concentrations at about 1.8 hrs after a 500 mg single oral dose of ETH (Micromedex, 2006). Zhu et al. (2002) showed C_{max} of 1.97 µg/ml at 1.50 hrs in healthy subjects and C_{max} of 1.35 µg/ml at 2 hours in TB patients after oral administration of ETH.

In this study, pre-dose ethionamide plasma concentrations were below the detection limit for 79% of the subjects at base line level. Similarly, samples drawn at 12 hrs, 16 hrs and 24 hrs post-dosing did not exhibit any detectable ETH concentrations as indicated in Tables 4.6 and 4.7. Ethionamide concentrations were quantifiable up to 8 hours post-dosing, after which decline in serum concentrations occurred. With the combination of all subjects, plasma levels at 2 hours were considerably greater compared to other levels from zero to 24 hours, consistent with data from other preceding studies that have illustrated the ETH kinetics in the plasma (Conte et al., 2000). Thus, C_{max} attained was highest at 2 hrs, indicating that samples obtained at 2 hours post-dosing are possible to capture the maximal level than samples taken at other times. Our study showed that drug concentrations in 92.8% of patients were below the expected MIC (10.0 to 40.0 mg/ml) for *M. tuberculosis* in MDR-TB (Mpagama et al., 2013).

Area under the curve (AUC) which indicates the whole amount of drug reaching the systemic circulation is directly comparative to the drug dose. In adults, increase in dose of the drug increases AUC. Thus, in clinical setting, dose increases should produce higher serum concentrations. Ethionamide is speedily and extensively distributed all over the body, resulting in a higher volume of distribution in children and adults (WHO, 2009). C_{max}, AUC_{0-24}, and $AUC_{0-\infty}$ were performed to determine

the bioavailability. The median values of AUC_{0-24} and $AUC_{0-\infty}$ were calculated to be 3.55 µg.h/ml and 3.91 µg.h/ml respectively in this study. AUC values found in our study, are higher than most of the values shown in the previous studies. Auclair et al. (2001) in healthy volunteers observed the value of AUC extrapolated to infinity to be 10.0 µg.h/ml both with food and in fasting condition. Study done by Zhu et al. (2002) showed 2.80 µg.h/ml for AUC_{0-10} in TB patients, 3.95 µg.h/ml for AUC_{0-12} in TB patients and 8.00 µg.h/ml for AUC_{0-48} in healthy volunteers. Thus, lower AUC estimates were observed in patients with tuberculosis compared to their healthy counterparts. It can be concluded that bioavailability was found to be lower in patients suffering from tuberculosis because they absorbed ETH gradually than the healthy subjects (Zhu et al. 2002). The observed decrease in AUC of our patient could be as a result of first-pass metabolism that might have been undergone by ethionamide. The low C_{max} and low AUC signify a decrease in the rate and extent of ethionamide absorption in our patients.

Elimination rate constant (Ke) has an effect on AUC, T_{max} and C_{max}. Increase in the elimination rate, corresponds to a decrease in values of AUC, $T_{max,}$ and $C_{max,}$ and vice versa. In this study, the values of Ke were constant, while the elimination half-life (T½) was 0.30 hr^{-1} and 2.31 hrs, respectively. The values reported by Zhu et al. (2001) for Ke and T½ was 0.43 hr^{-1} and 1.63 hrs for TB patients and 0.36 hr^{-1} and 1.94 hrs for healthy volunteers. The ethionamide T½ was estimated to be below 3 hrs (Jenner et al., 1984; Peloquin et al., 1991; Auclair et al., 2001). These values were in line with the present findings.

ETH is metabolized hepatically and excreted in urine; about 1-5% is excreted as unaltered active drug and the remains being excreted as metabolites (Arbex et al., 2010). Clearance (CL) is one of the functional parameter for assessment of removal of drug from the body. The quicker the CL, the lesser will be the volume of distribution (Vd) and elimination half-life ($T_{1/2}$). A decrease in $T_{1/2}$ results to a corresponding decrease in accumulation of drugs in the body, and the clearance will

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be increased. The oral clearance value observed in this study was 63.63 L/hr. Auclair et al. (2001) showed oral clearance value of 64.5 L/hr in healthy subjects under fasting conditions. The values for oral clearance are, to some extent, above the 51.2 L/hr that was reported previously (Peloquin et al., 1993). A study by Ahmad et al. (2009) has reported a clearance value of 32.59 L/hr after oral administration of ethionamide in healthy volunteers. Thus, slight differences in clearance value were observed in this study, as compared to the rate of clearance in previously reported studies. In this study, rapid ETH removal from the body could be the reason why samples drawn after 8 to 12 hours did not display measurable ETH levels for most of our study subjects during post dosing. Similarly, pre-dosing ETH levels in plasma were beneath the detection limit in most of our study subjects. Another study by Jenner and colleague reported a lesser value of about 1.1 L/Kg subsequent to the administration of ethionamide intravenously (Jenner and Smith, 1987). Hence, bioavailability is the main issue that accounts for the large values obtained for PK parameters after oral administration of ETH (Auclair et al., 2001).

The volume of distribution is an important parameter used to determine the loading dose. Volume of distribution per body weight reported in this study was 4.24 L/kg. Volume of distribution estimated by Zhu et al. (2002) was 5.07 L/kg for patients with TB and 2.38 L/kg for healthy subjects, whereas Auclair et al. (2001) reported 2.4 L/kg in healthy subjects under fasting conditions. The volume of distribution relates plasma level to the quantity of drug in the body (Ahmad et al., 2009). These estimates are relatively similar to the reported values in this present study. Thus, ETH is not significantly bound to circulating plasma proteins (30%) and the volume of distribution is large enough for ETH to penetrate the brain barrier to achieve concentrations in the cerebral-spinal fluid equivalent to that in the plasma.

The mean residence time (MRT) is an essential PK parameter that gives an account of the residence time of individual molecules in the body. MRT estimation in this study

was 3.69 hrs, whereas Ahmad et al. (2009) estimated MRT value to be 2.24 hrs. This was in line with our recent findings.

Ethionamide pharmacokinetics in HIV-negative and HIV-positive patients

No studies of ETH pharmacokinetics in MDR-TB patients with and without HIV-infection have been described in the literature. We were able to effectively establish and calculate the required pharmacokinetic parameters in both study groups by using the plasma concentration values found in our analysis. In our study, comparison of PK parameters of ETH between HIV (+) and HIV (-) patients showed no differences in AUC, Cmax, Tmax, and half-life. There was no difference in exposure to ETH. Oral bioavailability and metabolism were similar in both patients. Mean residence times, $T_{1/2}$ and total clearance was similar in both populations. The results generated in this study (Table 4.9), showed that there were no statistical significant differences in the PK parameters between HIV (-) and HIV (+) patients with MDR-TB.
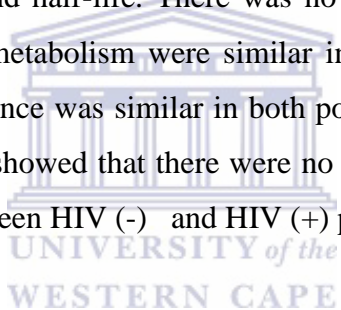
Effects of HIV-infection on pharmacokinetics of ethionamide

There is limited information which addressed the consequence of HIV infection on the ETH pharmacokinetics in sub-Saharan Africa. The extent and rate of absorption of ETH as characterized by the AUC, C_{max} and T_{max}, was alike in HIV (-) and HIV (+) patients as indicated in Table 4.9. HIV-positive patients demonstrated C_{max} value of 3.7 mcg/ml. This was similar to those cited in the literature on non-HIV-infected patients, where standard C_{max} range is 1–5 μg/ml, that is, at or above the standard minimum inhibitory concentration (0.3 to 1.2 mg/ml) (Heifets et al., 1991; Zhu et al., 2002). In this study, area under the concentration from zero hour to infinity (7.9 μg/ml) and AUC_{0-24} (7.7 μg/ml) was comparable to previous pharmacokinetic studies using identical dose.

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In this study, HIV (+) patients was not severely affected by HIV infection that could have altered the plasma concentrations of the ETH taken by them. Sahai et al. (1997) who have conducted extensive analyses on the PK studies in HIV-infected patients from North America suggested that malabsorption of anti-mycobacteria drugs increases as HIV disease becomes more advanced (as determined by lower CD4 cell counts and the presence of diarrhoea). Most of our HIV (+) patients had an excellent immunological and virological profile because the CD4 count level of all the HIV (+) patients was >100 cells/mm^3 and the viral load of 70.5% of HIV (+) patients was less than 60 copies/ml (Tables 4.4 and 4.5). There were no obvious side effects or drug interactions of ARV or MDR-TB drugs that could have affected ETH absorption rate in our HIV (+) patients. For instance, the side effect of protease inhibitors (e.g. diarrhoea) could have reduced the bioavailability of ETH; and the side effect of ETH (e.g. GIT disturbance) could have affected the ETH absorption rate and drug bioavailability (Weyer, 2005).
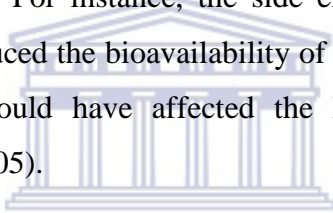
Gurumurthy et al. (2004a) reported that drug malabsorption is more likely to occur when concurrent GIT infection/diarrhoea or advanced immunodeficiency with or without diarrhoea is present. On the contrary, none of our HIV (+) patients had GIT infection (e.g. diarrhoea, vomiting etc.) that could have influenced ETH absorption rate. In addition, all HIV (+) patients in our study were on ARV therapy and were also receiving co-trimoxazole for pneumocystis jiroveci pneumonia (PJP) prophylaxis, resulting in less immune-suppression and less associated opportunistic infections. Another study of adult TB patients in Nairobi, Kenya, produced some interesting findings. This study was performed where as many as 90% of patients with AIDS were reported to have GIT dysfunction (Choudhri et al., 1997). The PK characteristics of anti-TB drugs, according to the presence or absence of HIV infection and diarrhoea, were studied. Their findings showed that neither diarrhoea nor HIV-infection was responsible for the inter-patient differences experienced in C_{max} or AUC. Their mean C_{max} was not significantly different for HIV-infected

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patients and non-HIV-infected patients or for those with or without diarrhoea. In addition, no significant association between the presence of HIV or diarrhoea and PK properties was seen for any of the drugs used.

Our findings are in support of the views of Sahai et al. (1996) and Choudhri et al. (1997) which reported that neither diarrhoea nor HIV-infection accounted for the inter-patient differences experienced in C_{max} or AUC.

A decrease in the volume of distribution of ETH distribution (Vd: 1.99 L/kg) in our study, may be connected with the short half-life (2.88 hrs), observed. A drug half-life is an indication of the extent of the drug's distribution or elimination and is dependent on both Vd and CL (Mehvar, 2004).
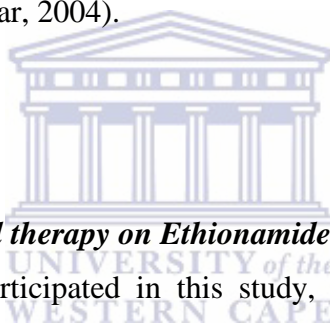
Influence of antiretroviral therapy on Ethionamide pharmacokinetics
HIV (+) patients who participated in this study, had been taking anti-TB drugs, together with antiretroviral (ARV) therapy comprising lamivudine, efavirenz and stavudine at least two weeks before the study. In this present study, ARVs administered to HIV-infected patients did not affect metabolism, distribution or renal elimination of ETH.

Research done by Coyne et al. (2009) on the pharmacology of 2nd-line anti-TB drugs and interactions with ARV agents, found that no studies had been performed for lamivudine and stavudine (a NRTI), and their interactions with ethionamide are complicated to forecast. Nevertheless, they recommended the use of standard ethionamide doses. One of the consequences of pharmacokinetic interaction such as hepatotoxicity was predicted when efavirenz (a NNRTI) was used in conjunction with ethionamide, but no studies has been done to verify this (Coyne et al., 2009). Hence, Coyne and colleagues recommended that efavirenz should be avoided if possible or if

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used, the concentrations of both efavirenz and ethionamide should be adjusted appropriately, and psychiatric morbidity with efavirenz should be closely monitored.

During the course of this research, patients' treatments were monitored in a designated hospital for potential drug-drug interactions and drug-adverse effects during concomitant administration of anti-TB drugs with ARVs to ensure optimal therapeutic outcome. None of our patients have serious underlying co-diseases (e.g. severe liver problem, cancer etc.) that would have triggered serious interactions with co-administered drugs. Therefore, PK drug interactions were not experienced among our study population during the period of the study and ETH PK as not influenced by ARVs taken by HIV (+) patients.
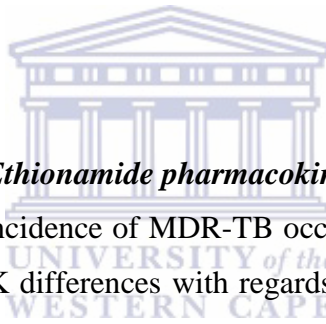
Effect of age and sex on Ethionamide pharmacokinetics

In the present study, the incidence of MDR-TB occurs more in the active population group (18 to 54 years). PK differences with regards to increase in age may decrease or increase the differences in drug response. Previous studies reported that considerable age-related influences, including reduction in gastric emptying, gastric acid secretion and small intestine absorptive capacity (Evans et al., 1981; Corazza et al., 1986). Perhaps, because of the consequences of the disease states, these findings have not been confirmed in healthy subjects (Gainsborough et al., 1993; Husebye et al., 1992).

In this study, ETH was rapidly absorbed after drug administration in all patients, regardless of their age. Thus, differences in age did not affect ethionamide absorption rate in this study. PK studies on the consequences of age on the absorption of drug have presented contradictory outcomes. Generally, absorption of a drug does not change significantly with age, but changes are found in the rate of absorption, than in the degree of absorption. Although a number of research has not revealed significant

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age-related differences in the absorption rates for different drugs (Gainsborough et al., 1993; Greenblatt et al., 2002). Through active transport mechanisms, the levodopa absorption is increased, whereas absorption of calcium and vitamin B12 is reduced (Blechman and Gelb, 1999; Mangoni and Jackson, 2004). A number of discrepancies obtained from the results of these studies may be as a result of dissimilar techniques of evaluating the absorption of a drug.

Ageing is linked to a decrease in the first-pass metabolism of a drug because of blood flow and a liver mass reduction (Anantharaju et al., 2002). A prodrug, e.g. ETH, needs to be activated in the liver, as a result, advancing age might decrease or slow its first-pass activation (Hilmer et al., 1991). Furthermore, due to age-related changes in body composition, non-polar compounds have a tendency to be lipid soluble and so their volume of distribution increases with age. The major consequence of the increased volumes of distribution is a half-life prolongation. Thus, increased volume of distribution and half-life were observed after administration of ETH in all our subjects.
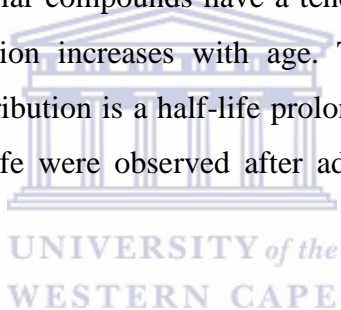
Hepatic drug clearance is dependent on the liver's ability to remove the drug from the blood and the quantity of liver blood flow. Therefore, clearance via the liver is dependent on the extraction ratio and the blood flow. The extraction ratio depends on the liver metabolizing capacity. In the elderly, reduction in renal function, predominantly glomerular filtration rate, influences the clearance of numerous drugs (Schwartz, 2003). Nevertheless, latest study has queried the significance of age-related reduction in kidney function as among the factors that affect PK. Older age is predisposed to reducing the clearance of a drug (on average), and likely expected to produce the least elimination rates of drugs (Schwartz, 2003).

Therefore, important PK changes, including volume of distribution of lipid soluble drugs increase and elimination half-life prolongation, decrease in hepatic and renal clearance, occur with advancing age. Limited studies have been done on the effect of

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age on PKs of ETH. So, supplementary studies are required for a proper understanding of the influence of age on ETH PKs.

Biological dissimilarities may exist among men and women that could lead to differences in drug responses. In our study, sex differences did not influence ETH PKs (i.e. drug absorption, distribution, metabolism, and elimination). ETH was rapidly absorbed after administration of drug in both women and men in our study. Huang et al. (2014) cited that complete absorption of drug was not significantly influenced by sex, but rates of absorption may be a bit slower in women. They concluded that bioavailability subsequent to oral drug administration, for CYP3A substrates specifically, may be fairly elevated in women than in men. In addition, Schwartz (2003) cited that biological dissimilarities existed among women and men that could give rise to differences in reactions to drug absorption, bioavailability and protein binding. They reported that part of the differences in sex could be as a result of differences in patient's body size because typically, men are larger than women. Auclair et al. (2001) observed comparatively high inter-individual differences in AUC, T_{max} and C_{max} of ETH and proposed that ETH first-pass metabolism and extensive range of body weights of their subjects could contribute to the variability. Auclair and his team concluded that, although body weights were not significant statistically in their analyses, they could exclude age and sex differences in the pharmacokinetics which might have added to the variability (Auclair et al., 2001).

On the contrary, absorption rate and extent of drug absorption are drug-specific; ETH was not included in the list of drugs whose absorptions were affected by sex differences in drug absorption (Fletcher, 1994; Soldin and Mattison, 2009). In 2000, Conte and his team published a study on the effects of gender on plasma concentrations of intrapulmonary ethionamide, confirming that ETH plasma levels were not influenced by gender. Thus, we found no sex influence on ETH rate of absorption. Our outcomes supported the observations of Conte et al. (2000) and

Soldin and Mattison, (2009) which reported that ETH plasma concentrations were not affected by sex.

ETH drug metabolism occurs predominantly in the liver, and it was extensively metabolised by both sexes in our study. Sex-related differences have been shown in the PKs of *cytochrome P450* (CYP450), with a higher activity in females for *cytochrome P3A4* (CYP3A4). However, even if there are sex-differences in PKs of drugs, only some drugs have shown significantly higher plasma concentrations in women (Soldin and Mattison, 2009). The influence of sex on ETH levels and its PK effects have not been studied extensively.

Differences in body size result in bigger volumes of distribution and quicker clearance of various drugs in men compared to women (Schwartz 2003). Conversely, in this present study, a larger volume of distribution was observed in females, while faster clearance was observed in males. Our findings are in line with that of Harris et al. (1995), who reported that drugs metabolized by CYP3A4 are extensively cleared by females. Individual patients' variability, including underlying diseases and other prescribed medications, might be reasons for this outcome. These findings did not affect the rate at which ETH was distributed in the body and its removal from the body.

Based on the PKs, no differences were observed in ETH Cmax, AUC, Vd and clearance, due to age or sex sufficient to warrant dosage adjustment in this study. Hence, further studies will be needed to confirm the consequence of age and sex on ETH PKs.

Effect of liver function on Ethionamide elimination
Impaired liver function greatly increases the risks of adverse drug effects and can result to substantial changes in the pharmacokinetics of various drugs metabolized

through the liver. According to Coyne et al. (2009), ETH extensive metabolism occurs mainly in the liver and the process of elimination decreases gradually with hepatic injury. In our study, ETH was predominantly metabolized hepatically and none of our patients was suffering from hepatic dysfunction. The liver enzymes (e.g. ALT, AST, GGT etc) levels in HIV (+) and HIV (-) patients were within the normal range for all patients. Hepatic function did not interfere with ETH metabolism and elimination. This indicates that ETH PK parameters were not influenced by liver dysfunction.

Chronic liver impairment affects all parameters of PK, because basically, drugs are metabolized by the liver: it is susceptible to drug toxicity. Most of the anti-TB drugs (isoniazid, pyrazinamide and ethionamide) used for MDR-TB treatment causes hepatotoxicity (Arbex et al., 2010). Arbex and his team showed that toxic hepatitis occurred in roughly 4.3% of the patients, particularly in patients with a liver disease or history of alcoholism. Their study highlighted the occurrence of liver changes about five months following the start of treatment with the drugs, and it is uncertain whether the changes are because of hypersensitivity or direct toxicity. Although one patient in our study was co-infected with hepatitis B virus and two patients were occasional alcohol drinkers, their liver enzymes (ALT, AST etc) were within the normal ranges, and the elimination rate was not affected. Surprisingly, both HIV (+) and HIV (-) patients in our study population had normal liver function.

Severe liver problem is one of the serious side effects of ARV therapy (lamivudine, efavirenz and stavudine) administered to the HIV-infected patients in our study population. Due to the fact that patients' therapy was closely monitored and reviewed at intervals in the hospital, liver toxicity was not an issue for the patients. Thus, PK parameters of ETH were not influenced by liver dysfunction.

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Effect of renal function on Ethionamide elimination

A statistical significant difference (p=0.03) was observed in the kidney function among the HIV (-) and HIV (+) patients, but this difference did not affect ETH PKs. For a drug that is mainly eradicated through mechanisms of renal excretory, injured renal function can change its pharmacodynamics and pharmacokinetics to a level that will warrant modification of the dosage regimen from that used in patients with normal kidney function (CDER and CBER, 1998). On the contrary, ETH is mainly eliminated via hepatic elimination, and renal impairment does not affect ETH elimination. Below 1% of the ETH amount emerged as unchanged drug in urine, while the remaining portion is removed as inactive metabolites in urine (Coyne et al., 2009). As indicated in Table 4.8, the median elimination half-life in HIV (-) patients was 2.3 hrs and 2.8 hrs in HIV (+) patients. Our findings were in line with the research outcomes of Conte et al. (2000) that stated that in humans, elimination half-life is about 2 to 3 hrs after ETH oral administration. Likewise, Zhu and colleagues observed that ETH half-life in TB patients after oral administration was at 1.63 hrs.

Our patients (HIV positive and HIV negative) were not severely affected by kidney dysfunction, which could influence ETH PKs or cause ETH dose adjustment. More than half of the study population (60%) had normal renal function and a lesser number (40%) had impaired renal function. As described in Table 4.3 above, greater number of HIV (-) patients had normal kidney function (that is, 72%) in comparison to the HIV (+) counterpart. Only 4.7% of the HIV (+) patients had moderate kidney malfunction. Estimated CrCl in HIV (-) patients was within the usual range and moderately decreased in our HIV (+) counterpart, with no interference in absorption of ETH, liver metabolism, protein binding, and distribution of drug, as indicated in Table 4.9. The renal clearance of ETH was not significantly different (p=0.77) for both HIV (+) and HIV (-) patients in our study. ETH PK parameter estimates in respect of CL and $T_{1/2}$ were independent of creatinine clearance (Zhu et al., 2002). None of our patients had severe kidney malfunction or end-stage kidney disease that would have warranted the dosage regimen to be changed from that used in patients

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with normal renal function (CDER and CBER, 1998). Hence, we found no effect of renal function on ETH Pk in MDR-TB patients. Our findings supported the views of CDER and CBER (1998) and Zhu et al. (2002), who reported that ETH PK parameter estimates were independent of creatinine clearance.

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Therapeutic implications

In South Africa, ethionamide is administered at a dose of 500 to 1000 mg orally per body weight once daily (DOH, 2013). However, in May 2016, WHO updated the drug-resistant TB treatment guidelines and incorporated a recommendation on the replacement of ethionamide with prothionamide, to decrease the toxicity of patients' drug therapy (WHO, 2016). The outcome of our research showed that most MDR-TB treatment was not producing the desired therapeutic concentrations and steady state concentration was not attained. This was observed when most of our study population did not show trough plasma concentrations at baseline, because previous ethionamide concentration had been eliminated completely from the body. This was confirmed by the short T1/2 (2.30 hrs) and short MRT (3.69 hrs). ETH plasma levels were below detectable levels after 8 to 12 hours in most patients. However, eight HIV infected patients showed detectable plasma concentration of ethionamide, whereas plasma levels were displayed at 16 hrs and 24 hrs in four patients. Hence, ETH steady state plasma concentrations were not reached. The resultant sub-therapeutic concentration could lead to low effect and increased resistance.
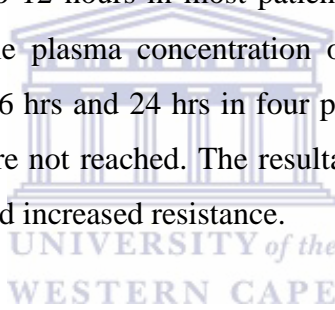
Auclair et al. (2001) acknowledged the significance of determining the circumstances that could promote or impair the attainment of satisfactory plasma concentrations of ETH. They stated that low ethionamide plasma levels may stop *Mycobacterium tuberculosis* total eradication, resulting in the development of drug resistance and therapeutic failure. In addition, in a study done by Ahmed et al. (2012), factors associated with reduced anti-tuberculosis drug serum concentrations in patients with HIV-TB co-infection highlighted that HIV-infected patients receiving TB treatment experienced reduced anti-tuberculosis drug serum concentrations. They suggested that further study was necessary to evaluate the role of higher initial doses of anti-TB drugs in this population.

Based on the result of the current study, the frequency of administration of ETH should be increased from once to twice daily in order to maintain therapeutic levels

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for 24 hours. Increasing the dose may lead to ETH toxic concentration. Zhu et al. (2002) stated that although in a clinical setting, an increase in ethionamide dose would yield elevated plasma concentrations, but care must be applied, because higher doses of ethionamide could increase gastrointestinal problems such as nausea and vomiting. Therefore, from the outcomes of our research findings, we suggest that increasing the duration of exposure of ethionamide is a preferred option, rather than increasing the dose. In doing so, ethionamide steady state concentration will be achieved.

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LIMITATIONS OF THE PRESENT STUDY

This study provides information about the pharmacokinetics of ETH in patients infected with MDR-TB; but, patients that participated in this study were relatively small in number. Unequal distribution of patients was observed in the HIV (-) and HIV (+) groups for comparison (25 HIV (-) and17 HIV (+)).

Moreover, few patients had renal dysfunction and none of the study population is suffering from liver dysfunction.

There was a small number of participants in each subgroup of HIV-infected patients with regard to CD4 counts, viral load and ART. Thus, the finding of this study on the effect of ARVs and HIV-infection on ethionamide pharmacokinetics is limited.

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CHAPTER SIX
CONCLUSIONS AND RECOMMENDATIONS

6.1. CONCLUSIONS

The aims of this study were:

- To determine ethionamide plasma concentration using the LC-MS method.
- To evaluate and compare the pharmacokinetics of ethionamide in MDR-TB patients with and without HIV infection.
- To assess the effect of lamivudine, stavudine and efavirenz on ethionamide pharmacokinetics.
- To find out whether age and sex do have any influence on ethionamide PK.
- To find out if there is any association between renal dysfunction and ethionamide elimination.

All our study objectives were achieved. Our study was capable to describe and evaluate ETH PKs in patients with MDR-TB and MDR-TB co-infected with HIV. This was successfully achieved by developing and validating an LC-MS analytical method for the determination of ETH concentrations in plasma. The LC-MS assay method was simple, highly sensitive, specific, and its chromatogram produced good-resolved ethionamide peak with excellent precision. This rapid, reproducible and accurate method makes this LC-MS analysis extremely appropriate for PK determination of ethionamide plasma concentrations in patients with MDR-TB. Therefore, we accept the null hypothesis. No evidence of significant difference was observed among the HIV (+) and HIV (-) patients with regard to any of the pharmacokinetic parameters studied.

Ethionamide PK is similar in MDR-TB patients with and without HIV-infection. HIV-infection does not affect ethionamide pharmacokinetic parameters. ETH steady state plasma concentrations were not attained.

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As mentioned above, owing to the little sample size and unequal distribution of patients in the different study subgroups, we could not definitely confirm that HIV infection does not influence ethionamide pharmacokinetics. Thus, we reject our first experimental hypothesis and accept the null hypothesis in this case. We found no proof of a statistically significant variation among the HIV (+) and HIV (-) groups.

According to the result from this study, antiretroviral therapy does not affect ETH PKs. ARV therapy, comprising lamivudine, efavirenz and stavudine, administered to HIV- infected patients did not affect metabolism, distribution and renal elimination of ETH. There were no obvious side effects or interactions of ARV or MDR-TB drug that could have affected the rate of ETH absorption in the patients. Therefore, we accept the null hypothesis and reject our first experimental hypothesis in this case.

Moreover, renal impairment does not influence ethionamide elimination. Consequently, we accept the null hypothesis.

In addition, age and sex do not have any influence on ethionamide PK. Thus, we reject our first experimental hypothesis and accept the null hypothesis. ETH was rapidly absorbed after drug administration in all patients, notwithstanding their age. Based on PKs, no differences were observed in ETH Cmax, AUC, Vd and clearance, due to age or sex, sufficient to warrant dosage adjustment to our analysis. Thus, differences in age and sex did not affect PKs of ETH in the present study. As a result, we accept the null hypothesis and reject our first experimental hypothesis.

6.2. RECOMMENDATIONS

Based on the above stated limitations, future studies should be considered which involved the following:

- Greater number of patients should be used in order to confirm the findings of the study with regard to the influence of antiretroviral drugs and HIV-infection on the pharmacokinetics of ethionamide.

- Further investigation is recommended to establish if therapeutic drug monitoring for ETH would maintain constant ETH concentration in the plasma, thereby ensuring that the doses of the anti-TB drugs used attain the obligatory lowest effective levels, devoid of reaching toxic concentrations in the plasma that can lead to unpleasant adverse effects.

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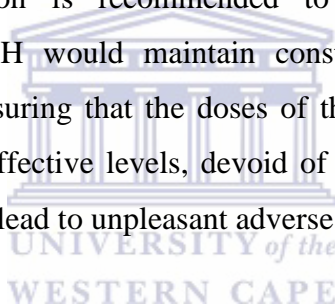
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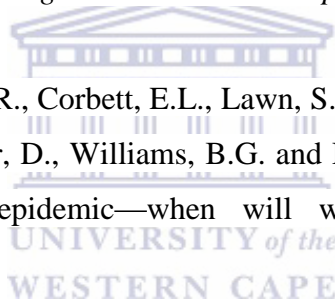
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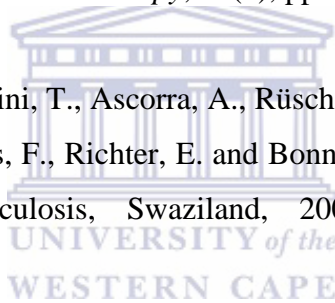
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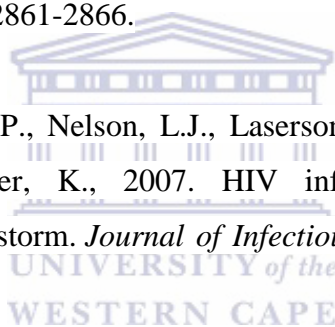
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