DRUG-DRUG INTERACTIONS BETWEEN ANTIRETROVIRALS AND FLUCONAZOLE IN HIV-INFECTED PATIENTS

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DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my original work and that I have not previously submitted it, in its entirety or in part, at any University for a degree.

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ABSTRACT

Background: HIV-positive patients have a significantly weakened immune system which makes them highly susceptible for opportunistic infections, requiring additional treatment. Cryptococcal meningitis and oropharyngeal candidiasis are treated with oral fluconazole. A great potential for drug-drug interactions (DDIs) between fluconazole and antiretrovirals (ARVs), efavirenz, nevirapine, and lopinavir/ritonavir, exists due to interference in common metabolic pathways. The outcome may result in the development of adverse drug reactions or drug resistance and treatment failure.

Aim: The primary aim of this thesis was to evaluate the effect of fluconazole on the pharmacokinetics of efavirenz, nevirapine and lopinavir/ritonavir in HIV-infected patients diagnosed with cryptococcal meningitis or oropharyngeal candidiasis.

Methods: A prospective study was conducted in 80 HIV-positive, treatment experienced adults (≥18 years old) treated in three different outpatient clinics in the Western Cape region. Patients were subdivided according to ARV regimen and the use of fluconazole. A sparse sampling design was used and corresponding ARV serum concentrations were determined by established HPLC and GC methods. Fluconazole serum concentrations were determined by a newly developed HPLC method. Patient characteristics, concomitant medications, clinical test data and ARV serum concentrations were included in a NONMEM generated, one-compartment, open pharmacometric model with first order elimination to detect any drug-drug interactions between fluconazole and the studied ARVs. The secondary outcome was to establish which patient characteristics influence ARV pharmacokinetics.

Results: From 80 outpatients, a total of 276 ARV serum samples (137 efavirenz, 67 nevirapine and 72 lopinavir) were collected for pharmacokinetic evaluation. Efavirenz clearance was correlated with race and concomitant use of rifampicin. No significant covariates were established in the nevirapine model. In the lopinavir model, concomitant use of clotrimazole and the antituberculosis combination isoniazid, pyrazinamide and rifampicin were identified as significant covariates.

Discussion: No significant effects of fluconazole on the pharmacokinetics of any of the studied ARVs were observed. Varying efavirenz plasma concentrations in different ethnic populations may be due to differences in gene expression particularly CYP2B6. Coloured

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patients had significantly lower efavirenz serum concentrations (56.8% decrease in clearance), which has not been previously described in the South African context. Although gender was not a significant covariate in the nevirapine model, female patients tended to have higher nevirapine serum concentrations. TB treatment in all patients receiving lopinavir consisted of a combination of isoniazid, pyrazinamide and rifampicin, each with different effects on CYP isoenzymes. The exact contributing factor of each drug in the ultimate decrease in lopinavir clearance (46.4%) can therefore not be established.

Conclusions: Given the limitations of the sample size in the present study, no statistical significant effect of fluconazole on the pharmacokinetics of the investigated ARVs could be demonstrated. A retrospective analysis of the data showed various co-factors that influence the pharmacokinetics of the investigated ARVs. This data needs to be confirmed in a prospective study as the identified covariates are appropriate in the management of HIV-infected patients in the South African context.

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ABSTRAK

Agtergrond: HIV-positief pasiënte het 'n aansienlike verswakte immuunstelsel, wat hul hoogs vatbaar tot opportunistiese infeksies maak, en dus, addisionele behandeling benodig. Cryptococcal meningitis en orofaringeale kandidiase word met orale flukonasool behandel. As gevolg van middeling in algemene metaboliese paaie is daar 'n groot moontlikheid van middel-middel interaksies tussen flukonasool en die antiretrovirale (ARV) middels, efavirenz, nevirapine, en lopinavir/ritonavir. Die uitkomste hiervan mag tot die ontwikkeling van nadelige middel-middel interaksies of middelweerstandigheid en mislukte behandeling lei.

Doel: Die primêre doel van hierdie tesis was om die effek van flukonasool op die farmakokinetika van efavirenz, nevirapine en lopinavir/ritonavir in HIV geïnfekteerde pasiënte met gediagnoseerde cryptococcal meningitis en orofaringeale kandidiase te evalueer.

Metodes: Die studie was met 80 HIV-positief, behandeling-ervare volwassenes (≥18 jaar) onderneem. Voorafgenoemde was in drie verskillende buitepasiëntklinieke in die Wes-Kaap behandel. Pasiënte was volgens ARV regimen en die gebruik van flukonasool, of dan nie, verder verdeel. 'n Beperkte steekproef ontwerp was gebruik, en ooreenstemmende ARV serum konsentrasies is deurgevestigde HPLC en GC metodes vasgestel. Flukonasool serum konsentrasies was deur 'n nuutontwikkelde HPLC metode vasgestel. Pasiëntkenmerke, gepaardgaande medikasie, kliniesetoets data en ARV serum konsentrasies was by 'n NONMEM genereerde, een-kompartement, oop farmakometriese model met eerste orde eliminasie ingesluit om enige middel interaksies tussen flukonasool en die bestudeerde ARVs op te tel. Die sekondêre uitkomste was om vas te stel watter pasiënt kenmerke ARV farmakokinetika beïnvloed.

Resultate: Uit 80 buitepasïente was 'n totaal van 276 ARV serum monsters (137 efavirenz, 67 nevirapine en 72 lopinavir) vir farmakokinetiese evaluasie gekollekteer. Efavirenz opruiming was met ras gekorreleer asook gepaardgaande gebruik van rifampisien. Geen betekenisvolle ko-variante was in die nevirapine model vasgestel nie. In die lopinavir model het die gepaardgaande gebruik van clotrimazole en die anti-tuberkulose kombinasie isoniazied, pyrazinamied en rifampisien, lopinavir opruiming verminder.

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Bespreking: In hierdie studie is geen betekenisvolle effekte van flukanosool op die farmakokinetika van enige van die bestudeerde ARVs waargeneem nie. Afwisselende efavirenz plasma konsentrasies in verskillende etniese populasies mag aan verskille in geenuitdrukking, veral CYP2B6, toegeskryf word. Kleurling pasïente het betekenisvolle verlaagde efavirenz serum konsentrasies getoon (56.8% verlaging in opruiming). Hierdie bevinding is nog nooit voorheen in die Suid-Afrikaanse konteks beskryf nie. Alhoewel geslag nie 'n beduidende ko-variant in die nevirapine model was nie, het vroulike pasïente geneig om hoer nevirapine serum konsentrasies te hê. TB behandeling, in alle pasïente wat lopinavir ontvang het, het uit die volgende kombinasie bestaan: isoniazied, pyrazinamied en rifampisien, elk met hul eie effekte op CYP isoensieme. Die presiese bydra van elke middel in die uiteindelike verlaging (46.4%) in lopinavir opruiming kan dus nie vasgestel word nie.

Gevolgtrekking: Gegewe die beperkings van die steekproef in die huidige studie, kon geen statistiese beduidende effek van flukonazool op die farmakokinetika van die betrokke ARVs gedemonstreer word nie. 'n Retrospektiewe analise van die data het gewys dat verskeidene ko-faktore die farmakokinetika van die betrokke ARVs beïnvloed. Hierdie data moet in 'n prospektiewe studie bevestig word omdat die geidentifiseerde covariates die bestuur van MIV-positiewe pasiente in die Suid-Afrikaanse konteks te verbeter.

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"I can do all things through Him who strengthens me." ~ Phillippians 4:13

ABBREVIATIONS

AAG	Alpha-1-acid glycoprotein
ACE	Angiotensin converting enzyme
ACN	Acetonitrile
ADE	Adverse drug events
AIDS	Acquired Immune Deficiency Syndrome
ALT	Alanine transaminases
ANOVA	Analysis of variance
ART	Antiretroviral therapy
ARV	Antiretroviral
AUC	Area under the curve
bd	bi-daily
BMI	Body mass index
cART	combination Antiretroviral Therapy
CD4	CD4 cell or T4 'helper' lymphocyte
$CHCl_3$	Chloroform
CH_3CN	Acetonitrile
$(C_2H_5)_2O$	Diethyl ether
CL	Clearance
C_{max}	Maximum plasma concentration
C_{nom}	Nominal concentration
C_{obs}	Observed concentration
CR	Creatinine
CSF	Cerebro spinal fluid
CYP	Cytochrome P450 enzyme system
DAD	Diode array detector
DDI	Drug-drug interaction

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DMSO	Dimethyl sulfoxide
DOH	Department of Health
EFV	Efavirenz
F	Oral bioavailability
FCS	Fetal calf serum
FCZ	Fluconazole
FDA	Food and Drug Administration
GC	Gas chromatography
GFR	Glomerular filtration rate
GIT	Gastro-intestinal tract
HIV	Human Immunodeficiency Virus
H_2O	Water
HPLC	High performance liquid chromatography
hr	hour
IL	Illinois
IRIS	Immune reconstitution inflammatory syndrome
IS/ISTD	Internal standard
K_a	Absorption rate constant
KBH	Karl Bremer Hospital
KH_2PO_4	Potassium dihydrogen phosphate
LDL	Lower than the detectable limit
LFT	Liver function test
LLOQ	Lower limit of quantification
LoF	Loss of function
LPV/r	Lopinavir/ritonavir
MeOH	Methanol
min	minute
MRC	Medical Research Council
Na_2HPO_4	Di-sodium hydrogen phosphate

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NaOH	Sodium hydroxide
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NRTI	Nucleoside reverse transcriptase inhibitor
NVP	Nevirapine
OI	Opportunistic infection
PD	Pharmacodynamic
PEP	Post-exposure prophylaxis
PI	Protease inhibitor
PK	Pharmacokinetic
qd	Once daily
SD	Standard deviation
sec	seconds
SS	Steady state
$t_{1/2}$	Half-life
TB	Tuberculosis
TBH	Tygerberg Hospital
TCNC	T.C. Newman Clinic
t_{max}	Time to maximum concentration
TMP-SMX	Trimethoprim/sulphamethoxazole – also known as co-trimoxazole
UK	United Kingdom
ULN	Upper limit of normal
ULQ	Upper limit of quantification
USA	United States of America
UV	Ultraviolet
V	Volume of central compartment
V_d	Volume of distribution
VL	Viral load
WHO	World Health Organization
WS	Working solution

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1.0 LITERATURE REVIEW

1.1 Burden of HIV/AIDS

The human immunodeficiency virus (HIV) has been known since the 1980s (Pomerantz *et al*., 2003). Evolutionary scientists, however, believe that the virus was present years before it became public knowledge. A cross-species transfer of the simian immunodeficiency virus from primates to humans has led to the development of one of the deadliest epidemics of humankind (Maher, 2010).

1.2 HIV and Associated Opportunistic Infections

In developing countries, HIV positive patients with late stage disease significantly contribute to the total burden of disease. There is an estimated 5.5 million South-Africans living with HIV; contributing 17% to the global HIV/AIDS epidemic (Jarvis *et al*., 2010). The compounding problem is late stage patients that are likely to develop a variety of opportunistic infections as the virus, characterized by a reduction in T-lymphocytes, renders the host immunocompromised and susceptible to a variety of pathogens. Opportunistic infections (OIs) are defined as diseases caused by pathogens, albeit bacteria, fungi, viruses or parasites, that would rarely result in disease in healthy individuals. People infected with the human immunodeficiency virus (HIV), however, have a significantly weakened immune system which compromises their ability to keep such infections under control. Although the occurrence of OIs has decreased significantly after the emergence of combination antiretroviral therapy (cART) in developed countries, OIs still pose a major hurdle with

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regards to morbidity and mortality in HIV-infected individuals in developing countries such as South Africa (Ruhnke, 2004). Furthermore, late presentations of disease as well as HIV-treatment failure are reasons why OIs remain troublesome and a significant cause of death in HIV-infected individuals (Willemot *et al.*, 2004).

Mycobacterium tuberculosis causes Tuberculosis (TB),the most deadly infection arising in patients living with HIV/AIDS (Sánchez *et al.*, 2010, Elsherbiny *et al.*, 2009). Globally in 2007, one out of three HIV-positive people were co-infected with TB (WHO, 2009). Tuberculosis was diagnosed in 27%-34% of HIV-infected adults admitted to tertiary hospitals in Kwa-Zulu Natal, South Africa during 2001 (Holmes *et al*., 2003). Since 2001 the number of patients co-infected with HIV and TB has increased, with Cape Town having the second largest patient population. Other OIs such as pneumocystis pneumonia (PCP), caused by the fungus *Pneumocystis jiroveci,* remains a common life-threatening infection seen in both treatment-naive and treatment-experienced patients (Enomoto *et al*., 2010). PCP typically affects patients with a CD4 count of less than 200, however, patients with a CD4 count of less than 300 and a history of previous OIs, are also at risk and may require prophylactic treatment (Hull *et al*., 2008).

The yeast, *candida albicans*, is present in the system of most people. In immunocompromised patients, however, the body's immune system cannot keep the fungus at bay resulting in disease (candidiasis). *Candida* can occur in the mouth, throat or vagina and is one of the more common opportunistic infections seen during the early stages of infection (Pappas *et al*., 2004). The parasite *toxoplasma gondii* causes an infection known as

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toxoplasmosis which frequently manifests in the brain resulting in encephalitis. Patients with CD4 count of less than 100 are at risk.

Oropharyngeal candidiasis (OPC) and cryptococcal meningitis are the two most prevalent infections seen in late stage HIV/AIDS patients (Gallant *et al*., 1994). Although OPC is usually treated topically, severe forms are treated systemically and this necessitates further treatment or prophylaxis with antifungal agents, such as fluconazole (FCZ). A great potential for drug-drug interactions (DDIs) therefore exists in treated HIV/AIDS patients which in turn increases a patient's probability of experiencing adverse drug events (ADEs) or treatment failure (Seden *et al*., 2009).

1.2.1 Oropharyngeal candidiasis

The most commonly encountered opportunistic infection in patients with HIV/AIDS, OPC, is reported to occur in approximately 90% of infected patients (Hamza *et al*., 2008). Moreover, OPC remains the most frequent HIV-associated oral disease in sub-Saharan Africa in patients with a CD4 count of < 250 cells/µl (Durden *et al*., 1997).

OPC is a fungal infection of the tongue and oral mucosa usually caused by *Candida albicans*, however, non-*Candida* species have recently also been found to result in OPC more frequently (Jenkinson *et al*., 2002). It is diagnosed by observing the following symptoms: white, furry patches on the tongue and oral cavity; painful or burning red patches on the tongue; an unpleasant taste in the mouth; decreased appetite and intolerance of certain foods.

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The development of oropharyngeal candidiasis in a patient is an indicator of progressive immunosuppression and therefore worsening of the condition.

OPC is generally treated with oral fluconazole, either given as single dose (750mg) or as 2-week therapy (150mg/day). Studies have found that there is no statistically significant difference in efficacy between these two regimens (Hamza *et al.*, 2008).

1.2.2 Cryptococcal meningitis

Cryptococcal meningitis (CM) is a life-threatening fungal infection commonly seen in patients with HIV and is one of the key indicators of the development of acquired immunodeficiency syndrome (AIDS) (Sloan *et al.*, 2009). The infection is diagnosed by performing a lumbar puncture. The disease generally affects adult patients and patients with a CD4 cell count of 100 cells/µl or less. The incidence of such life-threatening opportunistic infections has declined following large scale ART roll-out, but patients are still diagnosed with CM due to late presentation (Pappas, 2010).

Current South African guidelines outline three treatment categories for cryptococcal meningitis in HIV patients (McCarthy *et al.*, 2007):

- A 2 week induction phase with amphotericin B (IV) at a dose of 1-1.5 mg/kg/day
- The consolidation phase consists of oral FCZ, 400 mg given daily for 8 weeks

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- Secondary prophylaxis (long term maintenance) with oral FCZ 200 mg/day for life or until CD4 count is greater than 200 cells/mm^3 for more than 6 months on cART (at least 12 months on FCZ in total)

The prevalence of HIV in the Sub-Saharan Africa region is approximately 22 500 000 cases with a reported incidence estimated at 720 000 cases of cryptococcal meningitis per year, which demonstrates the impact of the disease (WHO, 2009; Sloan *et al*., 2009). The annual approximation of death due to HIV-related cryptococcal meningitis is 600 000 (Pappas, 2010). Research has also shown that in HIV infected individuals, the cryptococcal infection has a very high rate of relapse (30-50 %) following treatment (Ruhnke, 2004). Management of the infection in patients therefore routinely involves lifelong antifungal therapy in addition to the antiretroviral therapy. With the emergence of immune reconstitution inflammatory syndrome (IRIS), a condition seen in cART-treated patients, there is a paradoxical deterioration in the patients' clinical status as the recovering immune system responds to a previously acquired opportunistic infection which in turn worsens the symptoms of infection (Ruhnke, 2004). Management for this condition includes continuation of both antifungal therapy and cART.

1.3 Drug-drug interactions (DDIs)

Polypharmacy or the prescription of a cocktail of drugs to treat one or multiple pathologies has become common practice. Consequently the threat of DDIs has become more evident. Knowledge of the occurrence and the extent of such interactions are crucial to research, the health care provider and the patient to improve prescribing practices and avoid adverse drug reactions (ADRs) (Mehta *et al*., 2007; Lazarou *et al.,* 1998). The World Health Organization

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(WHO) defines ADRs as "any noxious, unintended and undesired effect of a drug, which occurs at doses used in humans for prophylaxis, diagnosis, or therapy". ADRs are a significant contributor towards hospital admissions (Pirmohamed *et al*., 2004). A meta-analysis of hospital admissions in the USA, between 1966 and 1996, shows that 45 770 patients were admitted to hospital due to an ADR and 17 753 (39%) of these admissions resulted in death (Lazarou *et al*., 1998). In the age of HIV/AIDS and tuberculosis, developing countries such as South Africa have also observed an increase in hospital admissions due to ADRs. A prospective, observational study of 655 patients admitted to the New Somerset Hospital in Cape Town, South Africa, found that a total of 96 patients (14%) experienced at least one adverse drug reaction; 41 patients were admitted to hospital because of the ADR, 41 patients developed an ADR after admission and 14 patients were hospitalized for other medical conditions (Mehta *et al*., 2007). The two drug classes that contributed mainly to hospital admission were cardiovascular and antiretroviral agents. Whereas ADRs that developed after patient admission were mostly attributed to drugs used to treat various opportunistic infections (antifungals, anti-tuberculosis drugs and antibiotics).

DDIs are a major cause of adverse drug reactions and the potential risk factors for developing DDIs are extensive (Pirmohamed *et al*., 2004). High pill burden, concomitant diseases, gender, race (genetic variation) and age are the main risk factors cited for developing DDIs (Evans-Jones *et al*., 2010; Lazarou *et al*., 1998). The relationship between experiencing a DDI and age is however disproportionate. Elderly patients are more likely to have altered drug plasma levels because of physical changes that affect drug disposition and metabolism (Routledge *et al*., 2003; Gurwitz *et al*., 1991). An increase in body fat content can result in an increased volume of distribution and changes in renal and hepatic function may affect the

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metabolism and excretion of drugs. All of these factors contribute to possible overdosing of elderly patients with standard dosages of a drug (Gurwitz *et al*., 1991).

DDIs are unavoidable and often overlooked especially in HIV-positive populations (Patel *et al.*, 2011). Adverse reactions caused by concomitant use of antiretrovirals and contra-indicated co-medication account for 5.2% of hospital admissions (Evans-Jones *et al.*, 2010). It is known that HIV-positive patients can develop a number of HIV-associated opportunistic infections, as discussed earlier, which requires additional treatment. Moreover, effective management of the disease requires life-long ART. HIV has therefore become a chronic illness and the HIV-patient population is ageing. With age comes a multitude of other illnesses that also require further treatment and therefore polypharmacy is commonly practiced in this patient population (Seden *et al.*, 2009; Tseng *et al.*, 1997).

Drug interactions are broadly defined as an event that occurs between co-administered drugs, drugs and food or drugs and lifestyle (such as smoking or drinking). DDIs specifically may affect the pharmacokinetic profile (absorption, distribution, metabolism and elimination) of a pharmaceutical agent as well as its efficacy and side effect profile (Robertson *et al*., 2005; Dresser *et al*., 2000). DDIs are not limited to oral administration of agents, but may also occur after drug administration via any other route i.e.: topical, rectal, inhalation.

Considerable variation exists in the reported prevalence of clinically significant DDIs (Patel *et al.*, 2011; Seden *et al.*, 2009; Stockley, 2002). This could be attributed to different definitions of clinical significant drug interactions, study methodology and the specific population studied (Stockley, 2002). DDIs are stratified into 4 different groups: drugs should not be co-administered; potential for interaction exists; no clinically significant interaction

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expected or too little or no clear data available on possible interaction (Patel *et al.*, 2011; Seden *et al.*, 2009; Robertson *et al.*, 2005). Moreover, DDIs are subdivided into either pharmacodynamics (PD) or pharmacokinetic (PK) interactions, according to their specific mechanism of interaction (Back *et al.*, 2003; de Maat *et al.*, 2003; Piscetelli *et al.*, 2001; Tseng *et al.*, 1997).

1.3.1 Pharmacodynamic Interactions

Pharmacodynamic interactions arise from an additive, synergistic or antagonistic effect that occurs at the sites of action in response to co-administration of drugs (de Maat *et al.*, 2003). An alteration in the pharmacological response (efficacy and/or toxicity) may result. The polyene antifungal amphotericin B is used mainly in the treatment of life-threatening systemic fungal infections (such as cryptococcal meningitis), as it has a narrow therapeutic index. Interactions between amphotericin B and antiretrovirals (ARVs) are pharmacodynamic in nature and may result in nephrotoxicity, blood dyscrasias and hypokalaemia (Albengres *et al.*, 1998). Other general examples of pharmacodynamic DDIs include: alcohol and antidepressants (synergism); ACE inhibitors and non-steroidal anti-inflammatory drugs (additive); thiazides and non-steroidal anti-inflammatory drugs (antagonism).

1.3.2 Pharmacokinetic Interactions

Most DDIs are pharmacokinetic in nature. This results in a change in either of the drugs blood concentration or its tissue distribution, thereby affecting the drug concentration at the targeted site of action. These effects are attributed to the drugs interaction with processes

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such as absorption, distribution, metabolism and excretion (Robertson *et al*., 2005; Back et al., 2003; Dresser *et al*., 2000). Pharmacokinetic DDIs have a number of possible outcomes. Drug levels of either the concomitant medications may be lowered thereby achieving sub-therapeutic levels which may lead to treatment failure as well as the development of drug resistance. Alternatively, DDIs may elevate the concentrations of co-administered medications resulting in drug toxicity and an increase in the severity and risk of developing adverse effects.

1.3.2.1 Drug interactions in absorption

Drug absorption is defined as the movement of a pharmaceutical agent across an epithelial barrier, from the gastrointestinal tract to the circulatory system. Concomitant administration of drugs or the combination of drugs and food can alter the gastrointestinal absorption of a drug resulting in decreased circulating drug concentrations (bioavailability) and drug effect (Welling, 1984). Absorption of a drug may be affected by various factors and a distinction is made between the total amount of drug absorbed and the rate of absorption (de Maat *et al*., 2003). According to de Maat *et al*. (2003) drugs that are taken as part of life-long or long-term therapy, HIV treatment being a case in point, the amount of drug absorbed is more important than the rate of absorption.

The bioavailability of a drug may significantly be augmented by the presence of food. This could be beneficial to increase the absorption of certain agents (ARVs such as lopinavir/ritonavir, atazanavir, nelfinavir; beta blockers; itraconazole) or to prevent unwanted effects such as gastric irritation (NSAIDs such as aspirin and ibuprofen; corticosteroids; penicillins) (Piscitelli *et al*., 2001; Tseng *et al*., 1997). Patients may be advised to take

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certain drugs (e.g. efavirenz, diuretics, nitrates) away from food in order to limit increased drug absorption and resulting plasma concentrations which may be associated with adverse effects (Robertson *et al*., 2005).

Chelation reactions between certain drugs and cations (e.g. metal ions and tetracyclines, cholestyramine and oral contraceptives, digoxin or Vitamin K) may decrease absorption of the specific pharmaceutical agent when taken concomitantly (Welling, 1984). Another factor that may influence drug absorption is the pH of the gastrointestinal tract. The absorption of a variety drugs, including two different antifungals ketoconazole and itraconazole, is pH dependent, whereas FCZ absorption seems to be unaffected by gastrointestinal pH (Piscitelli *et al*., 2001; Tett *et al*, 1995). The resulting absorption of ketoconazole and itraconazole then relies on the physiological pH of the gut as well as whether the concerned drug is an acid or a base (physiochemical properties). Furthermore, absorption of a drug may be altered by the use of antacids which could impair absorption of co-administered agents as well as decreasing gastric acidity (de Maat *et al*., 2003). Absorption of FCZ and nevirapine is unaffected by the concomitant use of antacids (Diflucan® package insert; Viramune® package insert). It is, however, recommended to take an antacid one to two hours before or after taking efavirenz or lopinavir/ritonavir as the co-administration of these combinations may lead to decreased absorption of the antiretroviral agents (Kaletra® package insert; Sustiva® package insert). A co-formulation of didanosine and magnesium antacid is known to decrease ciprofloxacin's (fluoroquinolone) area under the curve (AUC) by 80 percent (Sahai *et al*., 1993). This interaction can be avoided by taking the fluoroquinolone either 2 hours before or 6 hours after the antacid formulation. The new, enteric-coated didanosine formulation does not pose this problem however.

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1.3.2.2 Drug interactions in distribution

The most important considerations for distribution interactions between drugs are the degree of protein binding of each drug and its mechanism of distribution throughout the body. It is known that only the fraction of drug that is not bound to plasma proteins, albeit albumin, lipoproteins or glycoproteins, is pharmacologically active and able to elicit a response (de Maat *et al*., 2003). It is also this fraction that undergoes metabolism and is excreted. The fraction of drug bound to plasma proteins acts as a reservoir of that drug. In the event of an interaction between two drugs normally bound to the same plasma protein, some competition and displacement may occur (Robertson *et al*, 2005). Theoretically this could increase the unbound fraction of one of the drugs leading to higher plasma concentrations thereby enhancing the drug effect. However, homeostatic control ensures that changes in the unbound drug fraction do not alter plasma concentrations by increasing the elimination of unbound drug, thereby maintaining equilibrium (Robertson *et al*., 2005; de Maat *et al*., 2003). Clinically significant DDIs, as a result of protein binding, are uncommon and clinically not considered relevant (Sansom *et al.,* 1995). This is true unless displacement results in increased plasma concentrations of drugs that have a high hepatic extraction ratio or drugs that have a narrow therapeutic window, long half-life and relatively small volume of distribution (Vd) such as phenytoin or tolbutamide (de Maat *et al*., 2003; Stockley, 2002; Sansom *et al*., 1995).

The ARVs have varying degrees of plasma protein binding; nevirapine (60%), lopinavir/ritonavir (98-99%) and efavirenz (99%) (Cvetkovic *et al*., 2003; Smith *et al*., 2001). No significant interactions, with regards to protein binding, have been reported with the ARVs or FCZ (11-12%) respectively (Robertson *et al*., 2005).

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The distribution of certain drugs is also regulated by specific transporters such as P-glycoprotein (P-gp), a multi-drug resistant (MDR) protein (Zhang *et al.*, 2001). P-gp is an ATP-dependent efflux transporter widely distributed throughout the body. P-gp is located in hepatocytes, renal tubules, epithelium lining the small and large intestines, lymphocytes and endothelial cells of various barriers (blood-brain -, blood-testes – and maternal-fetal barrier) (Christians, 2004; de Maat *et al.*, 2003).

The protease inhibitors, including lopinavir/ritonavir, are substrates of P-gp and their distribution may be affected by the concomitant use of agents that also affect this efflux transporter (Table 1). Moreover, lopinavir/ritonavir and efavirenz also act as inducers of this efflux transporter and transport of co-administered agents and corresponding drug concentrations may be affected (Zhang *et al.*, 2001)

Table 1: Selected substrates, inducers and inhibitors of P-gp. Adapted from Zhang *et al.* (2001)

P-gp substrates		P-gp Inducers	P-gp Inhibitors	
Amytriptyline	Indinavir	Amytriptyline	Amiodarone	Haloperidol
Amoxicillin	Lopinavir	Dexamethasone	Atorvastatin	Ketoconazole
Atorvastatin	Morphine	Efavirenz	Clarithromycin	Itraconazole
Carbamazepine	Phenytoin	Lopinavir	Cyclosporin	Quinidine
Corticosteroids	Quinidine	Phenobarbital	Erythromycin	Ritonavir
Cyclosporin	Verapamil	Ritonavir	Grapefruit juice	Saquinavir
Digoxin		Rifampicin	Garlic	Simvastatin
Estradiol		St. John's wort	Green tea	Verapamil

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1.3.2.3 Drug interactions in metabolism

DDIs are often caused by the interaction of drugs in their common metabolic pathways. Metabolism of drugs results in the conversion of lipophilic drug compounds to a more ionized or polar form which can more easily be excreted (Attia, 2010). Metabolism of drugs occurs by two separate processes. Phase I reactions involve processes such as oxidation, addition of a hydroxyl group as an oxygen moiety to an organic molecule; reduction and hydrolysis, in order to convert the drug to a more polar compound (Guengerich, 2008; Zhang *et al.*, 2001). Phase II reactions, which are commonly referred to as conjugation reactions, involve the addition of amino acids, glucuronic acid, glutathione or sulphonates to the phase I functional groups resulting in metabolites of the specific drug (Attia, 2010; de Maat *et al.*, 2003). With rare exceptions, phase II metabolites are no longer pharmacologically active and can readily be excreted via the kidney.

Several enzyme systems are involved in drug metabolism, however, the majority of drugs are metabolised by via the cytochrome P450 isoenzyme system (CYPs) (Guengerich, 2008; Nebert *et al.*, 2002). CYPs are localised in the smooth endoplasmic reticulum of the liver and the gastrointestinal tract, the main metabolising sites, as well as the stomach, lungs, kidneys and the small and large intestines (Zhang *et al.*, 2001). This system is a superfamily of 57 genes that code for the various CYP enzymes (Guengerich, 2008). The enzymes are further subdivided into so-called families and subfamilies with regards to their amino acid sequence homology and the molecules that they metabolise (Nebert *et al.*, 2002). The most important human CYP isoforms with regards to drug metabolism are: CYP1A2, CYP2A6, CYP2B1, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 (Figure 1) (Guengerich, 2008; Back *et al.*, 2003). CYP3A4 is the most prominent isoenzyme involved in drug metabolism. It

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contributes 30% to the total amount of CYP enzyme and is responsible for the metabolism of roughly 50% of all marketed drugs (Figure 1) (Back *et al*., 2003; Zhang *et al*., 2001).

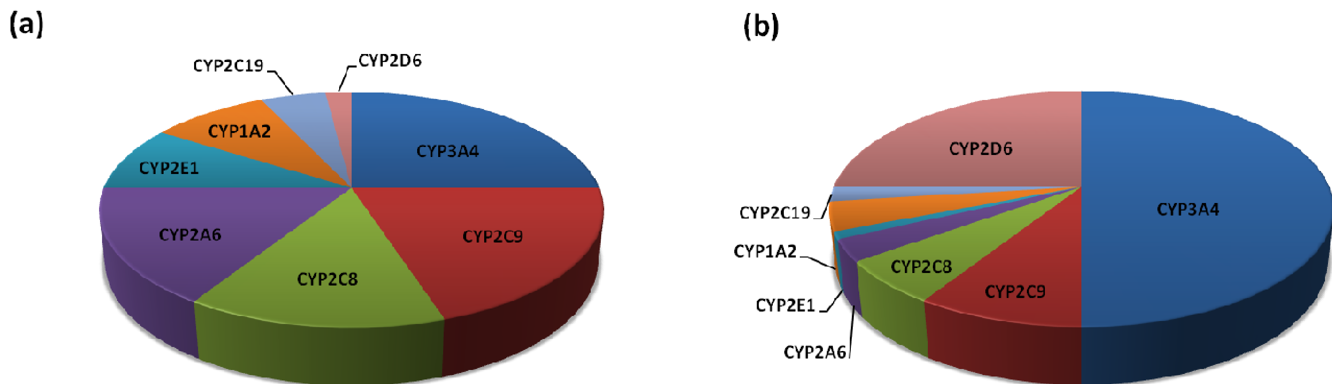

Figure 1: (a) Relative abundance of Cytochrome P450 isoenzymes in the human liver and (b) the relative contribution of specific isoenzymes to the metabolism and clearance of 403 marketed drugs. Adapted from Tozer *et al.* (2006).

A drug is a substrate of a CYP enzyme, when it relies on this enzyme system for metabolism. Moreover, drugs can be inhibitors or inducers of one or more CYP isoenzymes (Robertson *et al*., 2005; Back et al., 2003; de Maat *et al*., 2003). Inhibition and induction of drug metabolism are two distinct processes. Inhibitors of CYP isoenzymes result in increased drug plasma concentrations of co-administered agents and increase the risk of experiencing ADRs (Table 2). Inhibition is a direct and immediate effect of the co-administered agent on the responsible metabolising enzymes of the affected agent (Tseng *et al*., 1997). Inhibition is either competitive (co-medicated agents compete for the active site of the enzyme) or non-competitive (inhibitor binds to the enzyme-substrate complex resulting in a non-functional drug-enzyme complex) and reversible (once the co-medicated drug is discontinued) or irreversible (Piscitelli *et al*., 2001). Inhibition is commonly a rapid process and the full effect may be evident after a couple of days (de Maat *et al*., 2002; Tseng *et al*., 1997).

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Furthermore, the administration of multiple doses, HIV-infected patients a case in point, can lead to accumulation of the drug due to incomplete metabolism. This may result from a genetic deficiency of the particular CYP isoenzyme responsible for drug metabolism or due to the inhibitory interaction of a co-administered agent and toxicity may develop (Guengerich, 2008).

Induction of metabolic enzymes is a more complicated process. The outcome of CYP enzyme induction is an increase in transcription of CYP-encoding DNA, synthesis of the corresponding CYP enzyme and consequently increased activity of the metabolizing enzyme (Mohutsky *et al*., 2010). This is achieved via a signal transduction pathway once the enzyme inducer has achieved steady-state concentrations and therefore the time course of induction is lengthier and the affects may only be seen after 2-3 weeks (Tseng *et al*., 1997). Enzyme induction accelerates metabolism of the co-administered agent and results in decreased plasma concentrations and increased clearance from the body (Table 3). Certain drugs are also known to be auto-inducers. These drugs (eg nevirapine, rifampicin, phenytoin, carbamazepine) have the ability to increase the rate of their own metabolism, but only for a specific time period (Mohutsky *et al*., 2010). Hence, the specific drug is given at a lower dose when initialising therapy and the dose is subsequently increased to achieve appropriate plasma concentrations.

Induction of CYP3A isoenzymes, specifically, is mediated by a ligand-activated transcription factor known as the pregnane-X receptor (PXR), belonging to the nuclear hormone receptor family (Back *et al.*, 2003; Nebert *et al.*, 2002; Zhang *et al.*, 2001). When a drug molecule binds to the PXR, transcription of the associated CYP gene occurs which eventually leads to

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an increase in enzyme production and an associated decrease in the plasma concentration that is metabolised by the specific enzyme (Back *et al.*, 2003; Nebert *et al.*, 2002)

Table 2: Selection of CYP isoenzymes and their corresponding inhibitors.

CYP Isoenzyme	Inhibitor - ARVs	Inhibitor - Non-ARVs
2B6	efavirenz, ritonavir, nelfinavir	ticlopidine, curcumin
2C9	efavirenz, etravirine, delavirdine	fluconazole, amiodarone
2C19	efavirenz, etravirine, delavirdine	Omeprazole
2D6	ritonavir, delavirdine, maraviroc	quinidine, paroxetine, bupropion
3A4	lopinavir/ritonavir, other PIs, delavirdine	fluconazole, ketoconazole, erythromycin, grapefruit juice

Table 3: Selection of CYP isoenzymes and their corresponding inducers.

CYP Isoenzyme	Inducer - ARVs	Inducer - Non-ARVs
2B6	nevirapine	phenobarbital, rifampicin
2C9	efavirenz, lopinavir/ritonavir	Rifampicin
2C19	efavirenz, lopinavir/ritonavir	carbamazepine, rifampicin
2D6	~	dexamethasone, rifampicin
3A4	efavirenz, nevirapine	rifampicin, rifabutin, phenytoin, glucocorticoids, St. John's wort

1.3.2.4 Drug interactions in renal excretion

Firstly, unbound, active drug or drug metabolites are filtered through the glomerular membranes of the kidney upon which the drug reaches the lumen of the renal tubules. The rate of this filtration process is related to the flow of blood through the glomeruli.

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Prostaglandins produced in the kidney indirectly control the flow of blood through the kidney (Stockley, 2002). Drugs that inhibit prostaglandins, such as NSAIDs, may therefore alter blood flow and subsequent renal filtration of a drug. No clinically significant DDIs occur at this level.

Not all drugs rely on metabolism for excretion. Certain drugs, including FCZ (excreted mainly as unchanged drug in the urine), depend on renal clearance for their elimination. These drugs are mainly polar, non-lipophilic agents that are actively transported from the blood across tubular epithelium for excretion in the urine (Robertson *et al.,* 2005). Interactions may occur when co-administered agents inhibit, promote or compete for this transport mechanism. The trimethoprim-sulfamethoxazole (TMP-SMX) combination and probenecid are competitive inhibitors of renal tubular secretion (Piscitelli *et al.*, 2001). It is known that acyclovir and penicillin levels are elevated when co-administered with probenecid and the concomitant use of TMP-SMX and lamivudine results in increased plasma lamivudine concentrations (de Maat *et al.*, 2003; Piscitelli *et al.*, 2001). The aforementioned interaction is however not deemed clinically significant as elevated lamivudine concentrations are not correlated to the development of adverse effects (Piscitelli *et al.*, 2001). Furthermore, the acidity of a drug can also influence its excretion when used together with pH altering agents. Weak acids, such as salicylates, are more readily excreted when the urine is alkalanised.

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1.3.3 Drug-drug interaction studies

The armamentarium of therapeutic agents is constantly enlarging and evolving, therefore obtaining formal data with regards to potential DDIs for all drug combinations observed during clinical use is not possible. When information with regards to a particular drug combination is unavailable, predictions are made on the basis of extensive *in vitro* and/or *in vivo* pharmacokinetic and pharmacodynamic data (Tseng *et al*., 1997). Drug interactions may be similar within the same class of drugs or they may vary between drugs belonging to the same drug class (eg. simvastatin vs primvastatin) and therefore predicting DDIs may not be a simple procedure (Stockley, 2002).

Formal DDI studies during drug development are typically designed as randomized, controlled, cross-over studies in healthy volunteers. The data gathered from these studies provide information on the tested drug (A) and its potential DDI with the co-administered compound (B). More specifically, pharmacokinetic parameters describing the rate and extent of drug absorption, such as the maximum plasma concentration (C_{max}), time to maximum concentration (t_{max}) and area under the time-concentration curve (AUC), are determined from these studies. These parameters can then be compared for each patient when taking the test product alone (A) or concomitantly with another drug (A+B). Each patient in this case acts as his/her own control. A significant DDI is usually present if the 90% confidence interval for the ratio between both test conditions falls outside the 80-125% range.

DDIs, clinically significant or not, must be confirmed in patient cohorts for whom the drug is indicated (Fletcher, 2010). When comparing the pharmacokinetic parameters of a drug

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between healthy volunteers and affected patients, differences are commonly seen as a result of disease-related modifications (Tseng *et al*., 1997). Dickinson *et al.* (2008) report differences in pharmacokinetic parameters of the protease inhibitors (saquinavir, tipranavir and atazanvir) in healthy individuals and patients. When comparing atazanavir and tipranavir concentrations, HIV-infected patients had lower plasma concentrations than the cohort of healthy volunteers. Conversely, saquinavir concentrations were higher in HIV-positive patients, especially HIV-infected women (Fletcher, 2010; Dickinson *et al*., 2008). Another example is the interaction between rifabutin and lopinavir/ritonavir. Concentrations of rifabutin were lower in HIV-TB co-infected patients (AUC_{0-48} 4.42 µg.h/L) than in healthy volunteers (AUC_{0-48} 7.20 µg.h/L) when co-administered with lopinavir/ritonavir (Boulanger *et al*., 2009).

Furthermore, a DDI study between the antiretrovirals etravirine and raltegravir in healthy subjects only showed a modest effect of etravirine on raltegravir pharmacokinetics; 11% decrease in C_{max} and 10% decrease in AUC (Anderson *et al*., 2008). However, when this combination was investigated in four cases following co-administration in HIV-positive patients, it became evident that significant DDI may result (Mènard *et al.*, 2009). Mènard and colleagues observed that in all four patients a combination of standard etravirine and raltegravir (400 mg bd.) doses resulted in markedly reduced raltegravir trough concentrations (C_{min}) ranging from 5 – 30 ng/ml. The recommended therapeutic concentration for raltegravir ranges between 29 ng/ml and 118 ng/ml. Following therapeutic drug monitoring in these patients, a dosage adjustment of raltegravir to 1200 mg per day seemed to compensate for the DDI between etravirine and raltegravir in HIV-infected patients (Do *et al.*, 2011; Mènard *et al.*, 2009).

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Industry DDI studies are performed within a limited time period, whereas in a clinical setting, patients are often prescribed co-administered agents for an indefinite period of time (Fletcher, 2010). The conclusion drawn from all these studies is that controlled industry trials involve homogeneous, healthy subjects and the results obtained cannot always be generalised to the patient population. Moreover, DDIs may also be more prevalent or more extensive within HIV-positive patients.

The need exists for such studies to be performed in the target population in order to ascertain the complete potential of interaction between drug and disease or drug and co-administered agents. The present study focuses on potential DDI within a HIV-infected patient cohort in the Western Cape, South Africa.

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1.4 Antiretroviral agents

Effective treatment of the human immunodefiency virus (HIV) has become more complicated over the years as patients require antiretroviral regimens that consist of a combination of agents that target different stages of the viral replication cycle. Combination antiretroviral therapy (cART) involves concomitant administration of three or more agents from different pharmacological classes (Kis *et al*., 2009; Seden *et al*., 2009). Most of these agents are inducers or inhibitors, as well as substrates of the human CYP enzyme system in the liver and/or the gastrointestinal mucosa, and can therefore influence or alter the metabolism of co-administered agents resulting in DDIs (Kakuda *et al*., 2008). Numerous different classes of antiretrovirals exist (Table 4). The available treatment strategies for HIV-infected patients in South Africa are outlined in Table 5.

Table 4: Classes of antiretroviral drugs

Class	Agents available
Nucleoside reverse transcriptase inhibitors	zidovudine, didanosine, stavudine, lamivudine, tenofovir, zalcitabine, abacavir, emtricitabine
Non-nucleoside reverse transcriptase inhibitors	nevirapine, efavirenz, delavirdine*, etravirine*, rilpivirine*
Protease inhibitors	lopinavir/ritonavir, saquinavir, indinavir, nelfinavir, amprenavir
Fusion inhibitors/Entry inhibitors	maraviroc*, enfuvirtide*
Integrase inhibitors	raltegravir
CCR5 receptor antagonists	aplaviroc*, vicriviroc*
Maturation inhibitors	alpha interferon*

* Drugs not available in South Africa

Table 5: Standardised national antiretroviral therapy (ART) regimens for adults and adolescents. Adapted from the National Department of Health, South African Guidelines (2010)

1st line therapy		
All new patients requiring treatment	TDF + 3TC/FTC + EFV/NVP	EFV is preferred in TB co-infection NVP is preferred in pregnant women, women of child bearing age and women not on reliable contraception
Currently on d4T-based regimen with no side effects	d4T + 3TC +EFV/NVP	Remain on d4T if well tolerated. Switch early with any toxicity. Substitute TDF if at high risk of toxicity (older, high BMI, female, TB treatment)
Contra-indication to TDF renal disease	AZT + 3TC + EFV/NVP	
2nd line therapy		
Failing on a d4T or AZT-based 1st line regimen	TDF + 3TC/FTC + LPV/r	Virological failure followed by intensive adherence management. If repeat VL remains >1000 in 3 months despite adherence intervention, switch.
Failing on a TDF-based 1st line regimen	AZT + 3TC + LPV/r	Virological failure followed by intensive adherence management. If repeat VL remains >1000 in 3 months despite adherence intervention, switch.
Salvage therapy		
Failing any 2nd line regimen	Specialist referral	Virological failure on protease inhibitors is almost always due to non-adherence. Intensively explore and address issues relating to causes of non-adherence most often lead to resuppression. If VL remains high, refer where possible, but maintain on failing regimen.

***Abbreviations: 3TC** Lamivudine; **AZT** Zidovudine; **d4T** Stavudine; **EFV** Efavirenz; **FTC** Emtracitabine; **LPV/r** Lopinavir/ritonavir; **NVP** Nevirapine; **TDF** Tenofovir; **VL** Viral load

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1.4.1 Nevirapine

Nevirapine (Viramune®) belongs to the group non-nucleoside reverse transcriptase inhibitors (NNRTI's); one of the five existing classes of antiretroviral agents available in South Africa. Nevirapine was the first NNRTI widely introduced into clinical practice following approval by the US Food and Drug Administration (FDA) in 1996 (Pomerantz *et al.*, 2003). The drug is structurally classified as a dipyridodiazepinone and has a molecular weight (MW) of 266.3; the structural formula is given in Fig. 2 (Havlir *et al.*, 1995).

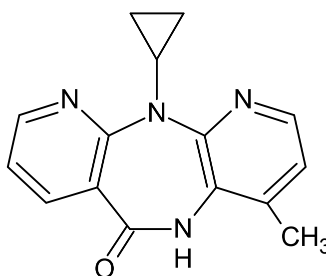

Figure 2: Chemical structure of nevirapine

1.4.1.1 Indications

Nevirapine is most effective when used in combination with other antiretroviral agents for the treatment of Human Immunodeficiency Virus Type 1 (HIV-1). The drug commonly forms the backbone of combination antiretroviral therapy (cART) in both treatment-naive and treatment-experienced HIV patients (Kappelhoff *et al.*, 2005). Because of its accessibility and cost nevirapine is extensively used in first line treatment regimens in resource-limited settings, such as South Africa (Elsherbiny *et al.*, 2009; Vrouenraets *et al.*, 2007; Sanna *et al.*, 2005).

Due to potential serious adverse events initiation of therapy with nevirapine, in adults specifically, is indicated in women with a CD4 count of less than 250cells/mm^3 and in men with a CD4 count of less than 400cells/mm^3 (South African Treatment Guidelines, 2010; Knobel *et al.*, 2008). This general guideline is followed unless the benefits to the patient outweigh the risks.

Furthermore, mother-to-child transmission of HIV is also a significant contributor to the incidence of disease in third world countries such as Africa (Moodley *et al.*, 2003; Luzuriaga *et al.*, 1997). Numerous studies have demonstrated nevirapine's efficacy in reducing vertical transmission of HIV (Luzurgia *et al.*, 1997). The HIV-Net 012 clinical trial run in Uganda showed that a single 200 mg nevirapine dose during labour and a single 2 mg/kg to their newborn within 72 hours of birth significantly decreases to risk of HIV-transmission (Kunz *et al.*, 2009). These results were compared to a similar study done with zidovudine, which however showed no significant effect. Not only is nevirapine superior in clinical efficacy, but the treatment is also markedly cheaper which makes the nevirapine regimen the better quality option, especially in developing countries (Vrouenraets *et al.*, 2007). It therefore also remains the drug of choice in women of child-bearing age (South African Treatment Guidelines, 2010).

1.4.1.2 Mechanism of action

Nevirapine inhibits the viral reverse transcriptase enzyme and hence the replication of the HIV Type-1 virus. Unlike the nucleoside analogues (NRTIs), which bind to the enzymes active site, nevirapine non-competitively targets the viral enzyme by binding to an allosteric site (Pomerantz *et al.*, 2003, Havlir *et al.*, 1995; Richman *et al.*, 1994). This results in a

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conformational change in the HIV reverse transcriptase, which renders the enzyme unable to convert single stranded viral RNA to double stranded DNA for incorporation into human DNA (Figure 3) (Dahri *et al.*, 2007; Smith *et al.*, 2001). This inhibition thus prevents further multiplication of the virus (de Clercq, 2004).

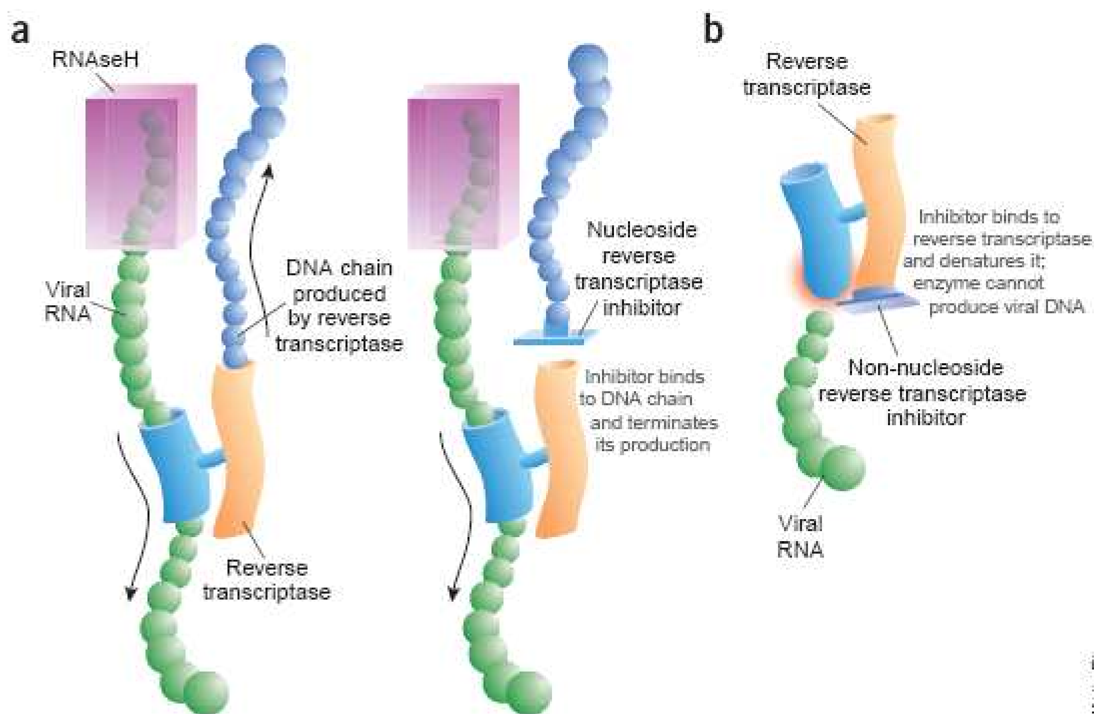

Figure 3: Mechanism of action of (a) NRTIs which require intracellular activation and (b) NNRTIs (mechanism described in text). Modified from R.J. Pomerantz *et al.*, 2003.

1.4.1.3 Pharmacokinetics

Nevirapine is administered as an oral dose in adults and utilised in combination with other antiretroviral agents. In order to account for the drugs auto-inducing capabilities on the CYP metabolising enzyme system, initiation with nevirapine consists of a two week lead-in period of a 200 mg once daily dose (South African Treatment Guidelines, 2010; Mohutsky *et al.*, 2010). Continuous therapy is then dosed as 200 mg nevirapine twice daily. Following oral

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administration, the drug is well absorbed (absolute bioavailability >90%) in both healthy subjects as well as HIV-1-infected patients (Smith *et al*., 2001). Absorption of nevirapine occurs almost completely within 4 hours after drug intake (Havlir *et al*., 1995). Absorption may be delayed by food intake, but bioavailability of the drug is not affected. Peak plasma concentrations of 5.74 µg/ml (5.00 -7.44 µg/ml) (median and interquartile range) are obtained within 2 hours following a 200 mg twice daily nevirapine dose (van Heeswijk *et al.*, 2000). The recommended therapeutic concentration for the drug is a trough concentration greater than 3.4 µg/ml (Dahri *et al*., 2007).

Nevirapine is a small, lipophilic molecule that is mainly unionized at physiological pH (pH=7.4) (Back *et al*., 2003). The drug is therefore widely distributed throughout the body including breast milk (Kunz *et al.*, 2009, Moodley *et al*., 2003). It crosses the placenta and is classified as a category B medication in pregnancy. Cerebrospinal fluid (CSF) penetration of nevirapine is moderate, concentrations reaching up to approximately 30% of the corresponding plasma concentration (Yazdanian *et al*., 1999). Nevirapine is 60% plasma protein bound; the majority being bound to albumin (Smith *et al*., 2001).

The elimination half life of nevirapine following a single 200 mg dose is 45 hours (van Heeswijk *et al*., 2000). This decreases considerably following multiple dosages, ranging from 25 to 30 hours for patients at steady state (Smith *et al*., 2001). Nevirapine undergoes extensive hepatic metabolism. The two main isoenzymes involved are CYP3A4 and CYP2B6 with limited contribution by the isoenzymes CYP2D6 and CYP2C9 (Elsherbiny *et al*., 2009; de Maat *et al*., 2003). Nevirapine is metabolised to 4 different metabolites namely 2-, 3-, 8- and 12-hydroxynevirapine, the 12-hydroxy form being the only active metabolite

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(Elsherbiny *et al*., 2009). The primary route of elimination is as glucoronidated metabolites (2-,3- and 12-hydroxynevirapine glucuronide) in the urine. Only a small fraction (±3%) of the drug is excreted unchanged in the urine.

1.4.1.4 Side effects

Nevirapine is relatively well-tolerated in most patients and has a reasonable safety profile (Pomerantz *et al.,* 2003; Bershoff-Matcha *et al.,* 2001; Havlir *et al*., 1995; Richman *et al.,* 1994). The two most serious adverse events associated with the drug are hepatotoxicity and severe hypersensitivity reactions. Following a retrospective investigation of the Boehringer Ingelheim databases, researchers identified particular risk factors for the development of hepatotoxicity. Sanna *et al.* (2005) and Stern *et al.* (2003) independently showed that gender and CD4 count on commencement of nevirapine treatment were the major determining factors. Their results showed that women with a CD4 count of more than 250 cells/µl were much more likely to have abnormal liver function tests and develop symptoms of hepatotoxicity. Moreover, male patients with a CD4 count of greater than 400 cells/µl were at higher risk for developing hepatic symptoms. It is estimated that between 6 and 30% of patients on a nevirapine containing regimen experience serious adverse effects involving the liver and this may lead to the discontinuation of therapy (Sulkowski *et al*., 2002).

A mild-to-moderate rash is the most common treatment-limiting side effect experienced in nevirapine-treated patients (Bersoff-Matcha *et al*., 2001; Havlir *et al*., 1995). The rate of nevirapine associated rash is estimated at 17% (Carr *et al*., 2000). The mechanism of nevirapine-associated rash remains unclear. It has been demonstrated, however, that taking a

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100 mg daily nevirapine dose for a two week introductory period decreases the risk of rash development (de Maat *et al.*, 2002).

1.4.1.5 Nevirapine drug-drug interactions

DDIs involving nevirapine are mainly due to interference with the CYP enzyme system, i.e. the CYP3A4 and CYP2B6 isoenzymes (Cohen *et al.*, 2007, Back *et al.*, 2003).

Rifampicin, a commonly utilised antimycobacterial agent, is a potent inducer of CYP3A and CYP2B6 (Elsherbiny *et al*., 2009; Ribera *et al*., 2001). The inducing properties of rifampicin overcome that of nevirapine's and the net result is a decrease in nevirapine plasma concentrations due to an increase in the ARVs metabolism. This interaction has been extensively described in cohorts of both healthy volunteers and HIV-infected patients. Results from a small Spanish study (n=10) showed significant mean reductions of 31% (maximum reduction of 41%) and 36% in the area under the time-concentration curve (AUC_{0-12}) and the C_{max}, respectively (Ribera *et al.*, 2001). There was no significant difference found when comparing the minimum nevirapine concentrations (C_{min}). Cohen *et al.* (2007) found similar results in a South African study (n=16) with significant decreases in both the AUC_{0-12} and C_{max} in those patients treated with a nevirapine-based regimen and in the continuation phase of antituberculosis therapy with rifampicin. In addition to this, the South African study found that almost 38% of the patients had a significant reduction in the C_{min}-values to below the recommended therapeutic trough concentration of 3.4 µg/ml. This is associated with an increased risk of drug resistance and ultimately treatment failure. Increasing the nevirapine dose to 300 mg twice daily has been suggested to overcome this problem (Ramachandran *et al.*, 2004). However, nevirapine pharmacokinetics is highly

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variable and this may then result in excessive plasma concentrations potentially resulting in adverse effects.

The use of nevirapine in HIV-infected individuals is often accompanied by other drugs used to treat specific co-morbidities. Significant interactions are seen between nevirapine and a variety of concomitant medications which can alter the pharmacokinetics of the co-administered agent which then results in altered drug levels. This includes, but is not limited to, the drugs listed in Appendix A. Drugs that are contraindicated with nevirapine use or require close monitoring, according to the drugs package insert, include: cimetidine, clarithromycin, fluconazole, ketoconazole, methadone, oral contraceptives, rifampicin, rifabutin, St. John's wort and warfarin.

The DDI between nevirapine and FCZ has not been extensively described and there are conflicting results found by various research groups (Wakeman *et al*., 2010; Geel *et al*., 2004; Kishimoto *et al*., 2000). In theory the competition for shared metabolic isoenzymes may result in elevated nevirapine levels as FCZ is an inhibitor of both CYP2C9 and CYP3A4 (Albengres *et al*.,1998). Increased nevirapine plasma concentrations may in turn lead to an increase in side effects including hepatotoxicity (Wakeman *et al*., 2010).

In vitro data suggest that inhibition of CYP isoenzymes by FCZ is the weakest when compared to other azoles such as ketoconazole, miconazole and itraconazole (Kishimoto *et al*., 2000; Albengres *et al*., 1998). It has, however, been established that due to the low degree of protein binding (11-12%) of FCZ, its *in vivo* plasma concentration is approximately 10 times greater than that of itraconazole (Albengres *et al*., 1998). Similar results were found

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during *in vitro* studies in rat liver microsomes. When comparing the effect of ketoconazole and FCZ on nevirapine metabolism using rat liver microsomes, the inhibitory constants (K_i-values) were 1.59 µM and 11.5 µM for ketoconazole and FCZ respectively (Kishimoto *et al.*, 2000). This shows that in vitro inhibition by ketoconazole is appreciably stronger. When looking at *in vivo* data from the same study however, nevirapine's area under the plasma concentration-time curve (AUC) increases almost 7.4 times when co-administered with 20 mg/kg FCZ and increases only 2.1 times when taken with the same dose of ketoconazole. Normal steady state plasma concentrations of a 400 mg daily dose and 100 mg daily dose for ketoconazole and FCZ respectively are 7.64 ± 3.87 µg/ml and 4.39 ±3.33 µg/ml (Force *et al.*, 1995). The conclusion drawn from this by Kishimoto *et al.* (2000) was that inhibition of ketoconazole is drastically decreased *in vivo* because of its small unbound fraction of drug, 3% compared to the 89% unbound fraction of FCZ.

Wakeham *et al.* (2010) conducted a pharmacokinetic study on 49 HIV-infected Ugandans. All the participants were treated with nevirapine 200 mg twice daily and further randomly subdivided into two groups; 22 patients were assigned a placebo and 27 patients were placed on active FCZ treatment. A total of four blood samples were collected over an 8 hour period and these samples were then analysed to determine the nevirapine concentration at each time point. The mean trough concentrations were 3.865 µg/ml and 5.141 µg/ml in the placebo and FCZ groups respectively. When comparing the maximum nevirapine concentrations (C_{max}), the FCZ group had a median value of 6.546 µg/ml (95% CI 6.040 - 7.974 µg/ml) whereas the placebo group had a mean value of 5.126 µg/ml (95% CI 4.739 -5.773 µg/ml). The corresponding increase in nevirapine AUC_{0-8} was approximately 29% and clearance of the drug was also significantly reduced (median values: 5.58 L/hr versus 4.34 L/hr). Altogether

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61 participants, 27 in the FCZ group and 34 in the placebo group, withdrew from the study due to significantly increased alanine amino transaminases (> 5X ULN). Furthermore, 11/27 and 20/22 patients on FCZ and the placebo, respectively, experienced a rash. One FCZ patient developed Stevens-Johnson syndrome.

Manosuthi *et al.* (2007) conducted a retrospective study in nevirapine-initiated patients with and without co-adminstered FCZ. The outcome was that concomitant use of nevirapine and FCZ led to significantly increased nevirapine plasma trough concentrations versus nevirapine alone (11 400 ± 6 100 ng/ml versus 6 500 ± 3000 ng/ml). It was also established that the nevirapine-FCZ combination was, however, well tolerated in most patients, despite have significantly higher nevirapine concentrations. Clinical hepatitis developed in only 2.4% of the nevirapine and fluconazole treated patients.

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1.4.2 Efavirenz

Like nevirapine, efavirenz (Sustiva®, Stocrin®) is a non-nucleoside reverse transcriptase inhibitor. Efavirenz is a trifluoro-derivative with a molecular weight (M_w) of 315.68 g/mol (Figure 4). The drug is a potent and selective inhibitor of HIV-1 and widely used in patient populations as a part of combination antiretroviral therapy. Efavirenz was granted FDA approval in September 1998 (Vrouenraets *et al.*, 2007). Prospective, controlled studies showed that regimens containing efavirenz, in combination with two nucleoside reverse transcriptase inhibitors (NRTIs) or a protease inhibitor (PI) were as effective in achieving viral load suppression as a PI-based regimen without efavirenz (Dahri *et al.*, 2007).

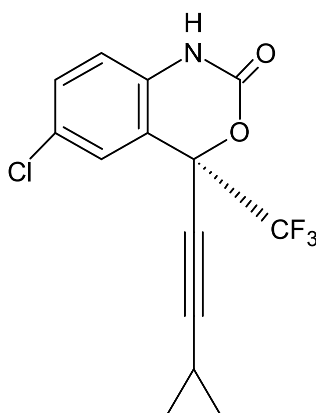

Figure 4: Chemical structure of efavirenz.

1.4.2.1 Indications

For the management of patients with HIV-1 infection, efavirenz is most often combined with agents belonging to the nucleoside reverse transcriptase inhibitor (NRTIs) or protease inhibitor (PIs) class. The drug is prescribed to both newly diagnosed and treatment-experienced patients (Sustiva® package insert). However, efavirenz, in many cases, is the drug of choice for initial therapy in treatment-naive patients as first-line therapy (Table 3) (Dahri *et al.*, 2007; Vrouenraets *et al.*, 2007).

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According to current South African treatment guidelines, adults are dosed with a 600 mg efavirenz coated tablet once daily. Efavirenz is teratogenic in non-human primates (Vrouenraets *et al*., 2007). It is therefore contraindicated during pregnancy or in women with child-bearing potential (16-45 yrs) and labelled as a category D agent in pregnancy by the FDA (Sustiva® package insert). Yet in daily practice efavirenz is still prescribed to pregnant women.

Efavirenz is given as post-exposure prophylaxis (PEP) to non-infected individuals who may have accidentally come into contact with the virus. This is often the case with health care workers treating infected patients.

1.4.2.2 Mechanism of action

Efavirenz, like all non-nucleoside reverse transcriptase inhibitors, acts by inhibiting the viral reverse transcriptase enzyme (Fig 3). The mechanism of action of efavirenz is similar to that of nevirapine which was discussed earlier.

1.4.2.3 Pharmacokinetics

Efavirenz is orally administered; available as a tablet (50 mg, 100 mg, 200 mg or 600 mg) or an oral solution (30 mg/ml). It is recommended for the drug to be taken on an empty stomach at bedtime in order to minimise possible central nervous system and psychiatric side effects (Lopez-Cortes *et al.*, 2006). The drug is 99% protein bound, the majority of this to albumin (Smith *et al*., 2001). Efavirenz is well absorbed from the gastrointestinal tract and peak plasma concentrations (C_{max}) are reached within three to five hours after taking the dose. In a

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study conducted with healthy volunteers the C_{max} ranged from 0.51 to 2.9 µg/ml following administration of efavirenz in doses of 100 mg up to 1600 mg (Sustiva® package insert). The suggested therapeutic window for efavirenz in HIV-infected is a plasma concentration between 1 and 4 µg/ml (Dahri *et al.*, 2007).

Efavirenz has a long half-life ($t_{1/2}$); 52 to 76 hours after a single dose. Half-life decreases to values between 40 and 55 hours during multiple dose administration (Smith *et al.*, 2001). Due to the long half-life, it takes approximately 6 to 10 days for a patient to reach steady state efavirenz plasma levels. Metabolism of efavirenz occurs in the liver via the CYP enzyme system. The primary isoenzyme responsible for this process is CYP2B6 with some involvement of CYP 3A4 (Ståhle *et al.*, 2004; Back *et al.*, 2003). The resulting metabolites are hydroxylated to form 8-hydroxyefavirenz (major) and 7-hydroxyefavirenz (minor), the products of primary metabolism (Ward *et al.*, 2003). Subsequent glucoronidation produces 8,14-dihydroxyefavirenz, the product of secondary metabolism, which is no longer active (Ward *et al.*, 2003). A large proportion of efavirenz (16 – 61%) is excreted as unchanged drug in the faeces; the remaining 14-34% is excreted as metabolites in the urine (Smith *et al.*, 2001). A very small percentage (± 1%) is excreted as unchanged drug in the urine (Vrouenraets *et al.*, 2007). Dosage adjustments in patients with renal insufficiencies are therefore not needed. Due to genetic differences in the expression of CYP proteins responsible for drug metabolism, large inter-individual variability occurs in efavirenz metabolism (Ståhle *et al.*, 2004). This may warrant therapeutic drug monitoring to assess an individual patients plasma drug concentration.

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1.4.2.4 Side effects

Efavirenz is tolerated relatively well in most adult patients when the drug is taken at the recommended dosage of 600 mg daily. Adverse effects commonly encountered with efavirenz based regimens include nausea, vomiting, dyspepsia, diarrhoea, rashes as well as certain nervous system effects (Adkins *et al.*, 1998). A multicenter, randomized trial investigated and compared the safety profile of efavirenz based regimens (efavirenz in combination with zidovudine and lamivudine or efavirenz in combination with only indinavir) to a regimen consisting of indinavir, zidovudine and lamivudine (Staszewski *et al.*, 1999). The researchers observed a significant difference between patients on the abovementioned regimens when investigating the incidence of nervous system effects and rash; efavirenz based regimens had a higher prevalence of both the adverse effects (48 and 54% respectively versus 21%). When looking at the incidence of nausea and vomiting, however, it was established that both efavirenz based regimens were superior to that of the comparator regimen. Other side effects experienced with efavirenz include elevated levels of triglycerides or cholesterol (hyperlipidemia), neutropenia as well as altered fat distribution (Fontas *et al.*, 2004; Zapor *et al.*, 2004).

Efavirenz, unlike other NNRTIs, is associated with various neuropsychiatric central nervous system adverse effects (Hawkins *et al.*, 2005). These effects include headaches, dizziness, impaired concentration, somnolence, insomnia, abnormal dreams, confusion and hallucinations (Hasse *et al.*, 2005; Hawkins *et al.*, 2005; Adkins *et al.*, 1998). In many cases these effects are minor, but they may lead to poor compliance and ultimately discontinuation of the drug (Zapor *et al.*, 2004). Neuropsychiatric symptoms normally appear within the first two weeks of therapy and resolve within a period of 6 to 10 weeks, though in certain patients,

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these effects may persist and result in severe depression, anxiety, suicidal tendencies, aggressive behaviour or paranoia depending on the plasma concentration of efavirenz (Hawkins *et al*., 2005).

Allergic reactions to efavirenz are relatively common, but the severity of the reaction varies considerably. The more harmless skin reactions, such as maculopapular rash, occur regularly (10%), whereas serious, potentially life-threatening allergic responses arise less frequently (<5 %) (Carr *et al.*, 2000; Adkins *et al.*, 1998). The more serious events are associated with the development of blisters, loss of skin or the formation of cutaneous sores (Montesorri *et al.*, 2004). This may be accompanied by fever and eventually lead to severe infections, sepsis and possibly death if left untreated.

1.4.2.5 Drug-drug interactions

Efavirenz is known to induce hepatic isoenzyme CYP3A as well as inhibit isoenzymes 2C9, 2C19 and 3A4 (Vrouenraets *et al*., 2007; Back *et al*., 2001). Co-administered agents that are metabolised via these pathways may have altered plasma concentrations due to interference in common metabolic pathways. Drugs such as methadone; cyclosporine, tacrolimus; atorvastatin and simvastatin; nifedipine, diltiazem and verapamil and hormonal contraceptives such as ethinyl estradiol are primarily metabolised by CYP3A. Induction of this isoenzyme by efavirenz may result in significantly decreased levels of these agents and dose adjustments may be necessary (Sustiva® package insert). On the other hand, drugs that rely on CYP2C9, CYP2C19 or CYP3A4 for their metabolism may have elevated plasma concentrations by way of efavirenz's inhibitory effect on these isoenzymes. Inhibition of

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CYP3A4, when compared to induction of the same enzyme by efavirenz, is a more rapid process and the effect can be seen after one or two co-administered doses (Flexner, 2004). Phenytoin and warfarin are drugs that fall into the latter category (rely on CYP metabolism) and care must be taken to avoid possible adverse effects. Furthermore, phenytoin and warfarin both potentially result in a bi-directional DDI with efavirenz (Sustiva® package insert). This means that the blood concentrations of both the co-administered drugs are affected by one another. Such an interaction is very difficult to predict and interpatient variability is high.

Co-administered drugs that induce CYP2B6 and CYP3A, the isoenzymes responsible for efavirenz metabolism, would increase the clearance of efavirenz and result in decreased efavirenz plasma concentrations (Elsherbiny *et al*., 2009; Ribera *et al*., 2001). The concomitant use of efavirenz and the antimycobacterial agent, rifampicin, is a commonly encountered combination. Rifampicin is a potent inducer of both the aforementioned isoenzymes and may therefore decrease efavirenz plasma concentrations which may result in treatment failure and the development of drug resistance (Elsherbiny *et al*., 2009). The Centre for Disease Control and Prevention have suggested an increase in the efavirenz dose from 600 mg daily to 800 mg daily to overcome the induction by rifampicin, however, Manosuthi *et al* (2006) found that such a dose increase was not necessary to achieve adequate virological suppression. Researchers investigating the use of the higher efavirenz dose have, however, found that patients experience higher incidences as well as severity of side effects (Manosuthi et al., 2006). Such dose adjustments are consequently not commonly done in the clinical setting. Still efavirenz remains the drug of choice for the antiretroviral backbone when treating HIV-TB co-infected patients (South African Treatment Guidelines, 2010).

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Other drugs that may induce efavirenz metabolism include carbamazepine, phenobarbital, phenytoin; and warfarin (Adkins *et al.*, 1998). According to the efavirenz package insert concomitant use of the following drugs is contraindicated: astemizole, midazolam, triazolam or ergot derivatives. A comprehensive list of efavirenz-associated DDIs can be seen in Appendix A.

Co-administration of voriconazole, a novel triazole antifungal agent, and efavirenz at the standard doses (EFV 600 mg daily and VCZ 200 mg twice daily) is contra-indicated (Catanzaro *et al*., 2004). Liu *et al.* (2008) evaluated the pharmacokinetics of both agents in healthy male subjects during a two phase study period. Results showed that mean steady state efavirenz concentrations increased significantly following concomitant use with voriconazole. The maximum plasma concentration (C_{max}) increased from 3.85 µg/ml to 5.26 µg/ml and the area under the plasma concentration-time curve (AUC) increased from 57.8 µg.hr/ml to 83.3 µg.hr/ml. This could most likely be explained by voriconazole's strong inhibition of CYP3A4. On the contrary, voriconazole levels significantly decreased when taken with efavirenz; C_{max} decreased from 3.06 µg/ml to 1.15 µg/ml with a corresponding decrease in AUC (26.3 µg.hr/ml to 5.71 µg.hr/ml) due to efavirenz's enzyme induction capabilities. The same pattern of interaction was found in numerous other studies including cohorts of HIV-positive patients (Catanzaro *et al*., 2004;). Guidelines now recommend adjusted doses of both agents (EFV 300 mg daily and VCZ 400 mg twice daily) when used concomitantly (Damle *et al.*, 2007).

When compared to voriconazole and ketoconazole, FCZ is a less potent inhibitor of CYP3A4, although the interaction between efavirenz and FCZ has not been well established

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(Dahri *et al*., 1998). In theory there is a potential for interactions as the drugs share a common metabolic pathway via CYP3A4 and 2C9, but FCZ has a greater affinity for fungal CYP than mammalian CYP isoenzymes. Robertson *et al.* (2005) and de Maat *et al.* (2003) concluded that there is no clinical significant interaction between these agents, although isolated case reports outline interactions between efavirenz and FCZ that have resulted in significantly altered plasma concentrations. A case described by Hasse *et al.* (2005) was a native Thai, HIV-positive woman diagnosed with cryptococcal meningitis. She presented at the local hospital with severe psychotic episodes. The patient was confused, experienced spells of dizziness and incidences of child-like behaviour and aggression. These neuropsychiatric symptoms were linked to toxic levels of efavirenz which was later confirmed after measurement of her plasma level; efavirenz measurements was 59 400 µg/ml which is approximately 30 times the normal limit. Following genetic testing the patient was found to be homozygous for the CYP2B6 G516T allele and therefore a poor metaboliser of drugs utilising the CYP2B6 pathway. In addition, the increase in drug efavirenz levels most likely was aggravated by a DDI between efavirenz and FCZ.

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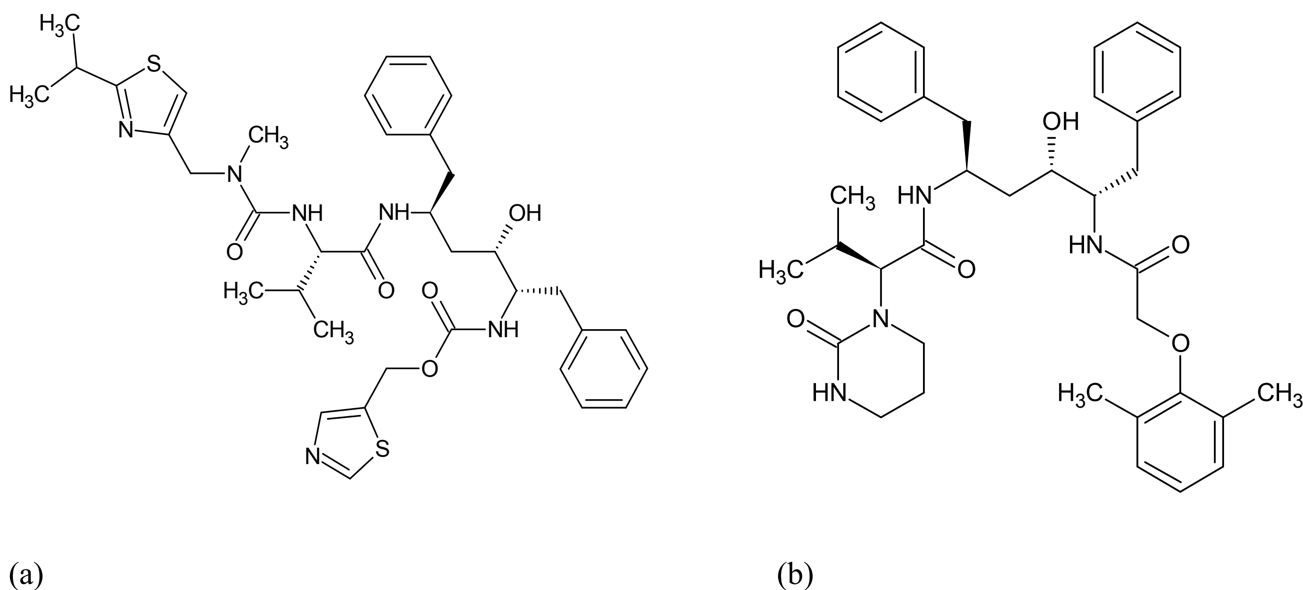
1.4.3 Lopinavir/ritonavir

Lopinavir (Kaletra®, Aluvia®) (Figure 5) belongs to the class of antiretroviral agents known as protease inhibitors and was approved by the FDA in 2000. Lopinavir is never used as a single protease inhibitor, but co-administered with a low, non-therapeutic dose of another protease inhibitor, ritonavir. The reason for combining these two agents is that ritonavir inhibits the rapid and extensive CYP 3A4-mediated metabolism of lopinavir (Cvetkovic *et al.*, 2003). This prevents lopinavir's rapid clearance from the body, enhancing its bioavailability and activity against the viral protease. Another advantage of the co-formulation is the decrease in pill burden and the knowledge that both agents are taken concomitantly. The lopinavir/ritonavir combination is available as a fixed-dose formulation of 200/50 mg (4:1 lopinavir/ritonavir ratio) for oral administration as a tablet.



(a) (b)

Figure 5: Chemical structure of (a) and lopinavir (b) ritonavir.

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1.4.3.1 Indications

Lopinavir/ritonavir is indicated for use in the management of HIV-1 infected patients in combination with additional antiretroviral agents. Lopinavir/ritonavir is commonly added to nucleoside analogues as first line therapy in both treatment experienced and treatment naive patients (Cvetkovic *et al.*, 2003). However in the African setting, lopinavir/ritonavir is more frequently prescribed as a part of salvage therapy in patients that have failed first line regimens (Table 5). According to the South African Treatment Guidelines (2010) virological failure is defined as a viral load of more than 1000 copies/ml after 3 months despite adherence intervention and may be caused by the development of drug resistance.

1.4.3.2 Mechanism of action

A polypeptide chain is formed following translation of viral RNA. This chain consists of essential proteins (reverse transcriptase-, protease- and integrase enzymes) that are needed by the virus to mature and produce new, infective virus particles. Before maturation can occur, the abovementioned proteins must be cleaved from the polypeptide chain in order to become functional (Pomerantz *et al.*, 2003). This is the responsibility of HIV-1 viral protease. In all retroviruses, the essential ingredient needed for the production of new virus particles is an active viral protease enzyme. The HIV-1 protease is homodimeric; comprised of a 99 amino acid sequence protein (Cvetkovic, 2003; Eron, 2000). The protease inhibitors, including lopinavir/ritonavir, are highly selective for the active site and thus potent inhibitors of the viral protease enzyme (Figure 6). Consequently preventing cleavage of the functional proteins needed for growth and maturation. The net result is thus production of immature, non-infective viral molecules (Pomerantz *et al.*, 2003).

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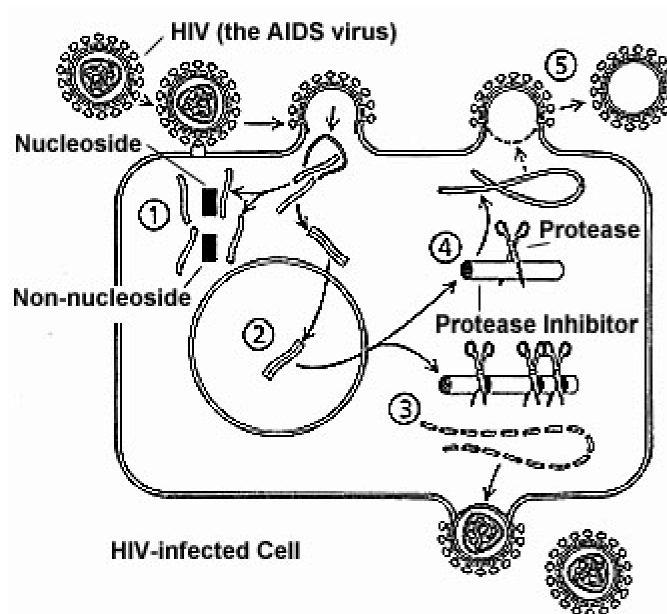

Figure 6: Mechanism of action of the protease inhibitor class of drugs.

1.4.3.3 Pharmacokinetics

The pharmacokinetic properties of the lopinavir/ritonavir combination have been investigated in both HIV-positive adult patients and healthy adults with no significant differences in the pharmacokinetics between these two populations (Cvetkovic *et al.*, 2003).

In a pharmacokinetic study performed in patients who were in steady state for lopinavir/ritonavir treatment at a dose of 400/100 mg twice a day together with food, mean peak plasma concentration (C_{max}) was 9.8 ±3.7 µg/ml (Bertz *et al.*, 2001). The time required to reach the maximum concentration was approximately 4 hours. Absorption and subsequent bioavailability of the drug can significantly increase when taken with a moderate-to-high fat meal (Gustavson *et al.*, 2000). Lopinavir is approximately 98-99% protein bound. Binding

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occurs with both albumin and alpha-1-acid glycoprotein (AAG); the majority of the drug being bound to the latter (Boffito *et al.*, 2003).

Both lopinavir and ritonavir undergo extensive first pass metabolism in the gut which leads to a decrease in plasma concentration (Kumar *et al.*, 2004). The main pathway of metabolism of this drug is via oxidative, hepatic metabolism via the CYP enzyme system. Lopinavir/ritonavir is almost solely metabolised by the CYP3A isoenzyme, with a small contribution from CYP2D6 which is involved in ritonavir metabolism (Kumar *et al.*, 1999). The half-life of the co-formulated drug is 5-6 hours, which results in twice daily dosing.

The largest percentage of the dose (80.1 - 85.1%) is recovered in the urine or faeces as metabolites (Kaletra® package insert). The remaining lopinavir is excreted as unchanged drug in the urine (±2.2%) and the faeces (±19.8%) (Cvetkovic *et al.*, 2003). Since the renal clearance of the drug is insignificant, no dosage adjustments are needed in patients with renal insufficiency. Seeing as the drug is metabolised and eliminated primarily by the liver, caution should be taken in patients with hepatic impairment or failure (Kaletra® package insert).

1.4.3.4 Side effects

Possible side effects with lopinavir/ritonavir are similar to those experienced with the majority of marketed drugs. This includes nausea, vomiting, diarrhoea, headache, rash, fever and abdominal pain (Dios *et al.*, 2002). An adverse effect commonly observed with protease inhibitors, is a change in the body fat composition known as lipodystrophy (Carr *et al.*, 1998;

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Danner *et al.*, 1995). The pattern of fat redistribution is similar to that seen in patients with hypercortisolism. In such cases fat tends to accumulate in the abdomen and trunk, which is referred to as a 'crix belly' or 'protease paunch', and a fat pad develops behind the neck. Furthermore, peripheral fat loss is characteristic in these patients. The limbs are normally thin accompanied by loss of fat in the face, especially the cheek area, which then results in an identifiable gaunt appearance associated with HIV stigmatisation.

Other troubling adverse effects associated with lopinavir/ritonavir therapy are the development of metabolic abnormalities such as increased hypercholesterolemia, hypertriglyceridemia as well as hyperglycaemia (Fontas *et al.*, 2004; Carr *et al.*, 1998). In certain patients this may result in clinical manifestation of diabetes mellitus. An increased risk of bleeding in patients with haemophilia has been observed and caution must be taken in patients with heart disease or a history of arrhythmias as the drug can cause a prolongation of the QT interval (Chinello *et al.*, 2007; Carr *et al.*, 1998). Moreover, lopinavir/ritonavir has been associated with abnormal liver function tests. Randomised control trials conducted to obtain FDA approval for the combination PI, illustrated that hepatotoxicity occurred in 1 - 9.5% of patients taking the drug (Canta *et al.*, 2005; Sulkowski, 2004). Patients with a history of hepatitis or abnormal liver functioning should be closely monitored as the condition may worsen when continuing with therapy (Canta *et al.*, 2005). The drug has been classified as a category C agent in pregnancy by the FDA.

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1.4.3.5 Drug-drug interactions

In certain circumstances DDIs could be advantageous. The fixed-dose co-formulated lopinavir and ritonavir is an example of utilising DDIs in order to favourably alter a drugs pharmacokinetic profile (Cvetkovic *et al.*, 2003).

The source of many of these DDIs could be due to sharing common metabolic pathways. As previously mentioned, the drug is metabolized mainly in the liver via CYP enzymes; particularly CYP3A, which is also responsible for the metabolism of approximately 50% of all marketed drugs (Back *et al.*, 2003). In addition to this, lopinavir/ritonavir is both a strong inhibitor of the CYP3A4 isoenzyme and an inducer of the CYP1A2, CYP2C9 and CYP2C19 isoenzymes (Yeh *et al*., 2006). Interaction studies between lopinavir/ritonavir and an array of co-medications have been performed in both small groups of patients as well as healthy volunteers (Decloedt *et al.,* 2011; Yeh *et al.*, 2006; la Porte *et al.*, 2004; Cato *et al.*, 1997). Concomitant use of lopinavir/ritonavir and agents that rely greatly on the abovementioned isoenzymes for their metabolism is contraindicated or must be monitored as elevated levels of the co-administered agent may result in serious adverse effects. The list of contraindicated drugs, according to the Kaletra® package insert, include: astemizole, ergonovine, ergotamine, flecainide, propafenone; midazolam, triazolam, pimozide and simvastatin. The herbal product, St. John's Wort, induces lopinavir/ritonavir metabolism. Its use together with lopinavir/ritonavir is not recommended, as it may drastically decrease plasma concentrations of the ARVs. This may possibly result in virological failure and ultimately drug resistance. Appendix A outlines various potential DDIs experienced with lopinavir/ritonavir use.

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Potential interactions due to the concomitant use of lopinavir/ritonavir and antimycobacterial agents such as rifampicin, which is a potent inducer of CYP3A4, must be taken into account prior to treatment (la Porte *et al.*, 2006). Numerous studies have illustrated that the aforementioned combination leads to sub-therapeutic levels of lopinavir/ritonavir and diminished virological response. According to Decloedt *et al.* (2011) doubling the lopinavir/ritonavir dose in healthy individuals was sufficient to surmount the increased metabolism of the protease inhibitor. Moreover, there is a decreased incidence of hepatotoxicity in the HIV-infected cohort when compared to healthy subjects (Decloedt *et al*., 2011). Subsequent follow up studies have, however, shown that these patients were more likely to develop hepatotoxicity associated with elevated lopinavir/ritonavir levels.

Co-administration of lopinavir/ritonavir and FCZ is common practice in HIV-infected patients as they are likely to develop a variety of opportunistic fungal infections. The potential mechanism of DDI is thought to be the inhibition of CYP3A4 by FCZ. Following ritonavir's approval by the FDA as both a single agent and a part of combination therapy, Cato *et al.* (1997) performed a randomized, cross over study in thirteen healthy volunteers to evaluate potential interactions between these two agents. In essence each participant acted as their control; receiving ritonavir followed by a wash out period and then crossed over to the ritonavir and FCZ combination. Serial blood samples were taken once patients reached steady state for the drug or drugs taken and these samples were then analysed to obtain the ritonavir concentration. Five volunteers (1 male and 4 female) discontinued the study after developing adverse reactions commonly seen with ritonavir. It was speculated that the increased ritonavir levels in women may be due to the difference in body size; these values were however not reported in the study. For the remaining eight volunteers that completed

the study, results showed that there were only minor differences in the resulting ritonavir plasma concentration and pharmacokinetic parameters. The 0-24 hour period C_{max} and AUC parameters were significantly different between the ritonavir alone (8.95 ± 2.41 µg/ml and 151 ± 36 µ.hr/ml respectively) and the ritonavir plus FCZ (10.25 ± 2.69 µg/ml and 169 ± 41 µ.hr/ml respectively). There was no significant difference in the time to maximum ritonavir concentration (t_{max}) in either group, 3.9 ± 1.1 hr (ritonavir alone) versus 4.1 ±1.0 hr (ritonavir plus FCZ). The conclusion drawn from this study by Cato *et al* (1997) was that the interaction between ritonavir and FCZ is minor with no clinical relevance or need for dose adjustment.

The potential for DDIs to occur between co-formulated lopinavir and ritonavir and FCZ exists as lopinavir, like ritonavir, is metabolized mainly by CYP3A4. FCZ is a moderate inhibitor of CYP3A4 and concomitant use may lead to increased levels of lopinavir/ritonavir. This combination has been evaluated in clinical practice, results mostly showing that there is no significant interaction between these agents. The 'boosting' of lopinavir with ritonavir may assist the drug from overcoming any possible interactions.

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1.5 Antifungal agents

The antifungal therapies currently available fall into three broad categories: systemic preparations for systemic infections as well as oral and topical preparations for mucocutaneous infections (Katzung, 1998). Furthermore, antifungal agents can be divided into three distinct classes: polyenes consisting of amphotericin B and nystatin; the fluorinated pyrimidine analogue, flucytosine and the azole antifungals (Dismukes, 1988).

The azole antifungals are synthetic compounds and can be further classified, according to their structure, into imidazoles and triazoles (Ringel, 1990). The imidazoles consist of one or more 5-membered rings, known as the azole ring, containing two nitrogen atoms, whereas the triazoles contain three nitrogen atoms in the azole rings (Dismukes, 1988). Structural differences between the two categories lead to differences in the absorption and efficacy of the drug.

1.5.1 Fluconazole

Fluconazole (Diflucan®) is a synthetic azole-derived antifungal agent. FCZ (M_w 306.3 Da), belonging to the triazole group, contains three nitrogen atoms in the five-membered azole ring (Figure 7).

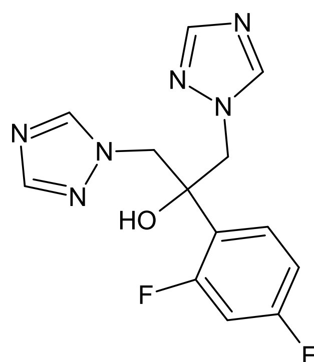

Figure 7: Chemical structure of fluconazole.

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1.5.1.1 Discovery

FCZ has been in use since 1990 following approval from the Food and Drug Administration (FDA) (Terrell, 1999). Since then FCZ has been utilized for its broad-spectrum of applications in the treatment of both superficial and systemic fungal infections. More recent research has reported the use of the newer triazole antifungals, such as FCZ and itraconazole, for a variety of indications rather than ketoconazole as discussed below (Terell *et al.*, 1992; Saag *et al*, 1988). This is due to the triazoles broader spectrum of activity and reduced toxicity when compared to ketoconazole (Dismukes, 1988).

1.5.1.2 Indications

FCZ has shown activity against a variety of pathogens both *in vitro* and *in vivo*. Susceptible organisms illustrating in vitro activity against the agent include: *Candida albicans*; non-*albicans Candida* species such as *Candida glabrata*; *Histoplasma capsulatum; Coccidioides immitis; Blastomyces dermatitidis* and *Cryptococcus neoformans* (Chen *et al.,* 2007). Good activity has especially been seen in a variety of *Candida* species as well as *Cryptococcus neoformans* in clinical situations. FCZ, therefore, remains a valuable and low-cost drug alternative for the treatment of oropharyngeal candidiasis, amongst others, and cryptococcal meningitis (Zobios *et al*., 2008).

The dosage for the consolidation phase of active cryptococcal meningitis is 400mg daily oral FCZ for a period of 8 weeks (McCarthy *et al*., 2007). This is then followed by long term maintenance or prophylactic therapy where FCZ is dosed at 200 mg daily. There are two schools of thought about the time period of prophylactic treatment. The first is that maintenance therapy is necessary until the patient has a CD4 count that is greater than 200

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cells/mm^3 for more than 6 months on antiretroviral therapy following a minimum of 12 months treatment with FCZ. The second argument is that prophylactic therapy with 200 mg daily FCZ is a part of lifelong treatment, irrespective of the associated CD4 counts. Researchers supporting the first mentioned hypothesis argue that lifelong treatment with FCZ may result in the development of drug resistance (Hamza *et al*., 2008; Vanden *et al*., 1998; Johnson *et al*., 1995). Furthermore, resistance to the drug is also encountered in certain non-albicans Candida species such as *C. Krusei* and some isolates of *C.glabrata* (Chen *et al*., 2007).

Vaginal candidiasis, osophogeal candidiasis and urinary tract infections caused by *C. albicans*, candidemia and disseminated candidiasis are the other main indications of FCZ (Guo *et al.*, 2010; Wildfeuer *et al.*, 1997).

1.5.1.3 Mechanism of action

Many antifungal agents are known and target different components of the fungal cell. The mechanism of action for all azoles is similar (Terrell *et al.*, 1992). According to Kyle e*t al.* (2004) both imidazole and triazole antifungal agents have a fungistatic effect at low and therapeutic concentrations.

FCZ's antifungal properties have been attributed to the inhibition of the synthesis of an important fungal cell membrane component, ergosterol (Figure 8). This mode of action involves the selective inhibition of fungal sterol-14α-demethylase, a critical CYP isoenzyme required for the synthesis of ergosterol (Guo *et al.*, 2010; Terell, 1999; Kelly *et al.*, 1995).

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Disruption of this fungal CYP isoenzyme (sterol-14α-demethylase) leads to inhibition of the conversion of an ergosterol precursor, lanosterol, to ergosterol via a demethylation process (Ringel, 1990). This interference, attributed to the formation of a toxic sterol intermediate (methylsterols) which is inserted into the membrane instead of ergosterol, leads to an alteration in fungal cell membrane composition which gives rise to impaired cell membrane integrity and fungal cell functioning (Sheehan *et al.*, 1999). This ultimately results in the inhibition of fungal cell growth and further replication (Thompson *et al.*, 2009).

FCZ, as well as another triazole agent itraconazole, has an increased affinity for the fungal P450 enzyme system rather than its mammalian counterpart (Sheehan *et al.*, 1999; Grant *et al.*, 1990). FCZ, when compared with ketoconazole/the older imidazoles, therefore does not result in a large extent of toxicity due to the interaction with mammalian sterols (Ringel, 1990).

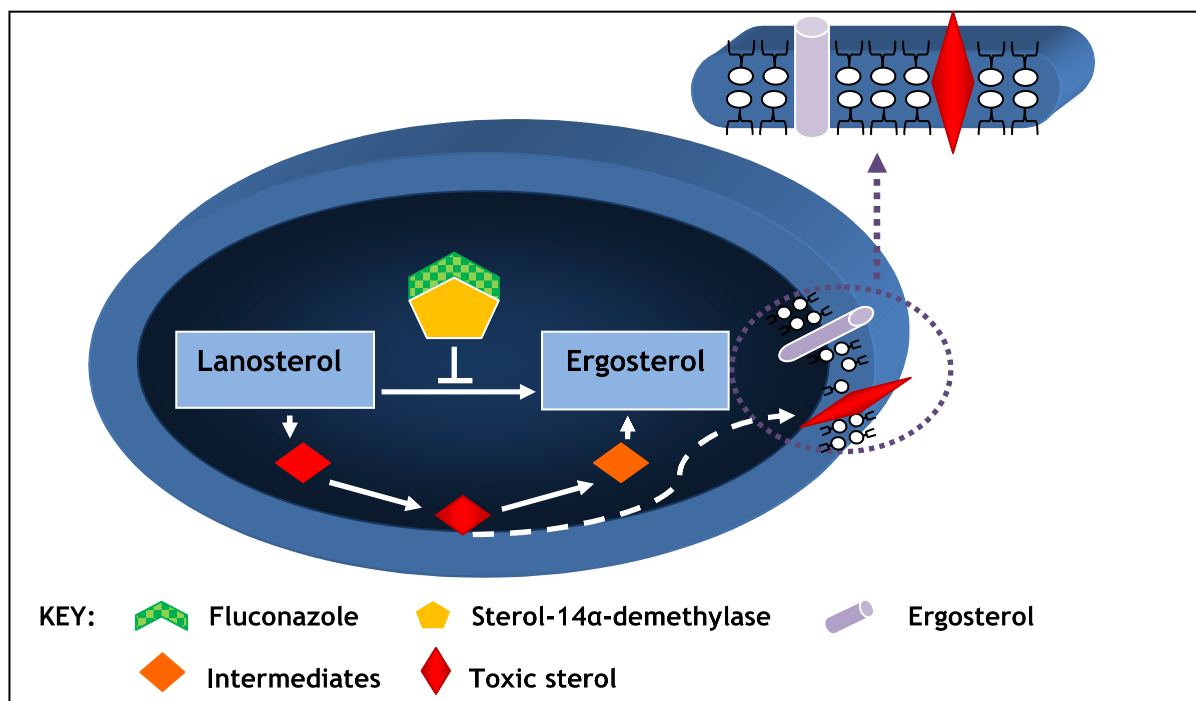

Figure 8: A schematic representation of fluconazole's mechanism of action.

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1.5.1.4 Pharmacokinetics

FCZ has been an important drug in the antifungal armamentarium because of its favourable pharmacokinetic profile. Within the African setting it remains the most prescribed new age antifungal. The triazoles, including FCZ, are highly hydrophilic molecules (Grudzien *et al.*, 2009; Brammer *et al.*, 1990). FCZ can therefore be administered orally as well as by an intravenous infusion. Following oral administration the drug is well and almost completely absorbed within 2 hours. FCZ is widely distributed throughout the body and has a bioavailability of >90% (Roos *et al.*, 2008; Chen *et al.*, 2007). This pattern of distribution has been observed in both healthy volunteers and HIV-positive patients (Wildfeuer *et al.*, 1997). Due to the small size of the molecule, FCZ shows excellent distribution into the central nervous system compartments. Numerous studies suggest that FCZ cerebrospinal fluid (CSF) concentrations are about 80% compared to the corresponding plasma concentrations (Wildfeuer *et al.*, 1997). Its absorption is not greatly affected by food or the absence of gastric acid, therefore FCZ can be taken away from food (Diflucan® package insert). The half-life ($t_{1/2}$) of the drug following oral administration ranges from 20 to 50 hours, with a mean $t_{1/2}$ of roughly 30 hours (Debruyne *et al.*, 1993). Steady state plasma levels are reached within 4-5 days. Following a 200 mg oral dose, steady state concentrations of approximately 4.6 mg/L ± 1.1 mg/L are achieved. The time to reach maximum plasma concentration ranges between 2.4 and 3.7 hours (Debruyne, 1997).

FCZ blood concentrations display a very low degree of interpatient variability because of its favourable PK profile and as distribution of the drug amounts to total body water which shows relatively little variation (Wildfeuer *et al.*, 1997). Consequently variation in plasma drug concentrations is limited (±20 % CV) in healthy individuals. A good linear relationship between the dose administered and the resulting plasma concentration has been demonstrated

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in both healthy subjects and immunocompromised patients (Debruyne, 1997; Wildfeuer *et al.*, 1997; Brammer *et al.* 1990). Roos *et al.* (2008) however, demonstrated that a larger degree of variation exists in patients diagnosed with HIV/AIDS as a result of disease interactions and DDIs (which will be discussed below).

FCZ is primarily cleared by renal elimination. Approximately 91% of the dose is excreted in the urine; 80% as unchanged drug and the remaining 11% as a combination of glucoronidated and nitrogen oxide drug metabolites (Chen *et al*., 2007; Debruyne *et al.*, 1993; Brammer *et al.*, 1990). The aforementioned metabolites are no longer active following hepatic metabolism by CYP3A4 isoenzymes. Because FCZ relies almost solely on renal elimination, dosage adjustments may be necessitated in patients with impaired renal function (Chen *et al.*, 2007; Wildfeuer *et al.*, 1997). Debruyne (1997) showed that the elimination half-life of FCZ could increase up to 98 hours in renal impaired patients with a creatinine clearance of less than 20 ml/min.

1.5.1.5 Side effects

When oral FCZ therapy was first introduced in the early 1990s, it was thought that the drug was devoid of serious adverse effects. Continuous research regarding FCZ and its safety has largely shown this to be true. As previously mentioned FCZ has a greater affinity for the fungal CYP enzyme system and therefore boasts with an improved safety profile. In most patients FCZ is well tolerated in dosages up to 1600 mg/day (Debruyne, 1997). It is however recommended that a maximum daily dose of 1600 mg should not be exceeded in order to avoid possible neurological side effects such as hallucinations, vivid dreams and insomnia. According to Grant *et al.* (1990) the incidence of adverse events in patients treated with FCZ is approximately 16%. For oral FCZ dosages of 100 mg up to 400 mg, the most common

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side effects experienced involved the gastrointestinal system (Chen *et al*., 2007). Nausea, vomiting, diarrhoea, abdominal pain, skin rash and headache are side effects frequently encountered in patients taking FCZ (Chen *et al.*, 2007; Sheehan *et al.*, 1999). The only caveat in FCZ's armour is the potential risk of developing hepatotoxicity in certain patients as well as the possibility of causing QT prolongation which may result in arrthymias (Guo *et al.*, 2010; Albengres *et al.*, 1998). Impaired hepatic functioning is normally identified following abnormal liver functions tests (LFTs).

Even though patients using FCZ experience less adverse effects than when treated with ketoconazole, plasma concentration levels should, in certain situations, be monitored due to its interaction with the CYP enzyme system.

1.5.1.6 Drug-drug interactions

A retrospective cohort study performed by Yu and colleagues (2005) at a tertiary care teaching hospital in Boston, Massachuchetts followed patients admitted to the hospital within a four year period between July 1997 and June 2001. The study included patients treated with oral FCZ, itraconazole or ketoconazole; during this period 3953 patients admitted were treated with FCZ. The researchers' findings were that 2716 patients on FCZ experienced a DDI and approximately 711 of these were serious DDIs. The population of HIV infected patients within this cohort was small (n=170) and only 4.3% of these patients experienced an interaction between FCZ and their antiretroviral therapy. The need for additional medications in HIV patients is common, however, and complex DDIs may result.

Significant DDIs between FCZ and co-administered medications have been observed in healthy volunteers and patients. From the drugs investigated in this pharmacokinetic study, FCZ is not highly bound to plasma proteins (11-12%) which explains that no interactions

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involving protein binding have been reported (Robertson *et al*., 2005). The main mechanism of drug interaction is due to FCZ's inhibitory effect on mammalian CYP enzymes, mainly CYP2C9, CYP2C19 and CYP3A4 to a lesser extent (Piscitelli *et al.*, 2001). In this manner FCZ would decrease the clearance of the co-administered agent resulting in increased plasma concentrations of these agents. Elevated drug concentrations may lead to serious adverse effects as well as discontinuation of therapy. The main DDIs experienced with FCZ, as indicated in the Diflucan® package insert, includes: certain benzodiazepines including diazepam, cyclosporine and tacrolimus, hydrochlorothiazide, midazolam, phenytoin, rifampicin and rifabutin, sulfonylureas, theophylline and warfarin. A more extensive list of potential DDIs with FCZ is presented in Appendix B.

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2.0 Gaps in research

- Data pertaining to ARV pharmacokinetic drug interaction studies is restricted in resource limited countries such as South Africa.

- Knowledge of DDIs between FCZ and the ARVs is limited; this includes the effect of FCZ on the pharmacokinetics of nevirapine, efavirenz and the lopinavir/ritonavir combination.

- Knowledge of patient characteristics or covariates that influence the pharmacokinetics of efavirenz, nevirapine and lopinavir/ritonavir is limited within specific, local patient populations.

- The use of pharmacometrics to model and simulate pharmacokinetic data of the investigated ARVs and their potential DDI with FCZ in the treated local patient population.

2.1 Aim of the study

The primary aim of this thesis was to evaluate the effect of FCZ on the pharmacokinetics of efavirenz, nevirapine and lopinavir/ritonavir in HIV-infected patients diagnosed with cryptococcal meningitis or oropharyngeal candidiasis. There is a large degree of inter- and intraindividual variation with regards to serum drug concentrations due to individual differences in the expression of metabolizing enzymes. Therefore a population pharmacokinetic approach has been applied to study the pharmacokinetics (alteration in PK)

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and potential DDIs between FCZ and the ARVs nevirapine, efavirenz, lopinavir/ritonavir in adult patients. In addition to this, population PK data of the investigated ARVs were also obtained.

The results of this study provide evidence based guidance for the combined antiretroviral-antifungal treatment in patients with cryptococcal meningitis and oropharyngeal candidiasis as well as information about relevant covariates/patient characteristics that may influence optimal therapy.

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3.0 Materials and methods

3.1 Clinical method

3.1.1 Subjects

A prospective study was conducted in HIV-positive, treatment experienced adult (≥18 years old) patients (N=80) which were subdivided into two separate groups. The first group (n=54), which will further be referred to as the control group, consisted of patients treated with one of the antiretroviral agents (ARVs) investigated; efavirenz, nevirapine or lopinavir/ritonavir, but not with FCZ. The cohort of patients (n=26) in the second group, also referred to as the treatment group, comprised of patients treated with one of the ARVs investigated as well as the antifungal, FCZ. FCZ was used in the patients for the treatment of oropharyngeal candidiasis (n=5), cryptococcal meningitis (n=21). Patients were recruited from three established infectious diseases outpatient clinics in the Western Cape area; Tygerberg Hospital, Karl Bremer Hospital and the T.C. Newman Clinic in Paarl. Patients under the age of 18 years and those with nausea and vomiting or severe anaemia were excluded from the study.

3.1.2 Protocol

This study was approved by the Health Research Ethics Committee at Stellenbosch University (N10/06/212) and conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, South African Guidelines for Good Clinical Practice (GCP) and the Medical Research Council (MRC) Ethical Guidelines for Research. The study also received approval from the Department of Health (DOH) as well as

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authorisation from the heads of the three respective clinics. The final version of the protocol is provided in Appendix C.

Written informed consent was obtained from each patient after details of the study, including the risks and benefits of the study, had clearly been explained to them in a language that the patient was comfortable with. Furthermore, all patients were informed that the study was entirely voluntary and they could leave the study at any point in time with no prior notice or reason. In addition to this they were also provided with the telephone number of one of the clinics doctors to answer any questions. The patient information and consent forms were available in English, Afrikaans and Xhosa (Appendix D-F). Detailed patient information and characteristics were recorded in 2 separate case record forms (CRFs) at each visit (Appendix G-H).

Patients were treated according to the regular standard of care. FCZ was prescribed by the patients' physicians.

3.1.3 Study design

The study was designed as a prospective, open, uncontrolled pharmacokinetic study with sparse sampling in an HIV-infected cohort. All participants had been at steady state for the respective antiretroviral regimens containing either 200 mg nevirapine twice daily, 600 mg efavirenz nocturnally or two tablets lopinavir/ritonavir (2 x 400/100 mg) twice daily. Patients recruited to the treatment group were also at steady state for FCZ. All patients had been at steady state for FCZ, in other words on treatment for more than 5 days.

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3.1.4 Sample collection

The blood sampling schedule for the purpose of the study was to collect four (4) random blood samples of 5 ml each (total 20 ml) from each study participant. These samples were collected over the span of two visits (2 blood samples per visit) to the outpatient clinic and a minimum time period of one hour between blood draws was maintained (Figure 9). When possible, these extra blood samples were taken at the time of routine clinical venepuncture so as to minimize discomfort to the patient.

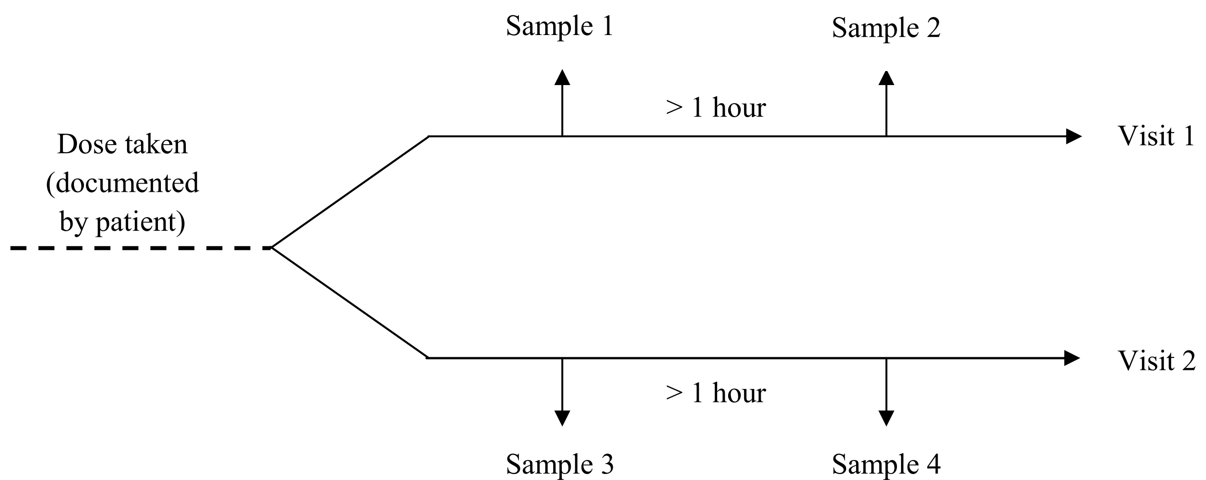

Figure 9: Flow diagram of blood sampling schedule in study participants.

There were a number of deviations from the planned sampling schedule due to loss to follow-up or unwillingness of a patient to complete the required number of blood draws. Following an interim analysis of the pharmacokinetic data, the protocol was amended to include serial blood sampling from consenting patients. This amendment was approved by the Ethics Committee. For these patients (n=4), blood samples were collected at times indicated in Figure 10 or as close to these times as possible.

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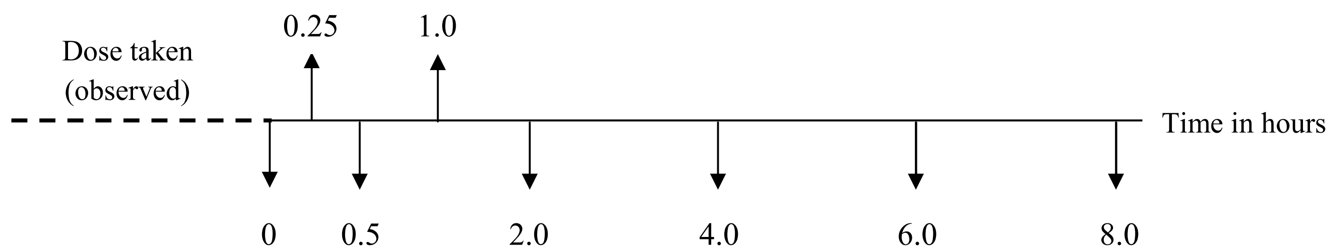

Figure 10: Flow diagram of serial blood sampling schedule performed in four patients.

Exact doses, dosing times, and blood sampling times were carefully recorded for each patient during each visit. Adherence during the week prior to blood sampling was assessed by means of a pill count.

Blood was collected in 7.5 ml Vacuette tubes containing serum separating clot activator. The blood samples were centrifuged at 1.789 g for 10 minutes to obtain serum. The serum was then promptly harvested and placed in a heated water bath (57°C) for an hour. After the serum samples were left to cool, they were stored in the -80°C freezer in the Division of Pharmacology, Stellenbosch University until they were shipped to the Therapeutic Drug Monitoring (TDM) Scientific Laboratory (Infectious Diseases), Wuerzburg, Germany for further analysis. All samples were analysed by validated high performance liquid chromatography (HPLC) or gas chromatography (GC) methods described below.

3.1.5 Statistical analysis

The identified independent variables were age, sex, race, body weight, height, BMI and concomitant medications. The mean values, standard deviation (SD), coefficient of variance, median and range for each parameter were calculated and the results presented in tables and

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graphs using Graph Pad Prism version 5.01 (GraphPad software, Inc, San Diego, California). Normality of patient characteristics was assessed by Shapiro-Wilks normality tests and the significant difference was then established by either an unpaired t-test or a Mann Whitney test. Contingency tables and chi-square tests were used to assess the categorical out A statistically significant difference was defined when $p < 0.05$.

A stepwise regression approach was used when modelling the data. This included a forward inclusion, by starting with no variables and adding any significant variables one by one, and backward elimination which is starting with all potential variables and then removing them one by one if not significant.

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3.2 Experimental method

3.2.1 Chemicals and reagents

All reference standards used are free bases.

3.2.1.1 Efavirenz

Efavirenz powder was purchased from Sequoia Research Products Ltd (Pangbourne, UK). The internal standard used, A86093, was purchased from Abbott Labs (Chicago, IL, USA). The following reagents were purchased from Merck (Darmstadt, Germany): potassium dihydrogen phosphate (KH_2PO_4); di-sodium hydrogen phosphate (Na_2HPO_4); HPLC grade methanol (MeOH), diethylether $(C_2H_5)_2O$ and HPLC grade water (H_2O). Fetal calf serum (FCS) was obtained from BIOCHROM AG (Berlin, Germany) and analytical grade acetonitrile (CH_3CN) was purchased from Sigma Aldrich (Steinheim, Germany).

3.2.1.2 Nevirapine

Nevirapine powder was purchased from Sequoia Research Products Ltd (Pangbourne, UK) and the internal standard, scandicaine from Astra Chemicals (Astra GmbH, Wedel, Germany). Analytical grade $(C_2H_5)_2O$, MeOH, dimethyl sulfoxide (DMSO), HPLC grade H_2O, sodium dihydrogen carbonate (Na_2HCO_3) and sodium carbonate (Na_2CO_3) were obtained from Merck (Darmstadt, Germany).

3.2.1.3 Lopinavir

Lopinavir powder was purchased from Sequoia Research Products Ltd (Pangbourne, UK). The lopinavir assay makes use of the same internal standard, A 86093 (Abbott Labs, Chicago, Illinois, USA) as used in the efavirenz analysis. FCS was purchased from

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BIOCHROM AG (Berlin, Germany) and analytical grade CH_3CN was purchased from Sigma Aldrich (Steinheim, Germany). All the other reagents were supplied by Merck (Darmstadt, Germany): $(C_2H_5)_2O$; KH_2PO_4; Na_2CO_3; $NaHCO_3$; MeOH; n-hexane and HPLC-grade H_2O.

3.2.1.4 Fluconazole

FCZ powder and the internal standard (IS), phenacetin, were both purchased from Sigma-Aldrich (Taufkirchen, Germany). Analytical grade CH_3CN was obtained from Sigma Aldrich (Steinheim, Germany). HPLC grade MeOH, sodium hydroxide (0.1N NaOH), chloroform ($CHCl_3$), K_2HPO_4 and HPLC grade H_2O were purchased from Merck (Darmstadt, Germany). FCS used to prepare the calibration curves and control samples were obtained from BIOCHROM AG (Berlin, Germany).

3.2.2 Preparation of calibration curves and quality controls

For the preparation of the standard samples an appropriate amount of the working solutions and the internal standard (ISTD) were added to blank serum samples to achieve a specific range of calibration concentrations (Tables 6, 7, 8 and 9). All stock solutions were stored at 4°C and were stable for at least 3 months and the long-term matrix stability is at least 6 months at 4°C. The solutions were warmed up to room temperature prior to use.

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3.2.2.1 Efavirenz

Table 6: Pipetting scheme for preparation of efavirenz calibration curves and control samples

No	EFV concentration (ng/ml)	Solution No	Volume pipetted (µl)	Volume of buffer (µl)	Volume of FCS (µl)	Volume of ISTD (µl)
LLQ	125	WS I	10	500	200	25
1	250	WS I	20	500	200	25
2	500	WS I	40	500	200	25
3	1 000	WS I	80	500	200	25
4	2 500	SS	20	500	200	25
5	5 000	SS	40	500	200	25
6	10 000	SS	80	500	200	25
ULQ	15 000	SS	120	500	200	25

EFV Stock Solution (SS) — 10 mg EFV/100 ml MeOH:KH_2PO_4 (1:1 v/v)
Working Solution I (WS I) — 10 ml SS/90 ml MeOH:KH_2PO_4 (1:1 v/v)
ISTD Solution — 10 mg Abbott ISTD A86093/100 ml MeOH:KH_2PO_4 (1:1 v/v)
Buffer — Sörensen-phosphate buffer (pH 7.0)

3.2.2.2 Nevirapine

Table 7: Pipetting scheme for preparation of nevirapine calibration curves and control samples

No	NVP concentration (ng/ml)	Solution No	Volume pipetted (µl)	Volume of buffer (µl)	Volume of NCS (µl)	Volume of ISTD (µl)
LLQ	150	WS I	75	500	250	25
1	250	WS I	125	500	250	25
2	500	WS II	25	500	250	25
3	1 000	WS II	50	500	250	25
4	2 500	WS II	125	500	250	25
5	5 000	SS	25	500	250	25
6	10 000	SS	50	500	250	25
ULQ	20 000	SS	125	500	250	25

NVP Stock solution (SS) — 5mg NVP/100 ml DMSO/MeOH (1:1 v/v)
Working Solution I (WS I) — 5 µg NVP/10ml DMSO/MeOH (1:1 v/v)
Working Solution II (WS II) — 50 µg NVP/10 ml DMSO/MeOH (1:1 v/v)
ISTD Solution — 4 mg scandicaine/100 ml H2O
Buffer — Sodium carbonate buffer (pH 9.4)

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3.2.2.3 Lopinavir

Table 8: Pipetting scheme for lopinavir calibration curves and controls samples

No	LPV concentration (ng/ml)	Solution No	Volume pipetted (µl)	Volume of Na-Ca buffer (µl)	Volume of FCS (µl)	Volume of ISTD (µl)
LLQ	150.00	WS I	15.0	500	500	25
1	250.0	WS I	25	500	500	25
2	500.0	WS I	50	500	500	25
3	1000.0	WS I	100	500	500	25
4	2000.0	SS	20	500	500	25
5	4000.0	SS	40	500	500	25
6	6000.0	SS	60	500	500	25
ULQ 1	10000	SS	100	500	500	25
ULQ 2	20 000	SS	200	500	500	25

LPV Stock Solution (SS) 10 mg LPV/100 ml H_2O/CH_3CN (1:1 v/v)
Working Solution I (WS I) 0.5 mg LPV/100 ml H_2O/CH_3CN (1:1 v/v)
ISTD Solution 5 mg Abbott ISTD A86093/100 ml MeOH
Buffer Sodium carbonate buffer (pH 9.4)

3.2.2.4 Fluconazole

The FCZ method described below was newly developed in the routine Therapeutic Drug Monitoring (TDM) Scientific Laboratory of the Infectious Diseases Centre of Würzburg Medical Centre in Würzburg, Germany.

Table 9: Pipetting scheme for preparation of fluconazole calibration curves and control samples

No	FCZ concentration (µg/ml)	Solution No	Volume pipetted (µl)	Volume of NaOH (µl)	Volume of FCS (µl)	Volume of ISTD (µl)
LLQ	0,25	WS I	12,5	200	250	25
1	0,5	WS I	25	200	250	25
2	1,0	WS I	50	200	250	25
3	2,0	WS I	100	200	250	25
4	4,0	SS	10	200	250	25
5	10,0	SS	25	200	250	25
6	20,0	SS	50	200	250	25
ULQ	40,0	SS	100	200	250	25

FCZ Stock Solution (SS) 10 mg FCZ in 100 ml MeOH
Working Solution I (WS I) 0.5 mg FCZ in 100 ml MeOH
ISTD Solution 5 mg phenacetin in 100 ml MeOH

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3.2.3 Instrumentation and chromatographic conditions

3.2.3.1 Efavirenz

For the determination of efavirenz serum levels a Beckman Coulter (Beckman Coulter GmbH, Stuttgart, Germany) HPLC system was used. The system consists of a System Gold 508 Autosampler, a System Gold Diode Array Detector Module 168 and a System Gold Solvent Module 125. The analytical column used was an XTerra™ RP18 column (2.1X150 mm I.D., particle size 5µm) from Waters Corporation (Eschborn, Germany).

The chromatographic separation was performed with a gradient elution. The UV signal was monitored at both 245 nm and 255 nm throughout the analysis. The two mobile phase components were as follows: mobile phase A consisted of 1/15 M potassium dihydrogen phosphate buffer (KH_2PO_4): acetonitrile (CH_3CN) at a ratio of 60:40 (adjusted to pH 6.7 with sodium hydroxide) and mobile phase B consisted of 1/15 M potassium dihydrogen phosphate buffer (KH_2PO_4): acetonitrile (CH_3CN) at a ratio of 30:70 (adjusted to pH 6.7 with sodium hydroxide). All solutions were filtered through a 0.45 µm membrane filter (Millipore Corporation, Billerica, MA, USA) prior to use.

The total run time for each sample was 30 min and a washing step was completed between each measurement in order to ensure complete elution of all substances. The analysis was performed at room temperature (22°C). The conditions for separation are outlined in Table 10.

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Table 10: Conditions for the mobile phase applied for analytical separation of efavirenz with regards to time, mobile phase and flow rate.

Time (min)	Flow rate (ml/min)	Mobile phase A[1]	Mobile phase B[2]	Duration (min)
0	0.20	70	30	2
2*	0.20	20	80	5
7~	0.25	20	80	12
19*	0.25	70	30	5
24~	0.20	70	30	6
30	0.20	70	30	STOP

[1] Mobile phase A: 1/15 M KH_2PO_4:CH_3CN (60:40), pH 6.7

[2] Mobile phase B: 1/15 M KH_2PO_4:CH_3CN (30:70), pH 6.7

* Change in mobile phase composition occurs within 30 sec

~ Change in flow rate occurs within 30 sec

3.2.3.2 Nevirapine

A Hewlett-Packard 5890 series II gas chromatograph was used for analysis. The system consisted of a 7673 autosampler and an HP 3396 series II integrator (Hewlett Packard GmbH, Waldbronn, Germany), equipped with a nitrogen–phosphorus-detector (NPD). Chromatography was performed with an RTX-5 Amine, 95% dimethyl – 5% diphenyl polysiloxane column, 15mX0.32µm 1.0µ film thickness from Restek Chromatography products (Restek GmbH, Bad Homburg, Germany). The carrier gas, helium 5.0, had a constant inlet pressure of 7.55 p.s.i and the injection mode was splitless (1 µl) for 0.3 min.

The temperature program was as follows: 120°C at time zero followed by a 10°C increase per min up to 310°C, the final temperature. This temperature was maintained for 2 min. The temperature of the injection port and the NPD detector was fixed at 300°C.

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3.2.3.3 Lopinavir

Lopinavir levels were determined by an HPLC analytical system consisting of the following: a Spark Holland™ Triathlon Autosampler (Emmen, Netherlands); a System Gold Solvent Module (Beckman Coulter, Krefeld, Germany); a System Gold 168 detector (Beckman Coulter, Krefeld, Germany) and a Jetstream II Plus Column Thermostat from Techlab GmbH (Erkerode, Germany). An XTerra™ RP18 analytical column (2.1X150 mm I.D., particle size 5µm) from Waters Corporation (Eschborn, Germany) was used.

Lopinavir concentrations were determined by means of a gradient method. The absorption was measured at both 220 nm and 254 nm. The two mobile phases were as follows: mobile phase A consisted of 20 mM potassium dihydrogen phosphate buffer (KH_2PO4): acetonitrile (CH_3CN) at a ratio of 80:20 (adjusted to pH 4.9 with H_3PO_4) and mobile phase B consisted of 20 mM potassium dihydrogen phosphate buffer (KH_2PO4): acetonitrile (CH_3CN) at a ratio of 30:70 (adjusted to pH 4.9 with H_3PO_4). All solutions were filtered through a 0.45 µm membrane filter (Millipore Corporation, Billerica, MA, USA) prior to use. The volume injected from each sample vial was 20 µl and the analysis was performed at room temperature (22°C).

The total run time for each sample was 30 min and a washing step was completed between each measurement in order to ensure complete elution of all substances. Lopinavir eluted after 18.7 min and scandicaine, the internal standard, after 20.1 min. Table 11 outlines the separation conditions.

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Table 11: Conditions for the mobile phase applied for analytical separation of lopinavir with regards to time, mobile phase and flow rate.

Time (min)	Flow rate (ml/min)	Mobile phase A[1]	Mobile phase B[2]	Duration (min)
0	0.20	65	35	10
10*	0.20	30	70	8
18~	0.25	30	70	5
23	0.25	65	35	2
27~	0.20	65	35	3
30	0.20	65	35	STOP

[1] Mobile phase A: 20 mM KH_2PO_4:CH_3CN (80:20), pH 4.9
[2] Mobile phase B: 20 mM KH_2PO_4:CH_3CN (30:70), pH 4.9
* Ratio of mobile phases increased linearly over 10 min period
~ Immediate change in flow rate

3.2.3.4 Fluconazole

The HPLC system consisted of a System Gold 508 Autosampler, a System Gold model 168 NM Diode Array Detector (DAD) and a System Gold 126 Solvent Module all from Beckman Coulter (Krefeld, Germany). The oven used was a Jetstream II Plus Column Thermostat from Techlab GmbH (Erkerode, Germany). An ASCENTIS Express™ liquid chromatography column (C18 2.1x100 nm, 2.7µ particle size), a Supelco product from Sigma-Aldrich (Steinheim, Germany) was used.

FCZ was measured by means of an isocratic method. The mobile phase was prepared by mixing filtered 20 mM potassium dihydrogen phosphate (K_2HPO4) buffer and acetonitrile (CH_3CN) at a ratio of 85:15 (v/v) with an adjusted pH of 3.0. The flow rate was set at 0.3 ml/min. The column was heated to 25°C and the volume injected from each sample vial was 1 injection of 20 µl. The UV absorption was measured at wavelengths of 210 nm and 260 nm. The total run time for each sample was 12 min and a washing step was done between

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each sample to ensure complete elution of all substances. The retention times of FCZ and the internal standard, phenacetin, were 4.3 and 8.6 min respectively.

3.2.4 Sample extraction

3.2.4.1 Efavirenz

Frozen serum samples were firstly thawed to room temperature. Following this the analytical samples were prepared by pipetting 25 µl of the internal standard, 500 µl of Sörensen phosphate buffer (41.3:58.7; 1/15 M KH_2PO4:1/15 M Na_2HPO_4; pH 7.0) and 200 µl of serum into a 15 ml screw cap test tube. After this 3 ml of diethyl ether were added to the test tube which was then vortexed for 5 min to ensure complete mixing of all substances. The samples were then centrifuged (Eppendorf Centrifuge 5804R, Hamburg, Germany) at 2.264 g for 5 min. The upper, organic layer was transferred to a clean conical tube which was evaporated to dryness under a gentle stream of nitrogen at 43°C. The addition of diethyl ether, mixing and drying under nitrogen stream following transferral to the conical vial was repeated. The resulting residue was either capped for storage in a refrigerator (4-5°C) until further analysis or reconstituted with 300 µl of a dilution mixture (1 1/15 M KH_2PO_4; 0.5 CH_3CN; 0.5 MeOH). The solution was vortexed at approximately 30 sec and centrifuged at 2.264 g for 5 min. In certain 'polluted' samples further separation was needed. In such a case the sample solution was transferred to a 1 ml plastic eppendorf tube (Eppendorf, Hamburg, Germany) and mixed in a microcentrifuge (Eppendorf Micro Centrifuge, Hamburg, Germany) at 15.399g for 5 min. The sample was then transferred to an autosampler vial with glass insert for analysis.

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3.2.4.2 Nevirapine

The sample preparation and extraction procedure of nevirapine were identical to the method described above for efavirenz, however, different extraction solutions were used. Sample preparation was done by spiking 250 µl of patient serum with 25 µl of internal standard and 500 µl of sodium carbonate buffer pH 9.4 (46 ml Na_2CO_3 and 204 ml $NaHCO_3$). Liquid-liquid extraction was performed following the method described above. The nevirapine residue was subsequently reconstituted with 300 µl of a DMSO/MeOH (1:1 v/v) solution.

3.2.4.3 Lopinavir

The sample preparation and extraction procedure of lopinavir were identical to the method described above for efavirenz and nevirapine, however, different extraction solutions were used. Samples were prepared by spiking 500 µl of patient serum with 25 µl of internal standard and 500 µl of sodium carbonate buffer pH 9.4 (46 ml Na_2CO_3 and 204 ml $NaHCO_3$). In addition to the previously described liquid-liquid extraction method, the lopinavir residues, once reconstituted with 220 µl of 20mM KH_2PO_4:MeOH:CH_3CN (1:0.5:0.5 v/v, pH 4.9), were washed with 1 ml n-hexane and then centrifuged for 5 min. The n-hexane was subsequently decanted.

3.2.4.4 Fluconazole

The frozen serum samples obtained from patients were firstly thawed to room temperature prior to the extraction procedure. Once thawed, the samples for HPLC analysis were prepared by aliquoting 25 µl of the IS, 200 µl of 0.1 N sodium hydroxide (NaOH) and 250 µl of patient serum into a 15 ml screw cap test tube. FCZ was subsequently extracted (liquid-liquid extraction) by adding 3 ml of chloroform to the test tube. The test tube was vortexed for 5 min to ensure complete mixing of all substances. Thereafter the samples were

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centrifuged (Eppendorf Centrifuge 5804R, Hamburg, Germany) at 2.264 g for 5 min which allowed for the complete separation of the two phases. After centrifugation the organic (lower) layer was transferred to a clean conical glass vial. The vials were then placed under a nitrogen stream (43°C) in order to evaporate the organic layer to dryness. The aforementioned procedure was then repeated for a second time in order to ensure optimum FCZ extraction. At this point the samples were either capped then stored in a refrigerator (4-5°C) for analysing at a later date or the samples were resolved and prepared for HPLC analysis.

Following extraction, the FCZ precipitate was resolved in 200 µl of potassium phosphate buffer. This was then again vortexed for approximately 30 sec and centrifuged at 2.264 g for 5 min before it was transferred to a 2 ml autosampler vial with glass insert.

3.2.5 Validation of the analytical method

The analytical laboratory provides a routine TDM service, specifically for ARVs and other anti-infectives. Although the bioanalytical method has not been strictly validated according to FDA and EMA guidelines, the laboratory is accredited and the assays have been sufficiently established for clinical use.

3.2.5.1 Specificity and selectivity

Serum samples were spiked with various substances to ensure that the signal measured by the detector comes from the substance of interest and that no other excipients, impurities, metabolites or concomitant medications, that may be present in the serum sample, interfere with the specific analyte. Three serum samples spiked with different concentrations of

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efavirenz, nevirapine, lopinavir/ritonavir and FCZ were analysed for interference with each of the compounds listed below.

Efavirenz, nevirapine, lopinavir/ritonavir and fluconazole

The following compounds were investigated for interference with the analytes: abacavir, adefovir, amprenavir, amoxicillin, aspirin, atorvastatin, dapsone, didanosine, efavirenz, fluconazole, folinic acid, ganciclovir, indinavir, itraconazole, lamivudine, lopinavir, methadone, methotrexate, nelfinavir, M8-metabolite of nelfinavir, nevirapine, oxazepam, paracetamol, pyrazinamide, pyrimethamine, ranitidine, rifampin, ritonavir, saquinavir, stavudine, sulfamethoxazole, sulfadoxin, tenofovir, trimethoprim, zalcitabine, and zidovudine.

No interfering peaks were observed at the retention times of efavirenz, nevirapine, lopinavir/ritonavir or FCZ.

3.2.5.2 Accuracy, precision and recovery

The accuracy and intra-day precision of each method were determined by measuring replicate serum samples of the test compound at various concentrations. The results are shown in Table 12.

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Table 12: Conditions for determining accuracy and precision of the analytical methods for the various test compounds.

	EFV	NVP	LPV/r	FCZ
No. of replicate plasma samples	8	6	6	8
No. of different concentrations	4	3	3	4
Sample concentration (µg/ml)	0.2	0.75	1.0	0.5
	2.3	2.5	5.0	4.0
	6.0	5.5	20.0	10.0
	8.5	-	-	20.0

Please see Appendix I for an example of fluconazole's interday precision.

Accuracy was calculated as the relative error of the nominal concentration (C_{nom}) and the mean value of the observed concentrations (C_{obs}) as follows: accuracy (%) = [(C_{nom}-C_{obs})/C_{nom}] x 100. Precision of each method was expressed in terms of relative standard deviation and was calculated from the observed concentrations as follows: precision (%) = [standard deviation (SD)/C_{obs}] x 100. Calculated accuracy (RE %) and precision (CV %) for each method are presented in Table 13.

The recovery of all the tested compounds was estimated by comparing the peak areas in extracted spiked serum samples to those of non-processed standard solutions. There is no difference in recovery between the calf serum matrix and that of serum samples. Recovery for efavirenz, nevirapine, lopinavir and FCZ was 96.2 ± 3.0 %, 97.8 ± 3.8 %, 95.1 ± 2.2 % and 94.9 ± 3.6 % respectively.

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Table 13: Accuracy and precision for the analytical methods of efavirenz, nevirapine, lopinavir and fluconazole using spiked serum samples: intra-day precision (C.V. %) and accuracy (RE %)

EFV				
No. of replicate serum samples	8	8	8	8
Sample concentration (µg/ml)	0.2	2.3	6.0	8.5
Mean concentration found (µg/ml)	0.202	2.275	5.969	8.049
C.V. %	2.6	3.2	2	3.8
RE %	-1.3	1.1	0.5	5.3
NVP				
No. of replicate serum samples	-	18	18	18
Sample concentration (µg/ml)	-	0.75	2.5	5.5
Mean concentration found (µg/ml)	-	0.7	2.37	5.159
C.V. %	-	4.8	4.6	4.1
RE %	-	6.6	5.1	6.2
LPV				
No. of replicate serum samples	-	8	8	8
Sample concentration (µg/ml)	-	1.0	5.0	20.0
Mean concentration found (µg/ml)	-	1.12	5.09	19.67
C.V. %	-	3.43	0.65	0.41
RE %	-	-12	-1.8	1.65
FCZ				
No. of replicate serum samples	8	8	8	8
Sample concentration (µg/ml)	0.5	4.0	10.0	20.0
Mean concentration found (µg/ml)	0.58	4.13	10.12	19.93
C.V. %	8.8	1.67	0.7	0.9
RE %	-16	-3.25	-1.2	0.35

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3.2.5.3 Calibration curves

The specific ranges of calibration concentrations for all of the investigated compounds are discussed above (Tables 6, 7, 8 and 9). The calibration curves, regression equations and correlation coefficients (r^2-values), as determined by the least squares analysis, are shown in Figures 11-14. In the following figures (Figure 11-14) the x-axis refers to the serum concentration and the y-axis indicates the amount/height quotient.

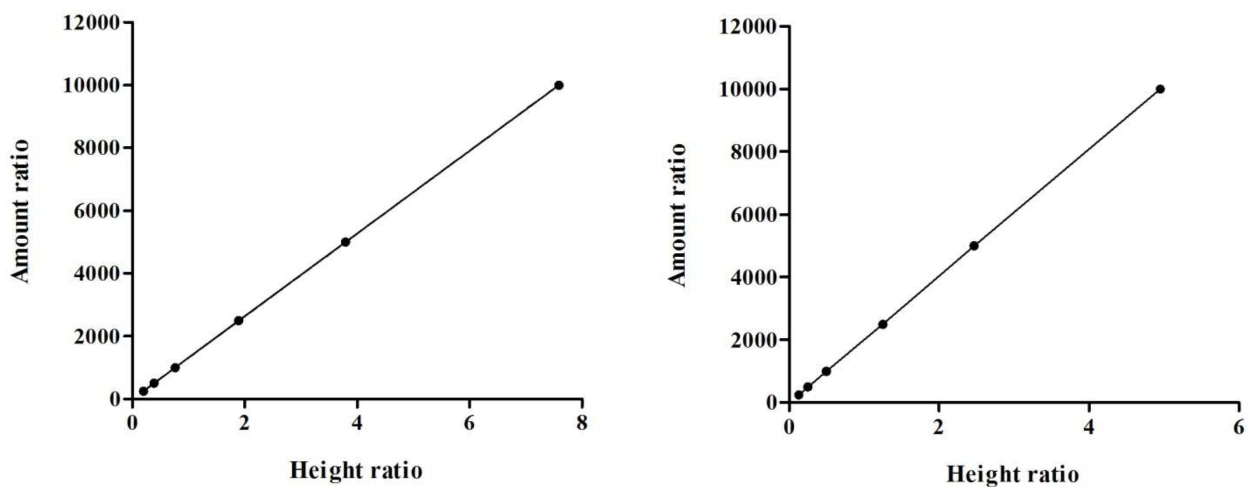

Figure 11: Calibration curves of efavirenz at (a) 245 nm, y = 1320.74 x – 6.67682, r^2 = 0.999 and at (b) 255 nm, y = 2024.11 x – 12.1730, r^2 = 0.999.

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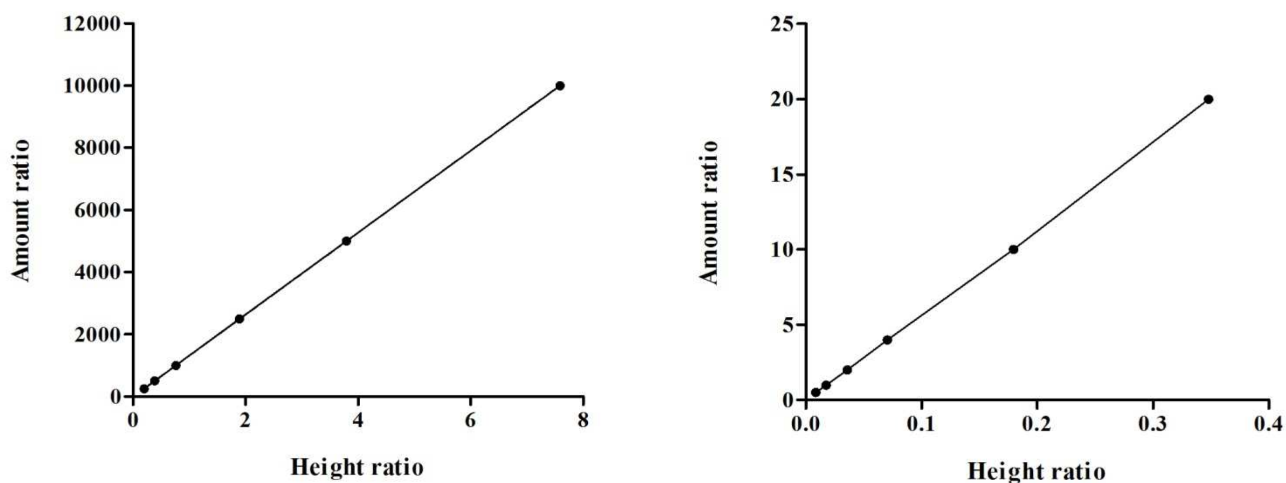
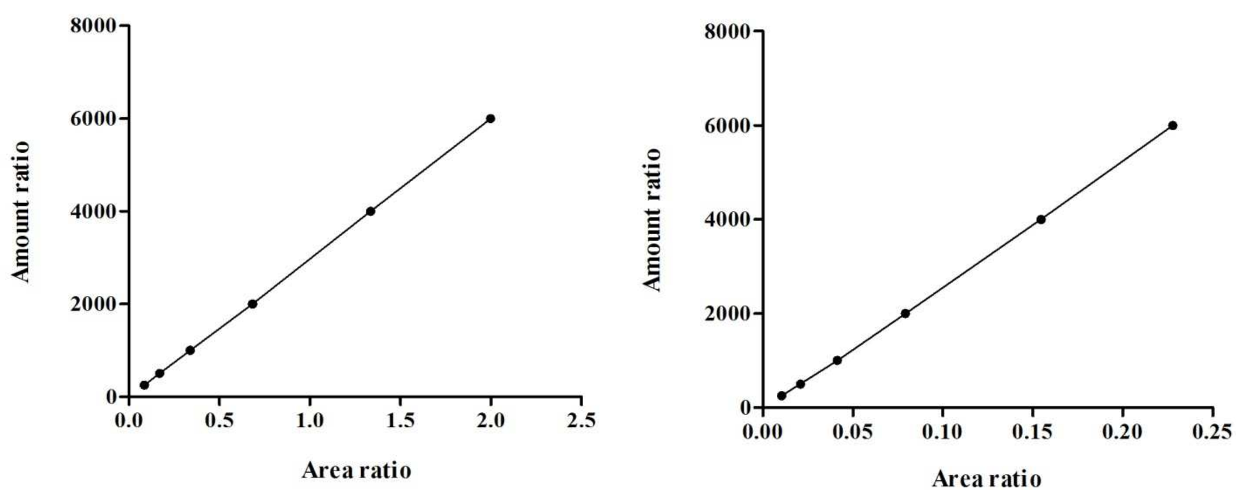


(a) **(b)**

Figure 12: Calibration curves of nevirapine at (a) 245 nm, y = 1320.74 x – 6.67682, r^2 = 0.999 and at (b) 255 nm, y = 2024.11 x – 12.1730, r^2 = 0.999



(a) **(b)**

Figure 13: Calibration curves of lopinavir at (a) 220 nm, y = 3007.14 x – 21.9032, r^2 = 0.999 and at (b) 250 nm, y = 26483 x – 67.7264, r^2 = 0.999

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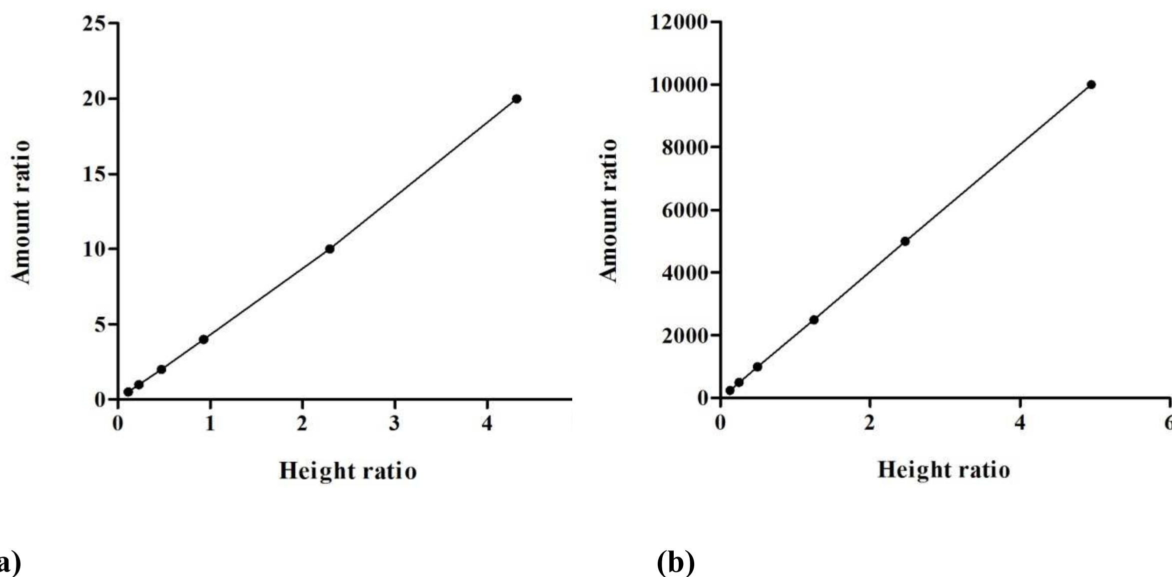

(a) **(b)**

Figure 14: Calibration curves of fluconazole at (a) 210 nm, $y = 4.6224\,x - 0.172663$, $r^2 = 0.999$ and at (b) 260 nm, $y = 58.4205\,x - 0.0911347$, $r^2 = 0.999$

3.2.5.4 Stability

Various storage conditions were investigated for each analyte to ensure stability during the analysis. Spiked serum samples were tested in the following conditions: 30 days at -20°C; 4 months at -20°C; 7 days at 4°C; 24 hours at room temperature (23-25°C); 60 min at 56°C and after four freeze-thaw cycles. The stability of the drugs was also tested at 57°C which is used as the viral inactivation step in sample preparation procedure. No degradation occurred for efavirenz, nevirapine, lopinavir or FCZ in any of the listed stability studies.

Furthermore, all the drugs of interest (efavirenz, nevirapine, lopinavir/ritonavir and fluconazole) were stable after extraction for at least 3 months at 4°C.

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3.3 Population pharmacokinetic analysis

3.3.1 Datasets used in the model development

Patients in the control and treatment group were administered either 600 mg efavirenz once daily, 200 mg nevirapine twice daily or 800/200 mg lopinavir/ritonavir twice daily. Patients in the treatment group were administered 200 mg FCZ mg once daily. The serum concentration-time data of efavirenz, nevirapine and lopinavir are based on a sparse sampling design, where most individuals had at least two blood samples taken. All available data were used for the population pharmacokinetic analysis.

3.3.2 Population Pharmacokinetic Model

Population pharmacokinetic models were built using a non-linear mixed-effects modeling approach. The first-order conditional maximum likelihood estimation in the NONMEM program (double precision, Version 7.1.2, ICON Development Solutions, Elliott City, MD) and NM-TRAN pre-processor were used. Models were run using the G-Fortran Compiler (GNU Compiler Collection) on a personal computer under the Microsoft Windows Vista operating system (Compaq Presario CQ70). PerlSpeaksNONMEM 2.3.1 (http://psn.sourceforge.net/) running activePerl 5.8.9 (ActiveState Software Inc., Vancouver, BC, Canada) were used to manage NONMEM batch files. The subroutines within NONMEM were linear mammillary models (ADVAN4 used with TRANS4 in the PREDPP library) to investigate a two-compartment, absorption model.

An open-compartment, open model with first order elimination provided the best fit to the sparse dataset. The structural pharmacokinetic model for the two-compartment model

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consists of the following parameters: absorption rate constant (K_a), oral clearance (CL) and volume of the central compartment (V). K_a is fixed to literature values. All individual V assumes the population mean value. CL was assumed to be log-normally distributed and exponential inter-individual variability terms were included in the pharmacokinetic parameters in the model (Equation 1). A proportional residual error model was used (Equation 2).

$$P_i = P\exp(\eta_i^p)$$ .... **Equation 1**

where:

P_i = the true parameter value for individual i

P = the typical value (population mean) of the parameter

η_i^p = the difference between the true value for individual, i, and the typical value for the population, with a mean of 0 and a variance of ω^2

$$C_{ij} = (\hat{C}_{ij}\varepsilon_{1ij} + \varepsilon_{2ij}) \bullet w$$ .... **Equation 2**

where:

C_{ij} = the jth measured observation (serum concentration) for individual i

$\hat{C}_{ij}$ = the jth model predicted value (serum concentration) for individual i

ε_{1ij} = the proportional residual error for the jth measurement for individual i, and is normally distributed with a mean of 0 and a variance of σ_1^2

ε_{2ij} = the additive residual error for the jth measurement for individual i, and is normally distributed with a mean of 0 and a variance of σ_2^2

w = the weighting factor defined as $\sqrt{C_{ij}^2}$

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Population pharmacokinetic parameters, without covariates, were estimated. Estimates of the pharmacokinetic parameters for each individual were subsequently obtained using Bayesian estimation and were further used in determining their relationship with dose and food state.

3.3.3 Covariate analysis

Covariate screening was accomplished using stepwise covariate model (SCM) in PerlSpeaksNONMEM (PsN). The hypothesis testing to discriminate among alternative hierarchical structural models based on the p-values for the forward inclusion and backward elimination at 0.05 and 0.01, respectively. Both linear and exponential relationships were explored. The resulting model that included all significant covariates was considered the "final" population pharmacokinetic model.

3.3.4 Model validation

A degenerate visual predictive check was performed by the simulation of the parameter estimates of the final model to generate 500 individual profiles. The median and 95% prediction intervals for the concentration at each time points were plotted and compared to the original data using Xpose4 package running on R (v. 2.13.0) (Figures 28-30).

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4.0 RESULTS

4.1 Patient population

Eighty HIV-positive patients were included in this study. The control group consisted of fifty four patients (n=54) who were taking one of the investigated ARVs as part of their treatment regimen. The treatment group consisted of twenty six patients (n=26) who were taking FCZ in addition to one of the investigated ARVs. Demographic characteristics (age, gender, race, weight, height, BMI) are presented in Table 14.

Table 14: Demographics and baseline characteristics of the study population

	Control group (n=54)	Treatment group (n=26)
Age (years)		
Mean, SD	39 ± 8	38 ± 8
Range	21 – 62	26 - 56
Gender, no. (%)		
Male	20 (37%)	8 (31%)
Female	34 (63%)	18 (69%)
Race, no. (%)		
Black	27 (50%)	14 (54%)
Coloured	27 (50%)	11 (42%)
White	0 (0%)	1 (4%)
Other	0 (0%)	0 (0%)
Weight (kg)		
Mean, SD	69.8 ± 15.4	61.8 ± 16.5
Range	40.4 - 112,0	38.6 – 107.6
Height (cm)		
Mean, SD	165 ± 8	164 ± 9
Range	151 – 186	147 - 183
Body Mass Index (kg/m^2)		
Mean, SD	25.8 ± 5.7	22.4 ± 4.8
Range	14.4 – 42.7	13.8 – 32.1
Adherence (%)		
Mean, SD	95 ±11	87 ±13
Range	59 – 113	56 – 104
Missing values (n)	7	3

SD standard deviation

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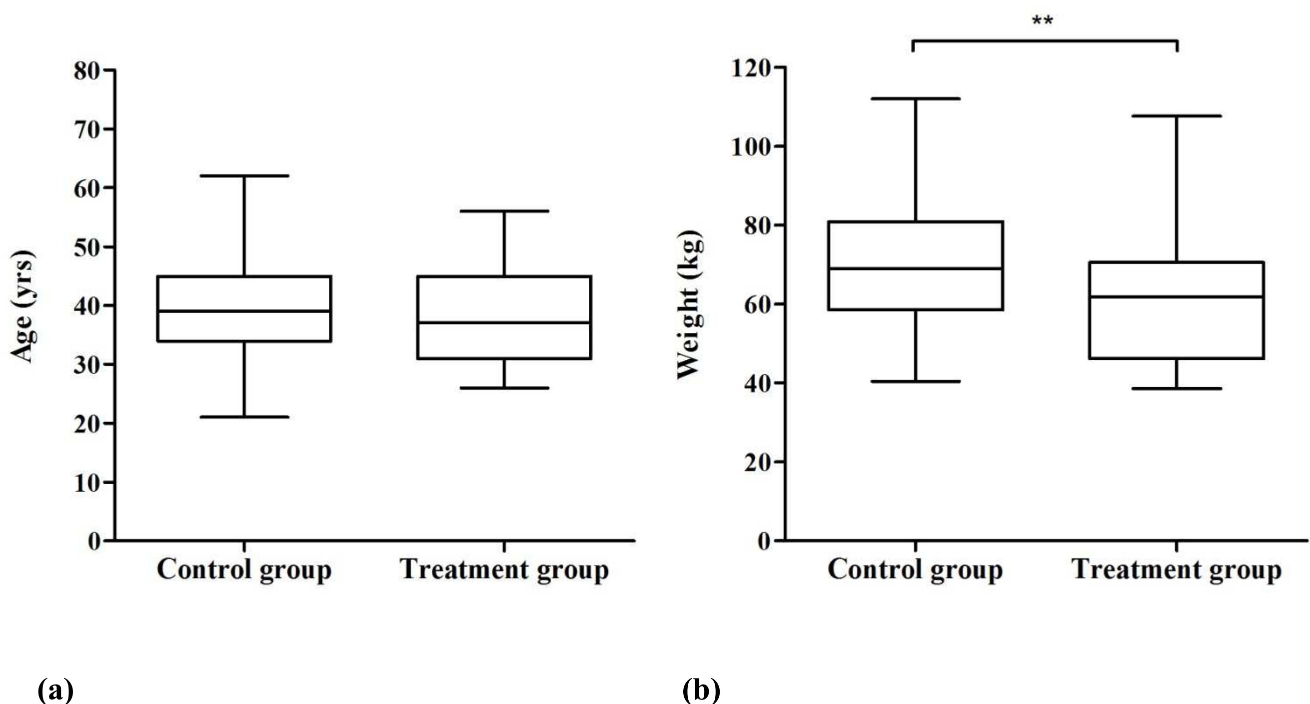
Fifty six patients (70%) were studied on two occasions as planned and twenty four patients (30%) were only studied on one occasion. The reason for the aforementioned was loss to follow-up (16 patients, 66.7%) or unwillingness to further participate in the study (8 patients, 33.3%).

An unpaired t-test was used to assess whether the two groups were significantly different ($p<0.05$) for patient characteristics that were normally distributed (age, weight and BMI) as verified by the Shapiro-Wilks normality test. The variable height was considered to be not normally distributed ($p=0.0004$, Shapiro-Wilks normality test), therefore the Mann Whitney non-parametric test ($p=0.96$) was used. Weight ($p=0.0022$) and BMI ($p=0.0006$) were significantly different between the two groups. The following box and whisker plots (Figure 15) illustrate the respective distributions; the whiskers indicating the maximum and minimum values of each parameter.



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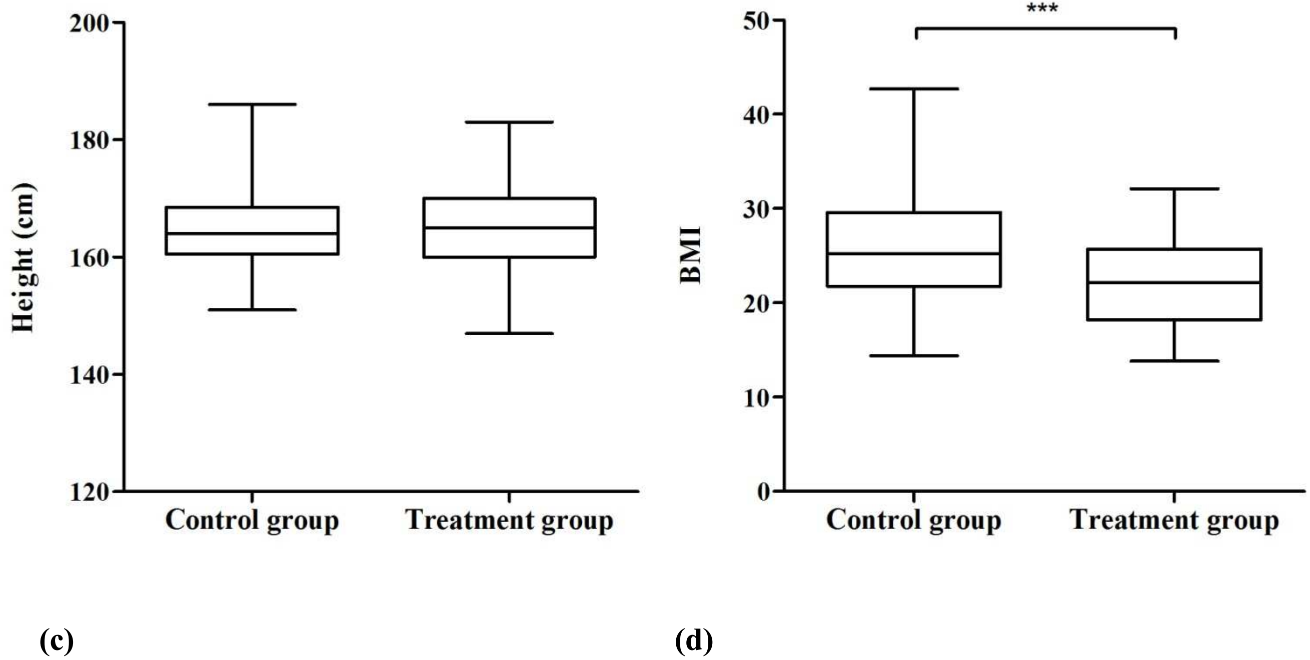

(c) (d)

Figure 15: Box and whisker plots of (a) age, (b) weight, (c) height and (d) BMI in the two groups. * $p < 0.05$

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Clinical Tests

CD4 count, viral load (VL), serum creatinine (CR), estimated glomerular filtration rate (GFR) as determined by the Cockroft & Gault formula (Cockroft *et al.*, 1976) and alanine aminotransferase (ALT) are presented in Table 15. Not all of the abovementioned clinical tests were performed for each patient; the number of missing values is also recorded in the Table 15.

Table 15: Parameters of clinical tests recorded for the study population.

Clinical Test	Control group (n=54)	Treatment group (n=26)	p-value
CD4 count (cells/µl)			
Mean, SD	412 ± 227	227 ± 175	< 0.0001*
Range	8 – 1001	4 - 672	
Missing values (n, %)	3 (3.2)	1 (2.0)	
Viral load (copies/ml)			
<40 (n, %)	60 (64.5)	23 (46.0)	0.0867
>40 (n, %)	25 (26.9)	22 (44.0)	
Range	LDL - 244 151	LDL - 800 000	
Missing values (n, %)	8 (8.6)	5 (10.0)	
Disease stage (WHO staging)			
Stage I (n, %)	3 (5.6)	0 (0.0)	
Stage II (n, %)	15 (27.8)	1 (3.8)	
Stage III (n, %)	28 (51.9)	2 (7.6)	
Stage IV (n, %)	8 (14.8)	23 (88.4)	
Serum creatinine (µmol/l)			
Mean, SD	64 ± 19	72 ± 23	0.0987
Range	36 - 124	36 - 129	
Missing values	18	8	
GFR (ml/min)			
<60 (n, %)	3 (3.2)	4 (8.0)	0.4135
>60 (n, %)	60 (64.5)	29 (58)	
Missing values (n, %)	30 (32.3)	17 (34)	
ALT (U/l)			
Mean, SD	48 ± 68	33 ± 20	0.6214
Range	11 - 320	9 – 84	
Missing values (n, %)	47 (50.5)	17 (34)	

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* Indicates significance ($p < 0.05$)

4.1.1 Control group

4.1.1.1 Patient Characteristics

Twenty patients (37%) in this group were male. Their mean age was 42 years (range: 26 to 62 years), mean body weight was 65.6 kg (range: 41.6 to 86.6 kg), mean height was 171 cm (range: 157 to 186 cm) and mean BMI was 22.5 kg/m^2 (range: 14.4 to 30.0 kg/m^2). Thirty four (63%) patients were female. Their mean age was 37 years (range: 21 to 52 years), mean body weight was 72.2 kg (range: 40.4 to 112.0 kg), mean height was 161 cm (range: 151 to 170 cm) and mean BMI was 27.7 kg/m^2 (range: 17.5 to 42.7 kg/m^2). Patients in the control group were staged as follows using the WHO staging of HIV/AIDS patients (WHO, 2009): three (5.6%) patients were stage 1; fifteen (27.8%) patients were stage 2, twenty eight (51.9%) patients were stage 3 and eight (14.8%) patients were stage 4.

The antiretroviral drugs used in these patients consisted of: efavirenz-based regimens (27 patients, 50%) of 600 mg once daily with an average duration of EFV-based ART of 3 years (range: 1-7 years); nevirapine-based regimens (18 patients, 33%) of 200 mg twice daily with an average duration of NVP-based ART of 2.5 years (range: 0.5 - 5 years) and lopinavir/ritonavir-based regimens (9 patients, 17%) of 400/100 mg twice daily with an average duration of LPV/r-based ART of 1.7 years (range: 1- 4 years). The exact regimens are outlined in Figure 16.

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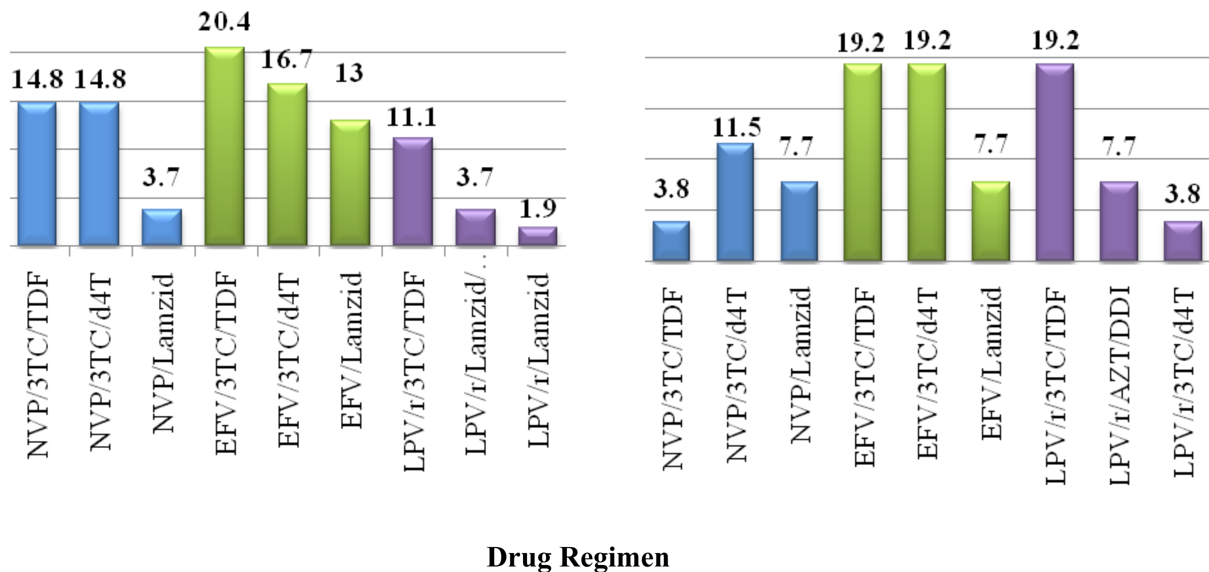

(a) **(b)**

3TC lamivudine; **d4T** stavudine; **DDI** didanosine; **EFV** efavirenz; **Lamzid** lamivudine/zidovudine; **LPV/r** lopinavir/ritonavir; **NVP** nevirapine; **TDF** tenofovir

Figure 16: Percentage of patients in the (a) control group and (b) treatment group receiving various efavirenz, nevirapine and lopinavir/ritonavir-based ARV treatment regimens

4.1.1.2 Concomitant diseases

Five (9.3%) patients had active TB and twenty two (40.7%) patients had at least 1 previous episode of tuberculosis, but were not currently on antimicrobial therapy. Hepatic failure was found in three (5.6%) patients and hepatitis in one patient (1.9%). Seventeen (31.5%) patients previously had at least one episode of oropharyngeal candidiasis and had received FCZ treatment at that time. The most prevalent concomitant diseases are shown in Figure 17.

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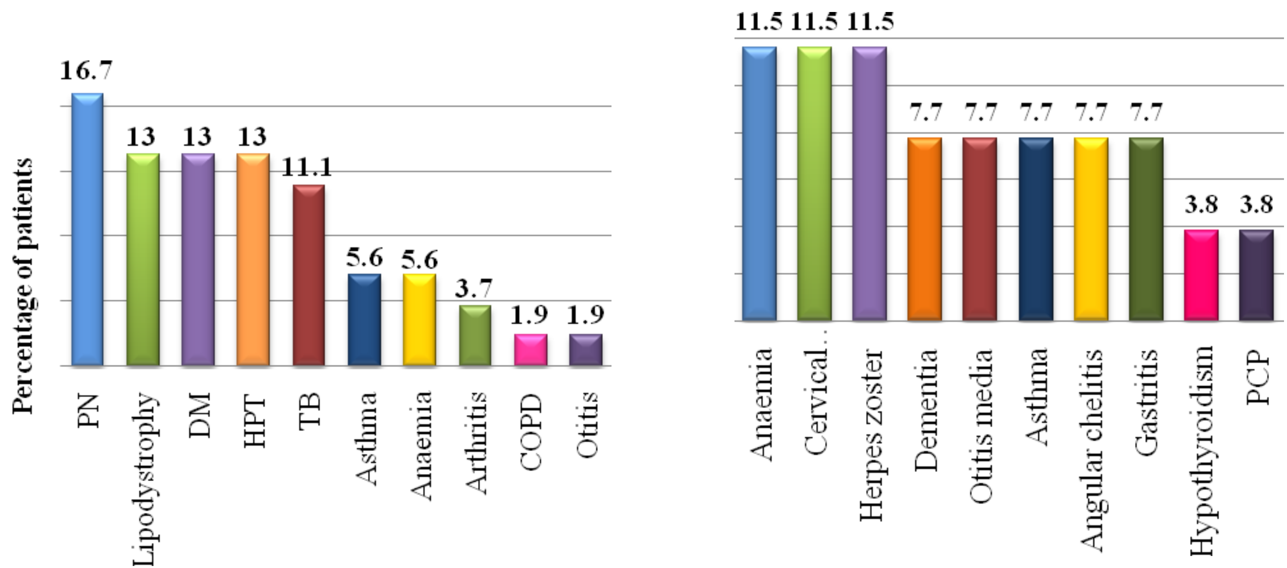

CONCOMITANT DISEASES

(a) **(b)**

COPD chronic obstructive pulmonary disease; **DM** diabetes mellitus; **HPT** hypertension; **PN** peripheral neuropathy; **TB** tuberculosis

Figure 17: Most prevalent concomitant diseases in (a) the control group and (b) the treatment group, given as a percentage of patients

4.1.1.3 Concomitant medications

All of the patients (n=54) were taking vitamin B complex, 6 patients were prescribed pyridoxine (vitamin B6) and 1 patient was given thiamine (vitamin B1) as a supplement. Twenty four (44.4%) patients were taking trimethoprim-sulfamethoxazole (TMP-SMX) as prophylaxis. Anti-TB drugs used in 5 patients included: rifampicin (80%), isoniazid (80%), pyrazinamide (80%), ethambutol (100%), streptomycin (20%), ciprofloxacin (20%) and moxifloxacin (20%). Nine (16.7%) patients were prescribed an antidepressant, amitryptiline. Antidiabetic agents used were metformin (9.3%), gliclazide (5.5%) and human insulin (3.7%). Hydrochlorothiazide (HCTZ), enalapril and amlodipine were given as

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antihypertensive therapy in 3.7%, 7.4% and 1.9% respectively. At the time of sampling three (5.6%) patients were taking amoxicillin and two (3.7%) patients' flucloxacillin.

4.1.2 Treatment group

4.1.2.1 Patient characteristics

Eight (30.8%) patients in this group were male. Their mean age was 41 years (range: 32 to 50 years), mean body weight was 65.0 kg (range: 41.4 to 107.6 kg), mean height was 171 cm (range: 163 to 183 cm) and mean BMI was 22.1 kg/m^2 (range: 13.8 to 32.1 kg/m^2). Eighteen (69.2%) patients were female. Their mean age was 37 years (range: 26 to 56 years), mean body weight was 59.0 kg (range: 38.6 to 84.4 kg), mean height was 160 cm (range: 147 to 174 cm) and mean BMI was 22.6 kg/m^2 (range: 15.1 to 30.1 kg/m^2). Patients in the treatment group were staged as follows using the WHO staging of HIV/AIDS patients (WHO, 2009): one (3.8%) patient was stage 2, two (7.6%) patients were stage 3 and twenty three (88.4%) patients were stage 4.

The ARVs used in the FCZ group consisted of efavirenz-based regimens (13 patients, 50%) of 600 mg once daily; nevirapine-based regimens (5 patients, 19.2%) of 200 mg twice daily and lopinavir/ritonavir-based regimens of 400/100 mg twice daily (8 patients, 30.8%). The exact regimens are outlined in Figure 16. Two patients receiving lopinavir/ritonavir-based regimens received a double dose of the ARV to compensate for co-adminstration. The duration of cART with efavirenz, nevirapine and lopinavir/ritonavir were as follows in the treatment group: 4 years (range: 1-8 years); 4 years (range: 1-6 years) and 1.75 years (range: 0.5-3 years).

4.1.2.2 Concomitant diseases

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None of the patients had active cryptococcal meningitis at the time of the study. Seven (26.9%) patients had active TB and twelve (46.2%) patients had at least 1 previous episode of tuberculosis, but were not currently on antimicrobial therapy. Drug induced hepatitis was found in one (3.8%) patient, one (3.8%) patient was diagnosed with hepatitis B and renal failure was observed in two (7.6%) patients. A weight loss of more than 10% of the patients bodyweight was seen in six (23.1%) patients. The most common concomitant diseases in the treatment cohort are shown in Figure 17.

4.1.2.3 Study drug

Oral FCZ was prescribed to the patients for treatment of either oropharyngeal candidiasis (5 patients, 19.2%) or maintenance therapy for cryptococcal meningitis (21 patients, 80.8%). Twenty five patients (96.2%) were dosed with 200 mg fluconazole per day and one patient (3.8%) received 100 mg FCZ per day. All patients had been at steady state for FCZ, in other words on treatment for more than 5 days.

4.1.2.4 Concomitant medications

TB treatment in these patients consisted of a combination of the following agents: rifampicin (85.7%), isoniazid (71.4%), pyrazinamide (57.1%), ethambutol (71.4%), streptomycin (14.2%), ciprofloxacin (14.2%) and moxifloxacin (14.2%). Dapsone was prescribed to 4 patients as prophylaxis for pneumocystis pneumonia (PCP) and twenty one (80.8%) patients were taking TMP-SMX as prophylaxis.

4.2 Drug serum concentrations

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Altogether 276 blood samples were collected from the 80 study participants for pharmacokinetic evaluation as follows: 137 efavirenz samples (36.5% efavirenz and FCZ); 67 nevirapine samples (32.8% nevirapine and FCZ) and 72 lopinavir/ritonavir samples (59.7% lopinavir/ritonavir and FCZ). The drug serum concentrations for each investigated ARV and the study drug, FCZ, is shown in Figures 18-21.

The study utilised a random sampling protocol. Drug intake was not observed in the majority of the patients, but the time of dose, as expressed by patient recall, was noted and the exact time of blood sampling was recorded. The time period between drug intake and blood sampling for each of the drugs can be seen in Figures 18-21. The therapeutic ranges (or minimum therapeutic plasma concentrations) have been included in Figures 18-21 for illustrative purposes, but it should be noted that for nevirapine and lopinavir/ritonavir the recommended therapeutic minimum trough concentrations are actually based on trough values.

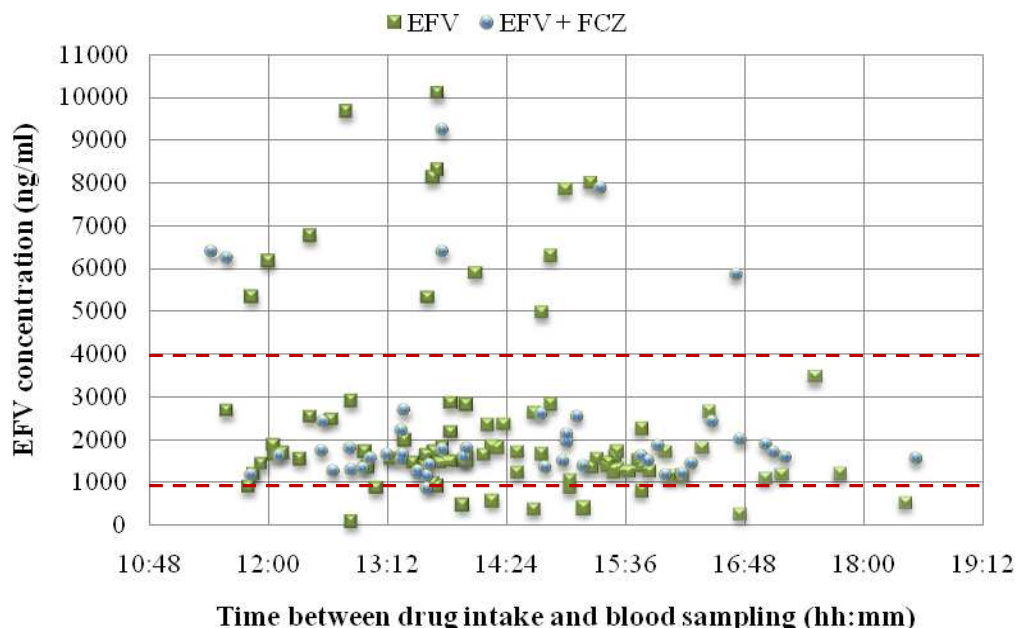

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Figure 18: Distribution of efavirenz plasma concentrations in both patient groups corresponding to the time between drug administration and blood sampling. Therapeutic range indicated by dashed red lines. Three outliers (concentrations >30 000 ng/ml) at 08:51, 10:35 and 12:30 hours post-dose have not been included in this figure.

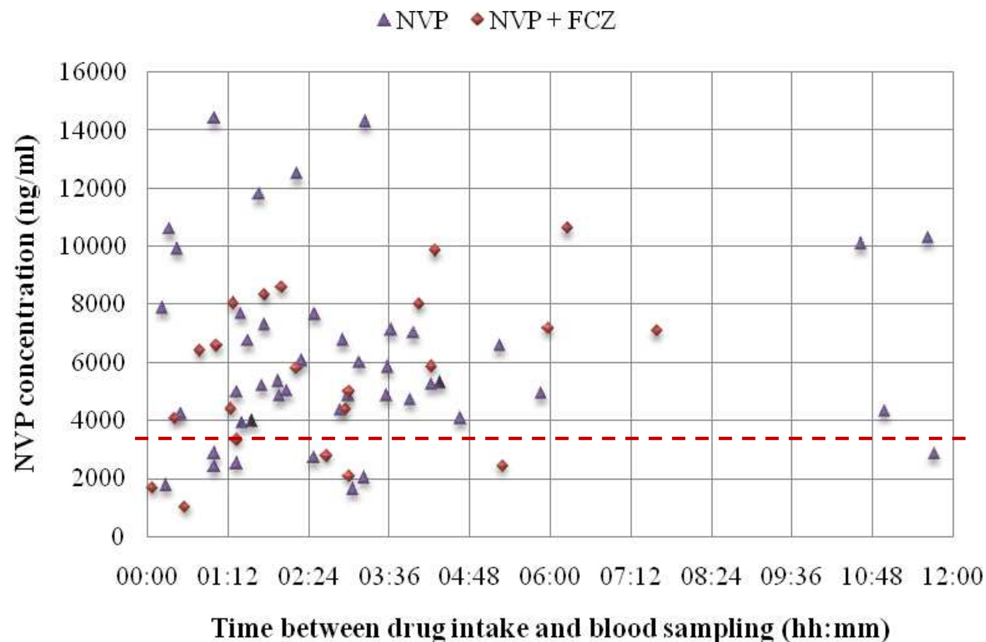

Figure 19: Distribution of nevirapine plasma concentrations in both patient groups corresponding to the time between drug administration and blood sampling. Recommended therapeutic minimum trough concentration indicated by dashed red line.

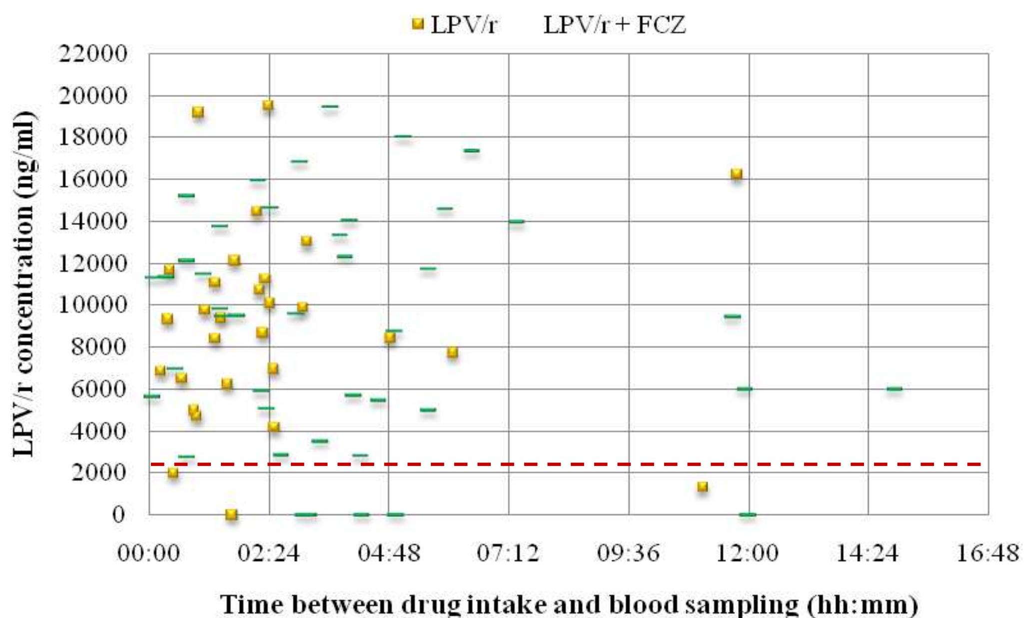

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Figure 20: Distribution of lopinavir/ritonavir plasma concentrations in both patient groups corresponding to the time between drug administration and blood sampling. Recommended therapeutic minimum trough concentration indicated by dashed red line.

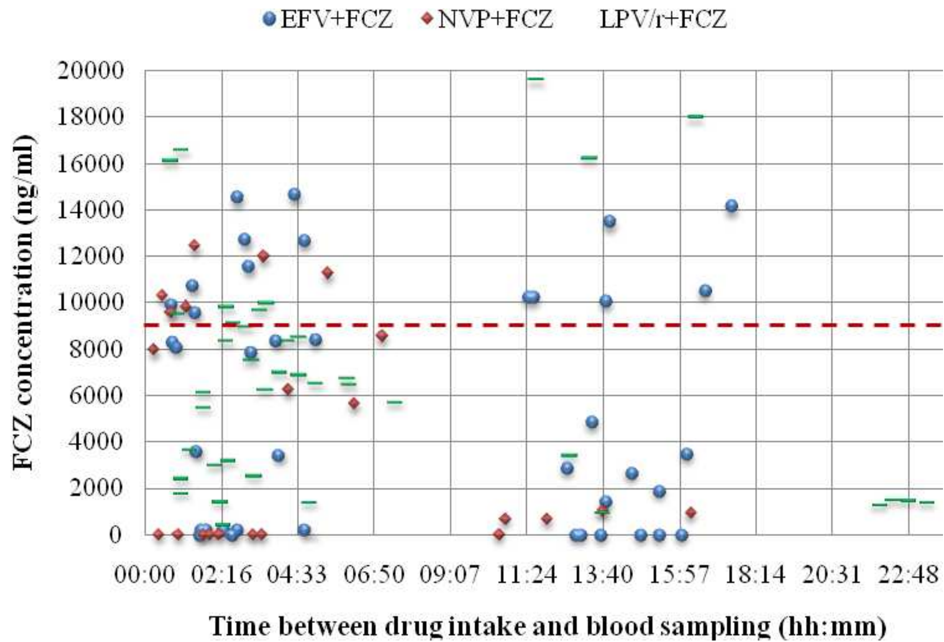
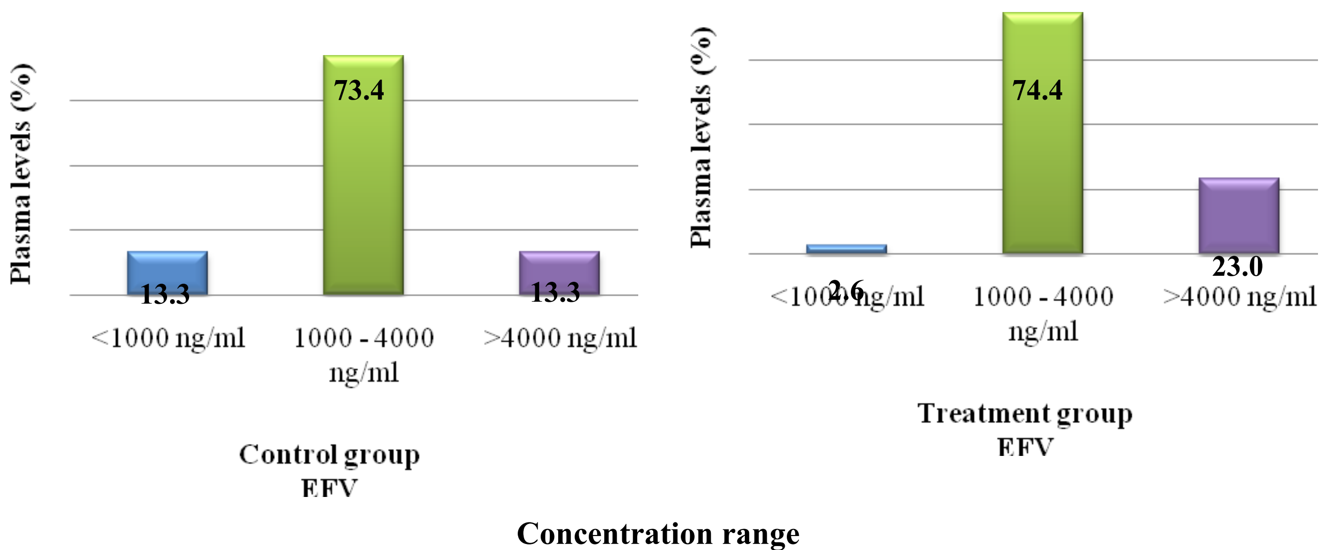


Figure 21: Distribution of fluconazole plasma concentrations in the treatment group, according to ARV regimen, corresponding to the time between drug administration and blood sampling. Recommended therapeutic minimum concentration indicated by red dashed line. One outlier (concentration >25 000 ng/ml) taken12:45 hours post-dose, has not been included in this figure.



(a) **(b)**

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Figure 22: Percentage of efavirenz plasma levels in (a) the control group and (b) the treatment group, subdivided according to the sub-therapeutic, therapeutic and toxic concentration range of efavirenz.

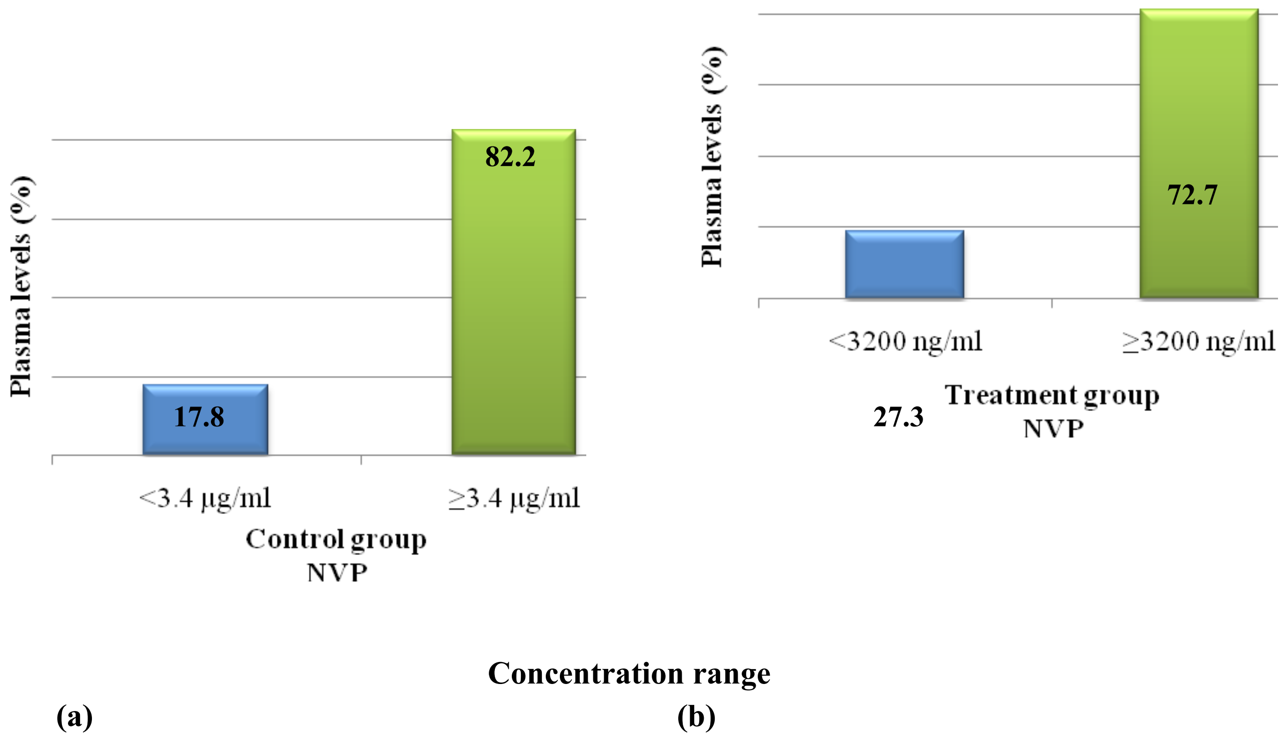

Figure 23: Percentage of nevirapine plasma samples in (a) the control group and (b) the treatment group, subdivided according to the sub-therapeutic and therapeutic nevirapine concentration ranges. The recommended therapeutic range is a trough value and is only used for illustrative purposes.

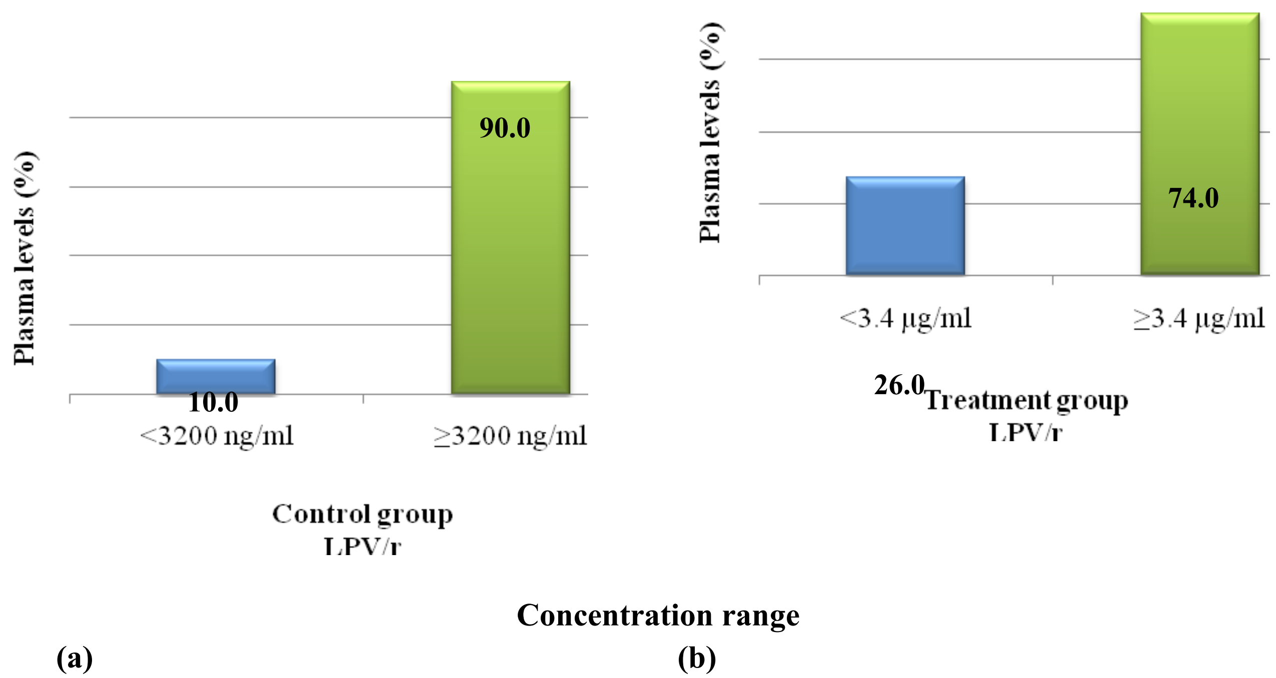

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Figure 24: Percentage of lopinavir/ritonavir plasma samples in (a) the control group and (b) the treatment group, subdivided according to the sub-therapeutic and therapeutic lopinavir/ritonavir concentration ranges. The recommended therapeutic range is a trough value and only used for illustrative purposes.

4.3 Population pharmacokinetics

4.3.1 Base Model

A one-compartment, open model with first order absorption was fitted to the efavirenz, nevirapine and lopinavir steady-state sparse concentration-time data. The structural pharmacokinetic model for the one-compartment model was parameterized on oral clearance (CL), volume of the central compartment (V), absorption rate constant (K_a) and F, the oral bioavailability which was fixed at 1. In order to resolve the issue of non-identifiability (parameters that cannot be estimated from the available data) due to the sparse sampling design, only the absorption parameters were assumed to be log-normally distributed and exponential interindividual variability terms were included in the pharmacokinetic parameters in the model. A proportional residual error model was used. The individual pharmacokinetic estimates for each individual were subsequently obtained using Bayesian estimation. The base model parameters are shown in Table 16 along with the final mode parameters following inclusion of the covariate model, which will be discussed in the next two sections.

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Table 16: Population pharmacokinetic model parameters of the base model and the final model incorporating covariate relationship

Parameter	NONMEM	Population Model	
	Designation	Base Model	Final Model
Efavirenz‡			
CL (L/h)	θ_1	12.0	15.8
V (L)	θ_2	891	860
K_a (h^{-1})	θ_3	0.66	0.66
CL (rifampicin)†	θ_6	~	0.0236
CL (race)†	θ_7	~	-0.432
Interindividual variability			
CL	ω_1	0.785	0.737
Proportional Error	θ_4	0.127	0.127
Additive Error	θ_5	126	126
Objective Function		1905.982	1900.322
Nevirapine			
CL (L/h)	θ_1	4.56	~
V (L)	θ_2	1700	~
K_a (h^{-1})	θ_3	1.66	~
Interindividual variability			
CL	ω_1	0.065	~
Proportional Error	θ_4	0.0284	~
Objective Function		-169.736	~
Lopinavir			
CL (L/h)	θ_1	4.23	4.27
V (L)	θ_2	147	1.72×10^5
K_a (h^{-1})	θ_3	0.85	0.85
CL ($2C8/9_{inhibitor}$ TMP-SMX)†	θ_6	~	3.16
CL (isoniazid & pyrazinamide)†	θ_7	~	-0.536
V (isoniazid & pyrazinamide)†	θ_8	~	-1.00
Interindividual variability			
CL	ω_1	0.36	0.28
V	ω_2	0.54	1.3
Proportional Error	θ_4	0.339	0.276
Additive Error	θ_5	0.043	0.043
Objective Function		1157.382	1130.41

†The covariate parameter was incorporated to the model such that: $(1 + \theta)$ CL or $(1 + \theta)$ V, where $\theta = 0$.
‡Efavirenz final model is based on the forward algorithm. The backward algorithm resulted in the base model.

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The distribution of nevirapine concentrations exhibited large variability that rendered the population pharmacokinetic model unstable. The individual concentrations were log-transformed in order to circumvent the problem with model convergence due to the large variability in the data.

4.3.2 Covariate Model

The concomitant medications were categorized based on their effect on specific CYP isoenzymes. All concomitant medications were characterized as inhibitor, inducer or substrate of a specific CYP isoenzyme or multiple CYP isoenzymes. These categories were set-up to simplify and reduce the number of co-administered drugs in the model. Only known inducers and inhibitors were introduced as categorical variables, substrates were not considered. Table 17 shows the designation for the concomitant medication that patients in the study had taken.

Concomitant medications, due to the multitude of drugs taken by the patients were coded, by their CYP effect, as dummy variables wherein drugs that are strong inducers or inhibitors of a specific CYP isoenzyme were coded as 1 in the CYP variable. This included the study drug, FCZ (Table 17).

Other categorical covariates included sex, race and stage. The dummy variables for sex were 1 for male and 0 for female; and for race, 0 for black patients and 1 for coloured patients. The severity of disease stage, according to WHO staging, had grades from 0 to 4.

The continuous variables included age, weight, height, body mass index, serum creatinine, glomerular filtration rate, and liver function based on ALT values.

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Table 17: Concomitant medications with known effects on cytochrome P450

Concomitant drug	Inhibitor	Inducer
TMP-SMX (Bactrim)	CYP2C8, 9	~
Amitryptiline	CYP2D6	~
Chlorpheniramine	CYP2D6	~
Ciprofloxacin	CYP1A2, 3A4, 5 ,7	~
Clotrimazole	CYP3A4, 5 ,7	~
Diclofenac	CYP2C9	~
Flucloxacillin	CYP3A4,5,7	~
Fluconazole	CYP2C9, 19, 3A4	~
Isoniazid	CYP2C8,9,19, 1A2, 2A6, 3A4,5,7	CYP2E1
Metformin	CYP2D6	~
Promethazine	CYP2D6	~
Pyrazinamide	CYP2C19	~
Ranitidine	CYP2D6	~
Rifampicin	~	CYP3A4,5,7, 1A2, 2B6, 2C8,9,19,2D6
Theophylline	CYP2D6	~
Tryptanol	CYP2D6	~

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4.3.3 Final Model

The model parameters for the final models are listed in Table 16, including the median population values, inter-individual variability and residual variability. The objective functions of the final model were 1900.322, -169.736, and 1130.41 for efavirenz, nevirapine and lopinavir, respectively. The stepwise covariate model algorithm in PsN was employed to automatically determine influential covariates using forward inclusion at p = 0.05 and backward elimination at p = 0.01.

In the efavirenz model, concomitant use of rifampicin and race were significant covariates of CL at the 5% level (p = 0.05) for the forward inclusion step. However, during the backward elimination process at p = 0.01 level, both covariates were not considered a significant. The covariate model equation was coded as $CL = CL_{base}(1+\theta_{i})$, where θ_{i} is the covariate dummy variable when coded as 0. The final efavirenz model was based on the forward algorithm as the backward algorithm resulted in the base model. The estimated clearance of efavirenz from the final model was 15.8 L/hr and after screening the covariates race and co-mediacted rifampicin accounted for a 56.4% and 2% change in efavirenz clearance respectively.

In the lopinavir model, TMP-SMX, clotrimazole and isoniazid had a significant influence on lopinavir clearance at both the 5% (p = 0.05) and 1% (p = 0.01) levels and were included in the final model. The relationship was defined such that $CL = CL_{base}(1+\theta_{i})$, where θ_{i} is the covariate dummy variable when coded as 0. In the forward algorithm, additional covariates that were significant at the 0.05 level were serum creatinine, CYP3A and body mass index. The corresponding objective function of the forward model was 1116.652. The estimated

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clearance of lopinavir from the final model was 4.27 L/hr. The concurrent use of antituberculosis agents accounted for a 46.4% change in lopinavir clearance.

The nevirapine data obtained in the present study comprised of a very extensive spread of drug serum concentrations. No significant covariates were observed in the nevirapine model and the estimated clearance, obtained from the base model, was 4.56 L/hr.

4.3.4 Model validation

The degenerate visual predictive check results are shown in Figures 25 to 27 for efavirenz, nevirapine and lopinavir, respectively. The results were generated from 500 simulations. The 90% prediction interval of the simulated results contained the individual concentration-time profiles from the pooled data relative to the time of their steady state dose. The simulated profiles were able to capture all the observed data, supporting the validity of the model.

Patients taking rifampicin had lower serum efavirenz concentrations, except for the three outliers, as shown by the red filled circles the left panel in Figure 25. In the right panel of Figure 25, coloured patients marked by red filled circles had an overall lower efavirenz concentration compared to black patients represented by black empty circles.

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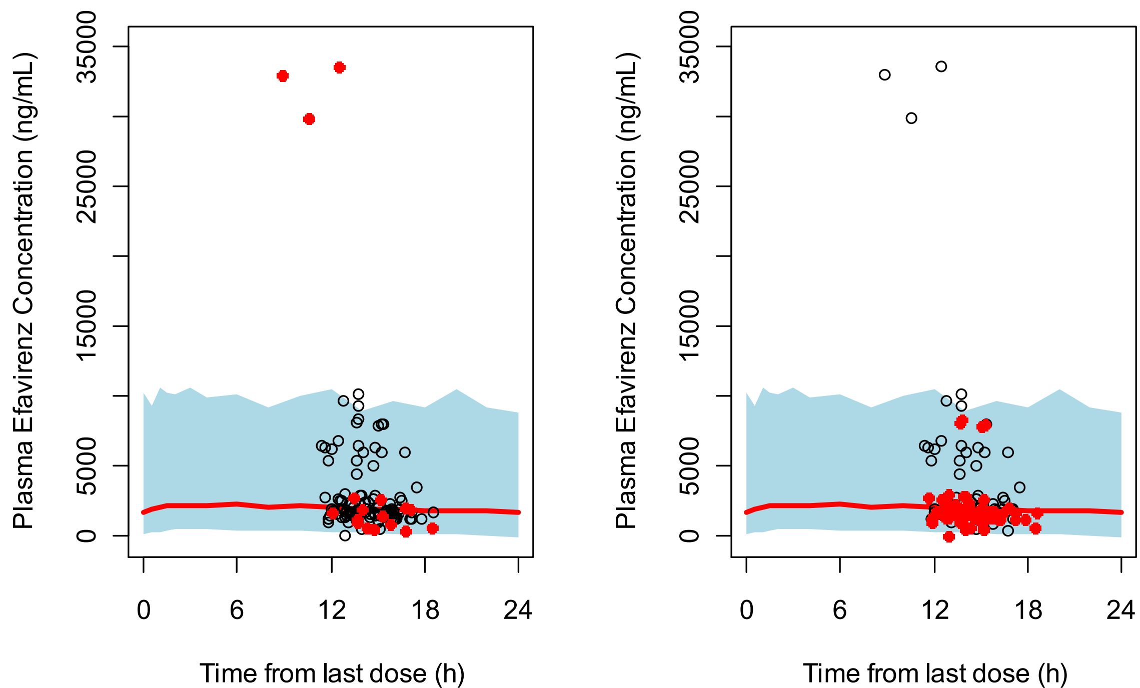

Figure 25: Degenerate visual predictive check showing the median (red line) and the 95% prediction interval (blue shade) of 500 simulated profiles for patients taking efavirenz. The left panel shows the actual efavirenz concentration from patients with (red solid circles) and without (black empty circles) rifampicin concomitant medication. The right panel shows the actual efavirenz concentration from black (black empty circles) and coloured (red solid circles) patients respectively.

Patients that were not taking TMP-SMX tended to have a smaller spread of serum lopinavir concentrations, as shown by the black empty circles the left panel in Figure 26. In the right panel of Figure 26, patients who were administered either isoniazid and pyrazinamide as part of their TB treatment, indicated by the red filled circles, had higher lopinavir concentrations. This was possibly due to their inhibiting effect on CYP3A isoenzymes. Because patients on isoniazid or pyrazinamide had higher peak lopinavir concentrations, and observed lower concentrations at the later time intervals, such as the 12-hour time point, this translates to the effect of the covariate on both V and CL parameters.

A simulation of 500 nevirapine concentrations is shown in a visual predictive check in Figure 27.

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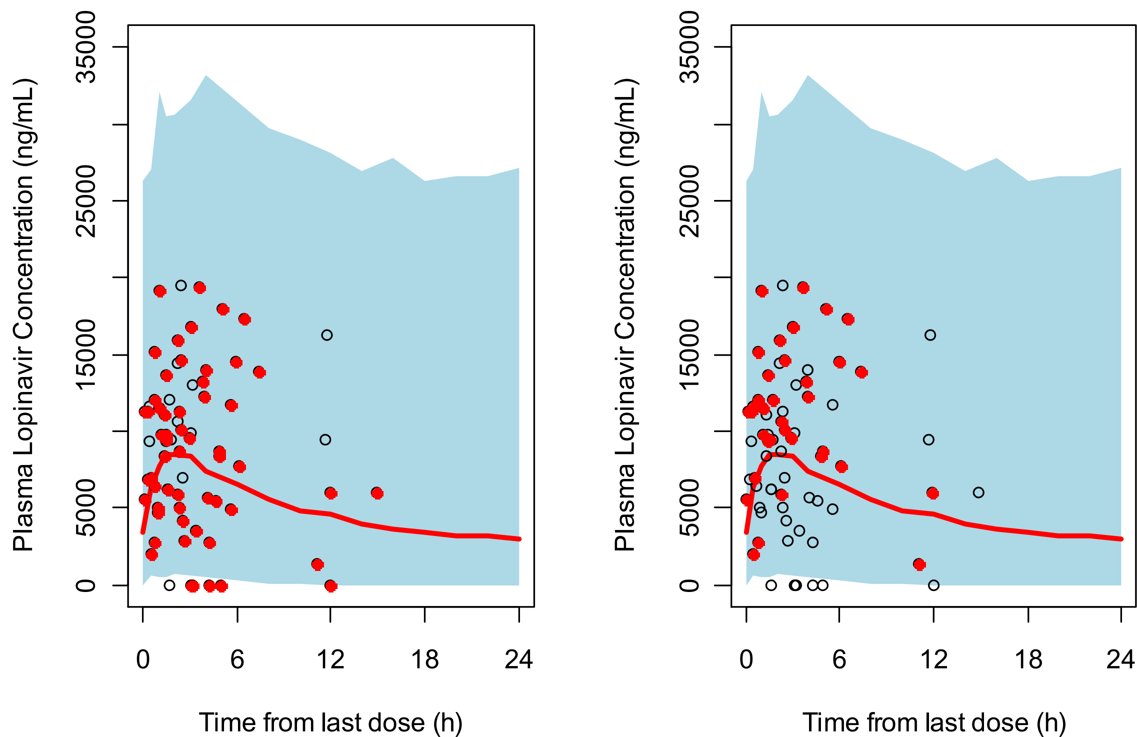

Figure 26: Degenerate visual predictive check showing the median (red line) and the 95% prediction interval (shaded blue) of 500 simulated profiles for patients taking lopinavir. The left panel shows the actual lopinavir concentration from patients with (red solid circles) and without (black empty circles) TMP-SMX concomitant medication. The right panel shows the actual lopinavir concentration in patients who had isoniazid and pyrazinamide as concomitant medication (red solid circles) and those that did not (black empty circles).

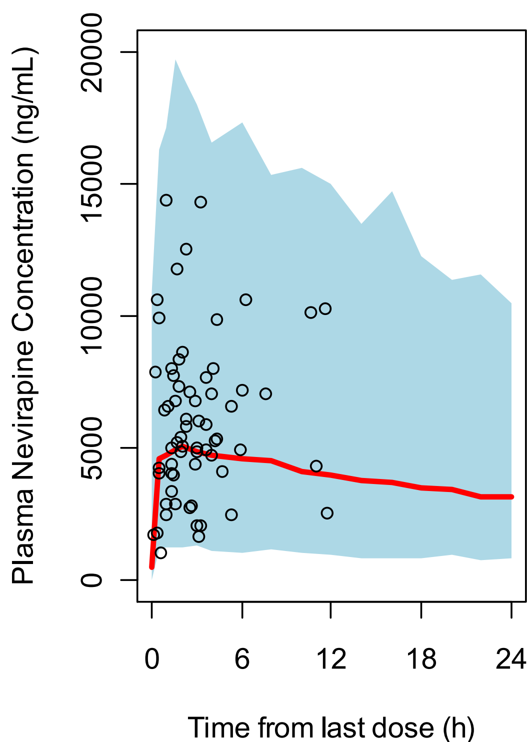

Figure 27: Degenerate visual predictive check showing the median (red line) and the 95% prediction interval (shaded blue) of 500 simulated profiles for patients taking nevirapine.

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5.0 Discussion

In developing countries, HIV positive patients with late stage disease significantly contribute to the total burden of disease. There is an estimated 5.5 million South-Africans living with HIV; contributing 17% to the global HIV/AIDS epidemic (Jarvis *et al*., 2010). The occurrence of opportunistic infections in these patients has decreased significantly after the emergence of combination antiretroviral therapy (cART), but additional treatment is still required (Seden *et al*., 2009; Ruhnke, 2004). Polypharmacy has therefore become a more common practice to manage one or multiple pathologies. The risk for experiencing DDIs, whether pharmacokinetic or pharmacodynamic in nature, is extensive due to high pill burden, concomitant diseases, age, gender and ethnicity (Evans-Jones *et al*., 2010; Lazarou *et al*., 1998). The outcome of DDIs may vary. Co-administration could result in decreased serum concentrations which can lead to treatment failure and potential drug resistance or an interaction following concomitant use may result in elevated blood concentrations and development of adverse drug reactions (Seden *et al*., 2009; Robertson *et al*., 2005; Tseng *et al*., 1997).

In this thesis the potential pharmacokinetic DDIs between the antifungal, FCZ (200 mg qd), prescribed as maintenance therapy and prophylaxis for cryptococcal meningitis and as treatment for oropharyngeal candidiasis, and any of the studied antiretroviral agents; efavirenz (600 mg qd), nevirapine (200 mg bd) and lopinavir/ritonavir (400/100 mg bd), was investigated. Theoretically pharmacokinetic DDIs between these agents primarily involve interaction in common metabolic pathways, particularly CYP3A4 and CYP2C9, the isoenzymes inhibited by FCZ.

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Furthermore, the population pharmacokinetic method used in this study for pharmacokinetic analysis also allowed for the evaluation of other significant co-factors which may affect the pharmacokinetics of the investigated ARVs.

5.1 Patient characteristics

In order to allow valid comparisons between the study groups, the control group and the treatment group should be comparable regarding drug regimens and patient characteristics. Age and height did not differ significantly between the two groups, however, body weight and body mass index were significantly higher in the control group. A larger body weight is associated with a greater volume of distribution and clearance, especially for highly lipophilic drugs, and this would result in lower serum concentrations of the drug (Guo *et al.*, 2010). When looking at weight and gender it is usually thought that males have a greater body weight than females. In this study, however, males and females in the both the control and treatment group actually had very similar average body weights (69.7 and 69.9 kg versus 60.8 and 61.0 kg, respectively). There was a tendency for patients in the control group to have lower, but not significantly different, efavirenz serum concentrations, which is consistent with the concept that an increased body weight is associated with decreased serum concentrations. The nevirapine and lopinavir serum concentrations, although not significantly different, tended to be greater in the treatment group which deviates from the aforementioned notion.

With regards to the clinical test results related to the HIV infection, patients in the treatment group had significantly lower CD4 values ($p<0.0001$), indicative of the patients' more

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pronounced severity of the illness. Once diagnosed, patients are clinically staged (I-IV) according to the World Health Organisation (WHO) disease staging system which is valuable in establishing the severity of illness and determining an appropriate treatment regimen (WHO, 2009). Twenty one out of twenty six patients in the treatment group had previously been diagnosed with cryptococcal meningitis. Cryptococcal meningitis is characterised as one of the AIDS-defining illnesses and patients are assigned as stage IV severity according to the WHO. It was therefore expected and re-established that the treatment group would have a higher degree of immune suppression (lower CD4 values) when compared to the control group (227 ± 175 cells/µl vs 412 ± 227 cells/µl). When comparing the severity of illness in the two study groups the following was ascertained; 5.6% of patients in the control were stage I, whereas no patients in the treatment group were; 27.8% of the control group were stage II versus only 3.8% of the treatment group; the largest percentage (51.9%) of the control group were stage III compared to the 7.6% of the treatment group; 14.8% of patients in the control group were stage IV versus 88.4% of the treatment group. This confirms that patients in the treatment group were more ill, as expected.

The viral load of a patient gives an indication of the number of HIV-RNA copies per millilitre plasma; therefore a lower than the detectable limit (LDL) viral load is desirable. Only 64.5 and 46.0 percent of patients were virally suppressed in the control and treatment group respectively (Table 15). This could be a possible indication of treatment failure due to poor adherence or the patients' may have developed resistance to one or more ARVs in their treatment regimen. Drug resistance is multifactorial and may be a result of a lack of compliance or sub-therapeutic drug levels originating from DDIs between co-administered

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drugs and therefore receiving a sub-therapeutic dose (Robertson *et al.*, 2005; de Maat *et al.*, 2003; Back *et al.*, 2000).

In this study, two patients in the control group had an ALT three times the upper limit of normal (3 X ULN); one patient as a result of non-ARV drug-induced hepatitis and the other due to alcohol-induced hepatitis. Continuous use and elevated concentration of co-administered drugs that may cause hepatotoxicity can worsen the condition. Drug-induced hepatotoxicity is an adverse drug reaction associated with a variety of drugs, including ARVs and FCZ. The majority of drugs must first be converted into a more ionized or polar form, via the two phases of metabolism, in order to be more easily excreted. The main metabolic pathway involves the CYP enzyme family. Hepatotoxicity may occur when reactive intermediates in this process accumulate and cause liver cell damage. All of the investigated antiretrovirals undergo hepatic metabolism via the CYP enzyme system. Furthermore, hepatotoxicity is listed as a side effect of nevirapine and lopinavir/ritonavir as well as the study drug, FCZ (Guo *et al.,* 2010; Canta *et al.*, 2005; Sanna *et al.*, 2005; Stern *et al.*, 2003).

5.2 Drug serum concentrations

Therapeutic drug monitoring of efavirenz and nevirapine has been the subject of great debate (Pretorius *et al.*, 2011). The deliberation is that steady state NNRTI drug levels do not vary greatly during a dosing interval as the drugs have considerably long half lives (40-55 hours and 25-30 hours, respectively) and that serum levels remain relatively constant (Back *et al.*, 2003; Smith *et al.*, 2001). It has been shown, however, that great inter- and intraindividual variability exists in NNRTI serum levels. This can partly be explained by genetic variations

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and polymorphisms in the expression of CYP isoenzymes. As shown in Figure 1, CYP3A4 is the most abundant and prominent isoenzyme involved in drug metabolism. Furthermore, this isoenzyme is also responsible for the metabolism of approximately 50% of all marketed drugs (Zhang *et al.*, 2001). CYP3A4 as opposed to other CYP3A isoenzymes (CYP3A5 and CYP3A7) is not subject to major genetic polymorphisms, but rather influenced by a variety of environmental factors (Wojnowski *et al.*, 2006). Altered serum concentrations of drugs that are metabolised by CYP3A4 may therefore be presumed to result from interindividual variation in the level of CYP gene expression. Gene expression may be influenced by homeostatic mechanisms, disease states and environmental factors such as smoking, diet and concomitant drug use (Lamba *et al.*, 2002).

A visual representation of the spread of drug serum concentrations for efavirenz, nevirapine and lopinavir/ritonavir (Figures 18-20) shows that a greater percentage of patients, in both the control and treatment group, had drug concentrations within or above the recommended therapeutic range (73.4, 82.2 and 90.0% versus 74.4, 72.7 and 81.0% respectively). When investigating non-adherence of the ARVs, defined as a serum concentration lower than the limit of quantification (<LLOQ), in the control and treatment group it was found that 0.6% and 5.1% had LLOQ serum concentrations, respectively.

One of the main mechanisms of pharmacokinetic DDIs is interference within common metabolic pathways involving the CYP enzyme system (Kakuda *et al.*, 2008; Tseng *et al.*, 1997). DDIs can result in altered serum concentrations of the associated ARVs. This is, however, not evident at first sight from the raw data obtained as the study made use of a

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random sampling schedule and the concentrations cannot be directly compared since there were different timeframes between dose intake and blood sampling. Furthermore, a straightforward comparison of ARV serum concentrations with the recommended therapeutic range would be misleading for nevirapine and lopinavir/ritonavir, since the therapeutic range refers to trough concentrations which were not obtained in this study (Pretorius *et al.*, 2011). The therapeutic range can therefore only be used as a reference point for illustrative purposes. To better establish a link between ARV serum concentrations and concurrent drug use or various patient characteristics, a population pharmacokinetic approach was therefore employed including modelling of the data.

Although the focus of this thesis was to investigate the effects of FCZ on the pharmacokinetics of ARVs, FCZ serum concentrations were measured by a newly developed HPLC method. This determination, in part, was to identify whether any observed DDI was directly related to FCZ serum concentrations and to establish whether the patients were adherent to their antifungal treatment. Moreover, verification of whether FCZ serum concentrations were in the recommended therapeutic range for maintenance/prophylactic treatment of cryptococcal meningitis and treatment of oropharyngeal candidiasis could also be done.

5.3 Population pharmacokinetics

To investigate the potential DDI between efavirenz, nevirapine or lopinavir/ritonavir and FCZ, a one-compartment pharmacokinetic model was developed, using a non-linear mixed effects modelling program (NONMEM), to establish whether differences in the random

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serum concentrations obtained for each of the two study groups could be as a result of an interaction between the aforementioned drugs. FCZ was incorporated as a categorical variable in the covariate model, along with all the other concomitant medications, according to their effects on specific CYP isoenzymes (Table 17).

5.3.1 Efavirenz

The efavirenz model delivered two significant covariates at the 5% level (p = 0.05); concomitant use of the antimycobacterial rifampicin as well as ethnicity. Rifampicin is a potent inhibitor of CYP3A4, which besides a sizeable contribution of CYP2B6, is partly responsible for metabolism of efavirenz (Ståhle *et al.*, 2004; Back *et al*., 2003). Concomitant use of this antimycobacterial agent and efavirenz was associated with only a minor (2%) and clinically not significant, increase in efavirenz clearance. This covariate was not significant in the backward elimination process, an automatic algorithm to remove non-significant variables, of the population pharmacokinetic model at the 1% level (p = 0.01). A DDI between efavirenz and rifampicin was expected as the drugs share a common metabolic pathway via CYP3A4 and CYP2B6. According to the literature, however, a more pronounced interaction between efavirenz and rifampicin has been demonstrated in other HIV-infected cohorts (Decloedt *et al.*, 2011; Elsherbiny *et al.,* 2009). Eventhough efavirenz is metabolised by CYP3A4, no DDI was found between this ARV and FCZ, a CYP3A4 inhibitor, which is consistent with literature from a number of studies (Robertson *et al.*, 2005; de Maat *et al.*, 2003; Dahri *et al.*, 1998).

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Of note, however, is that one coloured female patient in the treatment group had significantly elevated efavirenz levels on both sampling occasions; 33 023 ng/ml, 29 859 ng/ml and 33 622 ng/ml taken at 8.83, 10.52 and 12.50 hours post-dose. Corresponding FCZ serum concentrations were <LLOQ, 2 873 ng/ml and 2 663 ng/ml. Concomitant medications included FCZ, TMP-SMX and vitamin B complex. The patient was also diagnosed with abdominal TB and treatment consisted of rifampicin, isoniazid, ethambutol and pyrazinamide. Potential reasons for this elevation may be a DDI with FCZ and/or isoniazid or the patient may be a poor metaboliser of efavirenz, most likely for the CYP2B6 pathway. Elevated efavirenz levels have been correlated to an increased risk of developing central nervous system side effects, but this patient did not present with any adverse effects. Isolated case studies have been reported where the concomitant use of FCZ and efavirenz has led to increased concentrations of the ARV (Hasse *et al.*, 2005; Robertson *et al.*, 2005; de Maat *et al.*, 2003). The contribution of FCZ to the significantly elevated efavirenz levels in this study is, however, questionable. In this specific case the patient had a FCZ serum concentration that was <LLOQ at the time of first blood sampling which shows that the drug had not been taken for at least 4 to 5 days. Efavirenz levels were at their maximum at this point.

The most probable reason for increased efavirenz concentrations in the abovementioned patient could be a genetic variation in CYP2B6. Hasse *et al.* (2005) described a case of a female Thai woman, taking FCZ, found to be homozygous for the CYP2B6 G516T allele that had a plasma efavirenz concentration 30 times the normal limit. A pharmacogenetic study performed in HIV-infected patients in the International Medical Centre of Japan illustrated that patients homozygous for CYP2B6 *6/*6 (Q172H and K262R) had a significantly higher

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mean efavirenz plasma concentration when compared to patients heterozygous or without the *6 allele (Tsuchiya *et al.*, 2004).

Ethnicity was found to have the most significant effect on efavirenz pharmacokinetics. In the covariate model race accounted for a 56.8% increase in efavirenz clearance and subsequent decreased efavirenz concentrations observed in coloured patients. This data supports and adds important information to previously published data. Effects of race on efavirenz pharmacokinetics have been well documented and this effect can most likely largely be explained by differences in CYP2B6 expression in the different patient populations, as discussed above.

Stöhr and colleagues (2008) combined data from the Liverpool Therapeutic Drug Monitoring (TDM) register with the UK Collaborative HIV Cohort (CHIC) study to ascertain the effect of ethnicity on NNRTI plasma levels. The outcome of the study was that black patients, comprising 34% of the study population, had a 59% increase in efavirenz plasma levels ($p < 0.001$) showing a clear association between race and efavirenz plasma concentrations. During an exploratory analysis to correlate the effect of efavirenz plasma concentrations and the development of central nervous system adverse effects in European-American, African-American and Hispanic patients, recruited to the Adult AIDS Clinical Trial Group Study in the USA, Haas *et al.* (2004) found that a CYP2B6 G516T polymorphism was more common in African-American patients (20%) than in European-Americans (3%). This corresponded to significantly increased efavirenz plasma concentrations ($p < 0.0001$) and decreased drug clearance.

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A UK-based study of Ugandan and Zimbabwean adult HIV-positive patients reported similar findings when investigating the genetic link between race and CYP2B6 in efavirenz pharmacokinetics (Jamshidi *et al.,* 2010). It has been well documented that the polymorphisms resulting in a loss of function (LoF) of the CYP2B6 gene are associated with elevated efavirenz concentrations and corresponding decrease in clearance (Haas *et al.,* 2004; Tsuchiya *et al.,* 2004). The frequency of these LoF alleles in the UK study were found to be 65% in Zimbabweans, 39% in Ugandan Nilotics and 22% in Ugandan Bantus and may therefore influence efavirenz pharmacokinetics in these African populations.

The conclusion drawn from all of these studies is that varying efavirenz plasma concentrations in different ethnic populations may be due to differences in gene expression, particularly of CYP2B6. This thesis adds data that supports the hypothesis that race and efavirenz plasma concentrations are linked. Moreover, the present study illustrates a new finding that, in the South African context, coloured patients have significantly decreased efavirenz serum concentrations which may be related to a polymorphism of the CYP2B6 gene resulting in a gain of function as opposed to the LoF commonly seen in black patients.

Investigating the adherence, as measured by a pill count, shows no statistical difference between black patients (93.4%, n=41) and coloured patients (89.7, n=38) when taking both the control and treatment groups into account.

Currently efavirenz is uniformly dosed as 600 mg once daily. The present findings may, however, suggest that dosage adjustments according to race could be beneficial to avoid side effects associated with increased efavirenz levels in black patients or to ensure that coloured

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patients are achieving high enough plasma levels for viral suppression and prevent the development of drug resistance and ultimately treatment failure.

5.3.2 Nevirapine

The primary CYP isoenzymes responsible for metabolism of nevirapine are CYP3A4 and CYP2B6 (Elsherbiny *et al.,* 2009; de Maat *et al.,* 2003). As suggested by the literature, co-administration of nevirapine and FCZ was expected to be associated with a significant DDI, as a result of the inhibition of CYP3A4 by FCZ, resulting in elevated nevirapine concentrations. Kishimoto *et al.* (2000) described a 7.4 times increase in the AUC of nevirapine following concomitant use with FCZ, while Wakeman *et al.* (2010) reported a 29% increase in AUC and approximately 10% decrease in nevirapine clearance in HIV-positive Ugandan patients when nevirapine was co-administered with FCZ. The nevirapine serum concentration data in both patient groups in the present study showed substantial variation, ranging from 1 007 – 14 400 ng/ml over a time period of 0.08 – 11.66 hours post-dose. This created some problems in model convergence. To overcome the problem of extensive spread of nevirapine concentrations, they were converted to a log-scale with the base 10 to condense and collapse all the data which can then be handled by NONMEM and gives assurance that the model can converge. No significant covariates were, however, observed in this model.

Large inter- and intraindividual variability has been described in nevirapine concentrations because of the large extent of genetic variations in CYP2B6, CYP2C9 and CYP2D6 (Haas *et al.,* 2006). This may be the reason for the wide spread of concentrations seen as no DDI has

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been established between nevirapine and FCZ or any other co-administered agents in the present study.

In the aforementioned CHIC study, Stöhr *et al.* (2008) also investigated the effect of ethnicity on nevirapine plasma concentrations. They found that black patients, comprising 27% of the study population, had a significant increase (p = 0.002) of 39% in nevirapine levels. From the present study, however, no clear tendencies could be established between race and nevirapine serum concentrations.

It is estimated that between 6 and 30% of patients on a nevirapine containing regimen experience serious adverse effects involving the liver and this may lead to the discontinuation of therapy (Sulkowski *et al.*, 2002). A nested study within the Gilead study, protocol FTC-302, investigated the association between genetic polymorphisms of nevirapine metabolising CYP isoenzymes and the development of hepatotoxicity in 385 South African patients (Haas *et al.*, 2006). Contrary to previous reports and finding that CYP2B6 G516T polymorphisms influence efavirenz plasma levels, no association was found with nevirapine concentrations or with development of hepatotoxicity (Rotger *et al.*, 2005). Significant co-factors for the risk of developing hepatotoxicity was previously established in the Gilead study, these included female sex, increased body mass index, serum albumin concentration, plasma HIV RNA copies, lactate dehydrogenase level and aspartate aminotransferase level (Haas *et al.*, 2006). In the present study 83.3% of the patients on a nevirapine-based regimen were female. In keeping with the literature, nevirapine serum concentrations tended to be higher in female patients which are associated with an increased risk for hepatotoxicity.

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5.3.3 Lopinavir

The lopinavir model delivered the most extensive list of covariates that affected drug serum concentrations. Concomitant use of TMP-SMX, isoniazid or clotrimazole, an antifungal given to treat vaginal and oral candidiasis, was found to significantly affect lopinavir serum concentrations at both the 1% ($p = 0.001$) and 5% ($p = 0.005$) levels. Other covariates that influenced lopinavir pharmacokinetics at the 5% significance level in the present study included serum creatinine, body mass index and coadministration of CYP3A inhibitors, which was already accounted for by isoniazid and clotrimazole. These factors were however not included in the final model (Table 16).

TMP-SMX is a combination antibiotic administered as prophylaxis for a variety of infections, including urinary tract infections, bronchitis, traveler's diarrhoea and *Pneumocystis carnii* pneumonia (Wen *et al.*, 2002). Use of this drug was recorded in 56.3% of the study population. Trimethoprim and sulfamethoxazole are both inhibitors of CYP2C8 and 2C9, respectively (Wen *et al.*, 2002). Concomitant use of TMP-SMX and lopinavir resulted in a greater spread of serum lopinavir concentrations (Figure 28). A DDI between lopinavir and TMP-SMX was not expected as lopinavir is mainly metabolised by CYP3A4 with a limited contribution by CYP2D6 (Cvetkovic et al., 2003).

The antituberculosis drug isoniazid is a strong inhibitor of CYP3A4 which is responsible for the largest portion of lopinavir metabolism. Co-administration of CYP 3A4 inhibitors would be expected to result in increased serum lopinavir levels and a corresponding decrease in drug clearance. TB treatment in all patients receiving lopinavir, however, also included

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pyrazinamide, a CYP2C19 inhibitor, and rifampicin, a potent inducer of CYP3A4, 2B6, 2C8,9,19 and 2D6 (Table 17). The latter of which would result in decreased lopinavir serum concentrations and an increase in drug clearance via CYP3A4 induction. The final population pharmacokinetic model, however, shows that the abovementioned antituberculosis combination results in a 46.4% decrease in lopinavir clearance when compared to patients that were not taking antituberculosis agents (Figure 26). To tease out the contributing effect of each of these drugs is rather improbable. Furthermore, clotrimazole also contributed to the aforementioned decrease in lopinavir clearance which can be explained by its properties as an inhibitor of CYP 3A4, 3A5 and 3A7.

5.4 Study limitations and future work

There were a number of limitations in the study. The ideal study design for investigating potential DDIs between approved drugs is a randomised cross-over design. However such a design would not be feasible in a patient population that is receiving the investigated drugs as a part of their treatment regimen. A random, sparse sampling approach was utilized in order to recruit more patients and not to interfere with routine clinical venepuncture. Another caveat of the study is the limited sample size and number of blood samples, particularly in the nevirapine and FCZ treatment group. According to the literature the most probable DDI would result from the concomitant use of nevirapine and FCZ (Wakeman *et al.*, 2010; Manosuthi *et al.*, 2007). Although the use of a pharmacometric model contributed to the study design as predictions and simulations could be made based on all the data obtained from this study as well as data from various other studies and sources, the clinical significance of an interaction will require further data from a larger patient population.

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From a practical perspective future work would be to increase blood sampling, create a more rigid blood sampling time schedule and increase the sample size of groups. The covariates affecting ARV pharmacokinetics that were identified in the present study had not been pre-specified prospectively, but were only identified retrospectively. A confirmatory study would therefore be needed to verify the data. Two major concerns raised in the study, namely the racial difference in EFV clearance and the identified interaction between lopinavir and the anti-tuberculosis agents (INH/PZA), will require further investigation considering South Africa's patient population and the extent of HIV and TB co-infection. Finally, problems with adherence should be addressed at the patient and caregiver level. Improved methods of measuring adherence should be considered and further counselling might be necessary to keep patients motivated.

A next step in the pharmacometric evaluation of the outcome of the present study could be the development of a pharmacodynamic model of the data in order to correlate the link between patient characteristics, serum drug concentrations and CD4 and viral load data.

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6.0 Conclusion

Given the limitations of the sample size in the present study, due to practical reasons, no statistical significant effect of FCZ on the pharmacokinetics of the investigated ARVs could be demonstrated.

A retrospective analysis of the data showed various co-factors that influence the pharmacokinetics of the investigated ARVs. Efavirenz clearance was increased by 56.8% in coloured patients which was previously not known and resulted in lower serum concentrations. Concomitant use of efavirenz and rifampicin although significant, resulted in only a minor (2%) increase in efavirenz clearance. No significant covariates were established in the nevirapine model. Co-adminstration of combination antituberculosis agents including rifampicin, isoniazid and pyrazinamide, resulted in an overall decrease in lopinavir clearance by 46.4%.

The data obtained and the significant covariates identified in this study need to be confirmed in a prospective study as these results are appropriate for management of HIV-infected patients in the South African context.

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Appendix A

Drug-drug interactions encountered with nevirapine, efavirenz and lopinavir/ritonavir. Interactions are graded as follows: drugs should not be co-administered ●; potential for interaction Δ ; no clinically significant interaction expected ◊ and too little or no clear data on possible interaction ◊ . Adapted from www.hiv-druginteractions.org.

Coadministered drug	Nevirapine	Efavirenz	Lopinavir/ritonavir
Analgesics			
Aspirin	◊	◊	◊
Diclofenac	◊	◊	◊
Fentanyl	Δ	Δ	Δ
Ibuprofen	◊	◊	◊
Methadone	Δ	Δ	Δ
Morphine	◊	◊	Δ
Paracetamol	◊	◊	◊
Tramadol	Δ	Δ	Δ
Antiarrhythmics			
Amiodarone	Δ	Δ	●
Lidocaine	Δ	Δ	Δ
Quinidine	Δ	Δ	Δ
Antibacterials			
Amoxicillin	◊	◊	◊
Ciprofloxacillin	◊	◊	◊
Clarithromycin	Δ	Δ	Δ
Clavulanic acid	◊	◊	◊
Dapsone	◊	◊	◊
Erythromycin	Δ	◊	Δ
Ethambutol	◊	◊	◊
Isoniazid	◊	◊	◊

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Coadministered drug	Nevirapine	Efavirenz	Lopinavir/ritonavir
Antibacterials (cont)			
Metronidazole	◊	◊	Δ
Moxifloxacin	◊	◊	Δ
Penicillins	◊	◊	◊
Pyrazinamide	◊	◊	◊
Rifabutin	Δ	Δ	Δ
Rifampicin	●	Δ	●
Streptomycin	◊	◊	◊
Tetracyclines	◊	◊	◊
Trimethoprim-Sulfamethoxazole	◊	◊	Δ
Anticonvulsants			
Carbamazepine	Δ	Δ	Δ
Clonazepam	Δ	Δ	Δ
Phenobarbital	Δ	Δ	Δ
Phenytoin	Δ	Δ	Δ
Valproate	Δ	◊	Δ
Antidepressants			
Amitryptiline	◊	◊	Δ
Bupropion	Δ	Δ	Δ
Lithium	◊	◊	◊
Mirtazapine	Δ	Δ	Δ
Trazadone	Δ	Δ	Δ
Anti-diabetics			
Gliclazide	Δ	Δ	Δ
Insulin	◊	◊	◊
Metformin	◊	◊	◊
Pioglitazone	Δ	Δ	Δ
Antifungals			
Amphotericin B	◊	◊	◊

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Coadministered drug	Nevirapine	Efavirenz	Lopinavir/ritonavir
Antifungals (cont)			
Fluconazole	Δ	◊	◊
Itraconazole	•	Δ	Δ
Ketoconazole	•	Δ	Δ
Voriconazole	Δ	Δ	Δ
Antihistamines			
Loratidine	◊	Δ	Δ
Promethazine	Δ	Δ	Δ
Anti-coagulent			
Warfarin	Δ	Δ	Δ
Antiprotozoals			
Artemisinin	Δ	Δ	◊
Chloroquine	◊	◊	◊
Mefloquine	◊	◊	Δ
Primaquine	◊	◊	◊
Proguanil	◊	◊	Δ
Pyrimethamine	◊	◊	◊
Quinine	Δ	Δ	Δ
Antipsychotics/Neuroleptics			
Chlorpromazine	◊	◊	Δ
Clozapine	◊	◊	Δ
Haloperidol	Δ	Δ	Δ
Risperidone	◊	◊	◊
Antivirals			
Aciclovir	◊	◊	◊
Amantadine	◊	◊	◊
Oseltamivir	◊	◊	◊
Rimantidine	◊	◊	◊

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Coadministered drug	Nevirapine	Efavirenz	Lopinavir/ritonavir
Erectile dysfunction agents			
Sildenafil	Δ	Δ	Δ
Gastrointestinal agents			
Antacids	◊	◊	◊
Cimetidine	◊	◊	◊
Cisapride	Δ	●	●
Lansoprazole	◊	◊	◊
Metoclopramide	◊	◊	◊
Omeprazole	◊	◊	◊
Ondansetron	◊	◊	◊
Pantoprazole	◊	◊	◊
Prochlorperazine	◊	◊	◊
Ranitidine	◊	◊	◊
General aneasthetics			
Halothane	◊	◊	◊
Ketamine	Δ	Δ	◊
Herbals/Supplements/Vitamins			
Folic acid	◊	◊	◊
Garlic	Δ	Δ	Δ
Grapefruit juice	◊	◊	◊
Hops	Δ	Δ	Δ
Milk thistle	Δ	Δ	Δ
St. John's wort	●	●	●
Vitamin E	◊	◊	◊
Hypertension/Heart failure agents			
Amiloride	◊	◊	◊
Candesartan	◊	◊	◊
Enalapril	◊	◊	◊

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Coadministered drug	Nevirapine	Efavirenz	Lopinavir/ritonavir
Hypertension/ Heart failure (cont)			
Furosumide	Δ	Δ	Δ
Ramipril	◊	◊	◊
Sildenafil	Δ	Δ	●
Valsartan	◊	◊	◊
Illicit/Recreational			
Alcohol	◊	◊	◊
Cocaine	Δ	Δ	Δ
Gamma-hydroxybutyrate	◊	◊	Δ
Marijuana	◊	◊	Δ
MDMA (ecstasy)	◊	◊	Δ
Methamphetamine	◊	◊	Δ
Immune modulators			
Interferon alpha	◊	◊	◊
Interleukin-2	◊	◊	◊
Immunosuppressants			
Azothioprine	◊	◊	◊
Cyclosporine	Δ	Δ	Δ
Tacrolimus	Δ	Δ	Δ
Lipid lowering agents			
Atorvastatin	Δ	Δ	Δ
Fish oils	◊	◊	◊
Simvastatin	Δ	Δ	●
Other			
Allopurinol	◊	◊	◊
Caffeine	◊	◊	◊
Calcium	◊	◊	◊
Digoxin	Δ	Δ	Δ

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Coadministered drug	Nevirapine	Efavirenz	Lopinavir/ritonavir
Other (cont)			
Disulfiram	◊	◊	Δ
Levodopa	◊	◊	◊
Potassium	◊	◊	◊
Pramipexole	◊	◊	◊
Spironolactone	◊	◊	◊
Tamsulosin	Δ	Δ	Δ
Steroids			
Budesonide	◊	◊	Δ
Dexamethasone	Δ	Δ	Δ
Hydrocortisone (oral)	Δ	Δ	Δ
Hydrocortisone (topical)	◊	◊	◊
Prednisolone	Δ	Δ	Δ
Testosterone	Δ	Δ	Δ

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Appendix B

Drug-drug interactions encountered with fluconazole. Interactions are graded as follows: drugs should not be co-administered ●; potential for interaction Δ ; no clinically significant interaction expected ◊ and too little or no clear data on possible interaction ◊ .

Adapted from www.drugs.com/drug-interactions and www.medscape.com/drug-interactions

Coadministered drug	Fluconazole
Analgesics	
Aspirin	Δ
Diclofenac	◊
Fentanyl	●
Ibuprofen	◊
Methadone	●
Morphine	Δ
Paracetamol	◊
Tramadol	◊
Antiarrhythmics	
Amiodarone	●
Lidocaine	Δ
Quinidine	●
Antibacterials	
Amoxicillin	◊
Ciprofloxacin	Δ
Clarithromycin	◊
Clavulanic acid	◊
Dapsone	◊
Erythromycin	Δ
Ethambutol	◊
Isoniazid	Δ

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Coadministered drug	Fluconazole
Antibacterials (cont)	
Metronidazole	◊
Moxifloxacin	•
Penicillins	◊
Pyrazinamide	◊
Rifabutin	Δ
Rifampicin	Δ
Streptomycin	Δ
Tetracyclines	◊
Trimethoprim-Sulfamethoxazole	◊
Anticonvulsants	
Carbamazepine	Δ
Clonazepam	◊
Phenobarbital	Δ
Phenytoin	Δ
Valproate	◊
Antidepressants	
Amitryptiline	Δ
Bupropion	◊
Lithium	Δ
Mirtazapine	◊
Trazodone	•
Anti-diabetics	
Gliclazide	Δ
Insulin	◊
Metformin	Δ
Pioglitazone	Δ
Antifungals	
Amphotericin B	◊

Coadministered drug	Fluconazole
Antifungals (cont)	
Voriconazole	Δ
Antihistamines	
Loratidine	◊
Promethazine	Δ
Anti-coagulent	
Warfarin	●
Antiprotozoals	
Artemisinin	●
Chloroquine	Δ
Mefloquine	Δ
Primaquine	◊
Proguanil	◊
Pyrimethamine	◊
Quinine	Δ
Antipsychotics/Neuroleptics	
Chlorpromazine	●
Clozapine	Δ
Haloperidol	●
Risperidone	Δ
Antiretrovirals	
Efavirenz	◊
Lopinavir/ritonavir	◊
Nevirapine	Δ
Zidovudine	Δ
Anxiolytics/Hypnotics/Sedatives	
Diazepam	Δ
Lorazepam	◊
Zolpidem	◊
Zopiclone	◊

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Coadministered drug	Fluconazole
Beta blockers	
Atenolol	◊
Carvedilol	Δ
Metoprolol	◊
Propranolol	◊
Bronchodilators	
Salbutamol	◊
Salmeterol	Δ
Theophylline	Δ
Calcium channel blockers	
Amlodipine	Δ
Diltiazem	Δ
Verapamil	Δ
Contraceptives	
Estradiol	Δ
Ethinylestradiol	Δ
Levenorgestrel	Δ
Medroxyprogesterone (IM)	◊
Medroxyprogesterone (oral)	Δ
Cytotoxics	
Cyclophosphamide	◊
Doxirubicin	Δ
Tamoxifen	Δ
Vinblastine	Δ
Vincristine	Δ
Erectile dysfunction agents	
Sildenafil	Δ
Gastrointestinal agents	
Antacids	◊
Cimetidine	◊

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Coadministered drug	Fluconazole
Gastrointestinal agents cont.	
Cisapride	●
Lansoprazole	◊
Metoclopramide	◊
Omeprazole	Δ
Ondansetron	Δ
Pantoprazole	◊
Prochlorperazine	●
Ranitidine	◊
General aneasthetics	
Halothane	Δ
Ketamine	Δ
Herbals/Supplements/Vitamins	
Folic acid	◊
Garlic	◊
Grapefruit juice	◊
Hops	◊
Milk thistle	◊
St. John's wort	●
Vitamin E	◊
Hypertension/Heart failure agents	
Amiloride	◊
Candesartan	◊
Enalapril	◊
Furosumide	◊
Ramipril	◊
Sildenafil	Δ
Valsartan	◊
Illicit/Recreational	
Alcohol	◊

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Coadministered drug	Fluconazole
Illicit/Recreational cont.	
Cocaine	◊
Gamma-hydroxybutyrate	◊
Marijuana	◊
MDMA (ecstasy)	◊
Methamphetamine	◊
Immune modulators	
Interferon alpha	◊
Interleukin-2	◊
Immunosuppressants	
Azothioprine	◊
Cyclosporine	Δ
Tacrolimus	●
Lipid lowering agents	
Atorvastatin	●
Fish oils	◊
Simvastatin	●
Other	
Allopurinol	◊
Caffeine	◊
Calcium	◊
Digoxin	◊
Steroids	
Budesonide	Δ
Dexamethasone	Δ
Hydrocortisone (oral)	Δ
Hydrocortisone (topical)	◊
Prednisolone	Δ
Testosterone	Δ

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RESEARCH PROTOCOL

Title of project

Pharmacokinetic study to assess potential drug-drug interactions between antiretroviral therapy and fluconazole in patients with HIV/AIDS diagnosed with cryptococcal meningitis and/or oropharyngeal candidiasis.

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INTRODUCTION

Effective treatment of the human immunodefiency virus (HIV) has become more complicated over the years as patients require antiretroviral (ARV) regimens that consist of a combination of agents that target different stages of the viral replication cycle. Highly active antiretroviral therapy (HAART) involves concomitant administration of three or more agents from different pharmacological classes (O.Kis *et al*., 2009). Most of these agents are either inducers or inhibitors, as well as substrates of the human cytochrome P450 (CYP) enzyme system in the liver and/or the gastrointestinal mucosa, and can therefore influence or alter the metabolism of co-administered agents resulting in drug interactions (T.N. Kakuda *et al*., 2008).

In developing countries, HIV positive patients with late stage disease significantly contribute to the total burden of disease. There is an estimated 5.5 million South Africans living with HIV; contributing 17 % to the global HIV/AIDS epidemic (J.N. Jarvis *et al*., 2010). The compounding problem is the late stage patients that are likely to develop a variety of opportunistic infections as the virus, characterized by a reduction in T-lymphocytes, renders the host immunocompromised and susceptible to a variety of pathogens. Opportunistic infections (OIs) are defined as infections that would rarely cause disease in healthy individuals. Although the occurrence of OIs have decreased significantly after the emergence of HAART in developed countries, OIs still pose a major hurdle with regards to morbidity and mortality in HIV-infected individuals in developing countries such as South Africa (P. Willemot *et al.*, 2004, M Ruhnke, 2004).

Mycobacterium tuberculosis (TB) is thought to be the deadliest infection that arises in patients living with HIV/AIDS (M.S. Sánchez *et al*, 2010). Tuberculosis was diagnosed in 27%-34 % of HIV-infected adults admitted to tertiary hospitals in Kwa-Zulu Natal, South Africa during 2001 (C.B. Holmes *et al*., 2003). Since 2001 the number of patients co-infected with HIV and TB has increased, with Cape Town having the second largest patient population. Other OIs such as pneumocystis pneumonia (PCP), caused by the fungus *pneumocystis jiroveci,* remains a common life-threatening infection seen in both treatment-naive and treatment-experienced patients (T. Enomoto *et al*., 2010). PCP typically affects patients with a CD4 count of less than 200 cells/µl, however, patients with a CD4 count of less than 300 cells/µl and a history of previous OIs, are also at risk and may require prophylactic treatment (M.W. Hull *et al*., 2008). The yeast-like fungus, candida, is present in the system of most people, however, in immunocompromised patients the body's immune system cannot keep the fungus at bay resulting in disease (candidiasis). Candida can infect the mouth, throat or vagina and is one of the more common and less severe opportunistic

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infections seen during the early stages of infection. The parasite *toxoplasma gondii* causes in an infection known as toxoplasmosis which frequently manifests in the brain resulting in encephalitis. Patients with CD4 count of less than 100 cells/µl are at risk.

Oropharyngeal candidiasis and cryptococcal meningitis are the two most prevalent infections seen in late stage HIV/AIDS patients (J.E Gallant *et al*., 1994). This necessitates further treatment or prophylaxis with antifungal agents. A great potential for drug-drug interactions (DDIs) therefore exists in treated HIV/AIDS patients which in turn increases a patient's probability of experiencing adverse drug reactions (ADRs) (K. Seden *et al*., 2009).

Cryptococcal meningitis is a life-threatening fungal infection commonly seen in patients with HIV. The prevalence of HIV in the Sub-Saharan Africa region is approximately 22 500 000 with a reported estimate of 720 000 cases of cryptococcal meningitis per year, which demonstrates the impact of the disease (D.J. Sloan *et al*., 2009). Research has also shown that in HIV infected individuals, the cryptococcal infection has a very high rate of relapse (30-50 %) following treatment (M. Ruhnke, 2004). Management of the infection in patients therefore routinely involves lifelong antifungal therapy in addition to the antiretroviral therapy. With the emergence of immune reconstitution inflammatory syndrome (IRIS), a condition seen in HAART-treated patients, there is a paradoxical deterioration in the patients' clinical status as the recovering immune system responds to a previously acquired opportunistic infection which in turn worsens the symptoms of infection (M. Ruhnke, 2004). Management for this condition includes continuation of both antifungal therapy and HAART.

Cryptococcal meningitis is diagnosed by performing a lumbar puncture and is one of the key indicators of the development of acquired immunodeficiency syndrome (AIDS) (D.J. Sloan *et al*., 2009). The disease generally affects adult patients and patients with a CD4 cell count of 100 cells/µl or less. Current South African guidelines outline three treatment categories for cryptococcal meningitis in HIV patients (K. McCarthy *et al*., 2007):

- A 2 week induction phase with amphotericin B (IV) at a dose of 1-1.5 mg/kg/day
- The consolidation phase consists of po. fluconazole, 400 mg po. daily for 8 weeks
- Secondary prophylaxis (long term maintenance) with po. fluconazole 200 mg/day for life or until CD4 count is greater than 200 cells/mm^3 for more than 6 months on ART (at least 12 months on fluconazole in total)

The most commonly encountered opportunistic infection in patients with HIV/AIDS, oropharyngeal candidiasis (OPC), is reported to occur in approximately 90% of infected patients (O.J.M. Hamza *et al*., 2008). Moreover, OPC remains the most frequent HIV-associated oral disease in sub-Saharan Africa in patients with a CD4 count of < 250 cells/µl (F.M. Durden *et al*., 1997). OPC is treated with either single dose fluconazole (750mg po.) or 2-week therapy with oral fluconazole (150mg/day). Studies have found that there are no statistically significant differences between the efficacies of these two regimens.

Drug-drug interactions (DDIs) are typically classified as either pharmacokinetic or pharmacodynamic interactions. Pharmacokinetic interactions involve changes in blood concentrations of either agent due to the effect of interacting drugs on processes such as the absorption, distribution, metabolism and excretion of the drug (G.K. Dresser *et al*., 2000, S.M. Robertson *et al*., 2005). Most DDIs are pharmacokinetic in nature and are attributed to the interaction of drugs in common metabolic pathways. Pharmacodynamic interactions, conversely, arise from an additive/synergistic or antagonistic effect that occurs in response to co-administration of certain drugs (E. Albengres *et al*., 1998). An alteration in the pharmacological response (efficacy and/or toxicity) may result.

Pharmacokinetic DDIs have a number of possible outcomes. Drug levels of either the ARVs or the concomitant medication may be lowered thereby achieving sub-therapeutic levels which may lead to treatment failure as well as the development of drug resistance. Alternatively, DDIs may elevate the concentrations of ARVs or co-administered medication resulting in drug toxicity and an increase in the severity and risk of developing adverse effects.

The polyene antifungal amphotericin B is used mainly in the treatment of life-threatening systemic fungal infections (such as cryptococcal meningitis), as it has a narrow therapeutic index. Interactions between amphotericin B and antiretrovirals are pharmacodynamic in nature and may result in nephrotoxicity, blood dyscrasias and hypokalaemia (E. Albengres *et al*., 1998). Fluconazole is a triazole antifungal agent that inhibits the isoenzymes CYP2C9 and CYP3A4 of the human cytochrome P450 system. In theory it therefore decreases the metabolism and increases the plasma concentration of co-administered drugs that are metabolised by the CYP P450 system.

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ARVs are among the drug classes that have the greatest potential to develop DDIs (K. Seden *et al*., 2009). ARVs are potent inhibitors or inducers of liver enzymes (cytochrome P450 isoenzymes) which are responsible for the metabolism of a wide range of other drugs. Ritonavir is a protease inhibitor which is predominantly metabolised by CYP3A isoenzymes and, to a lesser extent, by CYP2D6. In addition to this, ritonavir is also a potent inhibitor of hepatic CYP3A4 as well as the ATP-dependent plasma membrane transporter, P-glycoprotein (Pgp) (A. Hsu *et al*., 1998). Ritonavir has a very high affinity for CYP3A and therefore other CYP3A inhibitors have little effect on ritonavir metabolism. Ritonavir, therefore, inhibits the metabolism of other agents metabolised by CYP3A leading to an increase in the corresponding concentrations (Table 1). The non-nucleoside reverse transcriptase inhibitors, nevirapine and efavirenz, are metabolised by CYP3A and CYP2B6 and CYP2B6 respectively (K. Dahri *et al*., 2007). Interference therefore exists between the metabolic pathways of the antiretrovirals and the antifungals, potentially resulting in drug-drug interactions.

The azole antifungal, fluconazole, is a potent inhibitor of both CYP3A4 and P-gp. Concomitant administration of fluconazole and the protease inhibitors (PIs), therefore, results in higher PI levels due to inhibition of their metabolism and delayed excretion (D. Pal et al., 2006). Fluconazole increases ritonavir concentrations, but the effect of ritonavir on fluconazole levels is unknown (Table 1). The effect of fluconazole on ritonavir-boosted lopinavir is also unknown. It has been shown, however, that ketoconazole decreases the boosted-lopinavir levels and the ketoconazole levels conversely increase (M.M.R de Maat et al., 2003, D. Pal et al., 2006). Efavirenz is both an inhibitor and an inducer of CYP3A4 and therefore its effect on concomitant agents is unpredictable (S.M. Robertson et al., 2005). Coadministration of efavirenz and fluconazole results in elevated efavirenz levels (M.M.R. de Maat et al., 2003). *In vitro* studies have shown that azole antifungals, including fluconazole, are expected to increase blood concentration of nevirapine, an inducer of CYP3A4 activity (E. Albengres et al., 1998).

Table 1: Known drug-drug interactions between fluconazole and the studied ARVs.

Inducer/Inhibitor

Substrate

	FLC	RTV	EFV	NVP	LPV/r
FLC		Increases fluconazole AUC[f,h] Effect unknown[g]	FLC AUC decreases[h] Effect unknown[e]	Fluconazole AUC expected to decrease[h]	Fluconazole AUC increases[d]
RTV	Increase in RTV AUC[c,f,j]		RTV AUC increases[b]	RTV AUC decreases[b] No significant change in levels[h]	
EFV	EFV AUC increases[e,h]	EFV AUC increases[b]		No clinical data available	No significant changes in EFV levels[b,h] EFV AUC decreases[d]
NVP	NVP AUC expected to increase[a,i]	No significant interaction/ change in levels[j]	No clinical data available		No significant changes in NVP levels[d]
LPV/r	Effect unknown[g]		LPV AUC decreases unless LPV/r dose increased[b,h]	Studies have found both no significant change as well as a decrease in LPV AUC[b,d,h]	

Legend: **AUC** Area under the drug concentration-time curve; **FLC** Fluconazole; **RTV** Ritonavir; **EFV** Efavirenz; **NVP** Nevirapine; **LPV/r** Lopinavir/ritonavir

[a] E. Albengres et al., 1998; [b] L.M. Catanzaro et al., 2004; [c] A. Cato et al., 1997; [d] R.S. Cvetkovic et al., 2003; [e] M.M.R. De Maat et al., 2003; [f] A. Hsu et al., 1998; [g] D. Pal et al., 2006; [h] S.M. Robertson et al., 2005; [i] K. Seden et al., 2009; [j] A.L. Tseng et al., 1997

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Drug-drug interactions between antifungals and antiretroviral agents become a concern when given concomitantly for a long period of time, as is the case when treating HIV positive patients with cryptococcal meningitis. For some antifungals and antiretrovirals, a relationship has been established between the plasma drug concentration and the resulting antifungal or antiviral (reduction in viral load) effect (J.W.C. Alffenaar *et al.*, 2010). Therapeutic drug monitoring (TDM) of both agents may be useful to predict or interpret the emergence of DDIs. TDM involves routine monitoring of plasma drug levels, thereby individualizing and optimizing drug therapy by dosage adjustments, improving dosing schedules or even altering the drug regimen. Information about the pharmacokinetics of these drugs and clinical co-variates that may have a possible influence on these parameters, including DDIs, is therefore important for the determination of the most appropriate dosing schedule in these patients.

GAPS IN RESEARCH

- Data pertaining to ARV pharmacokinetic drug interaction studies is restricted in resource limited countries such as South Africa.

- Knowledge of DDIs between fluconazole and the ARVs is limited; the effect of fluconazole on nevirapine levels and the lopinavir/ritonavir combination in specific.

- Pharmacokinetic studies that include patients with renal impairment are limited.

- More recently HIV is being diagnosed in patients over 50 years of age. It is therefore seen as a chronic condition requiring life-long treatment. Formal pharmacokinetic study data, however, is limited with regards to PK parameters in elderly patients.

AIM

Drug-drug interactions may lead to elevated or sub therapeutic drug levels, resulting in toxicity or treatment failure as well as the development of drug resistance. There is a large degree of inter- and intraindividual variation with regards to plasma drug concentrations due to individual differences in the expression of metabolizing enzymes. We therefore plan to use a population pharmacokinetic approach to study the pharmacokinetics (alteration in PK) and potential DDIs between fluconazole and the ARVs nevirapine, efavirenz, lopinavir/ritonavir in adult patients.

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The results of this study will provide evidence based guidance for the combined antiretroviral-antifungal treatment in patients with cryptococcal meningitis and oropharyngeal candidiasis as well as the covariates/patient characteristics that may influence optimal therapy.

SIGNIFICANCE

The data derived from this study will provide guidance to HIV/AIDS patients with regards to the appropriate dose and dosing schedule of co-administered ARVs and antifungal therapy, to identify patient populations at risk and to recommend an appropriate dosing schedule in such patients to avoid treatment failure or toxicity.

METHOD

Study design

This is a prospective, open uncontrolled study in a treatment population (ARVs + FLC) and a matched control group (ARVs only).

Inclusion criteria

Treatment population

- HIV-infected adults (≥ 18 yrs) diagnosed with cryptococcal meningitis and/or oropharyngeal candidiasis that are on antifungal therapy (fluconazole) and ARVs (at least one of the following: nevirapine, efavirenz, lopinavir/ritonavir) will be recruited from both outpatient and in-patient centres at Tygerberg Hospital (TBH), Karl Bremer Hospital (KBH) and the T.C. Newman Clinic (TCNC).
- Patients that have already been treated with fluconazole by another hospital and then referred to TBH because of the severity of the condition will also be recruited.

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Control group

- HIV-infected adults (≥ 18 yrs) that are currently on antiretroviral therapy (at least one of the following: nevirapine, efavirenz, lopinavir/ritonavir) will be recruited from both outpatient and in-patient centres TBH, KBH and TCNC.

Exclusion criteria

- Patients with an altered mental status as measured by the Glasgow Coma Score (<15).
- Patients whose medical condition is such as to make the drawing of blood inadvisable eg. patients with anaemia as well as patients with severe diarrhoea or dehydration.
- Patients who are unwilling to participate in the study or sign the consent forms.

Blood specimens

Blood will be collected in 7.5 ml Vacuette tubes containing serum separating clot activator. The antiretroviral and antifungal drug specimens will be centrifuged and serum will be stored in the Pharmacology Division's -70 °C freezer until being shipped to the Division of Infectious Diseases, University of Würzburg Medical Centre (Germany) for analysis. As this is a population pharmacokinetic analysis, there are no specific time points where blood must be taken and so each patient may have blood taken at various time points after medication intake.

Outpatients:
A total of four (4) blood samples (5ml each, total volume of 20ml) will be taken from the outpatients over a span of two clinic visits. The sampling schedule for all enrolled outpatients (treatment as well as their matched control group) will be divided into two categories according to the reason for fluconazole use; either cryptococcal meningitis or oropharyngeal candidiasis. The actual sampling methods for both groups are the same, only the time frame between clinic visits differ (see table 2). Once patients have been recruited, they will have a sample drawn on their arrival at the clinic after they have taken their medication. Before the patient then leaves the clinic he/she will have another blood sample taken. . At this first visit the patient will also be asked to only take his/her medication at the clinic on the date of the second visit, after a trough level blood sample has been drawn. The

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timeframe for the cryptococcal meningitis- and their matched control group is at least one month between blood draws at the clinic visit as these patients receive at least 6 months fluconazole therapy. The oropharyngeal candidiasis group and their matched controls have a one week period between clinic visits as they only receive a 14-day course of fluconazole.

Table 2: Sampling blood volumes and guidelines for blood sampling in the outpatient population.

	BLOOD SPECIMENS		
	Volume per sample (ml)	**Number of samples**	**Total volume of blood (ml)**
Treatment population	5	4	20
Control population	5	4	20

	SAMPLING SCHEDULES		
OPC pts and matched control	Sampling Day 1	Sample no.1	On arrival
		Sample no. 2	Before departure
	ONE WEEK*		
	Sampling Day 2	Sample no. 3	On arrival (before medication taken)
		Sample no. 4	Before departure
CM pts and matched control	Sampling Day 1	Sample no. 1	On arrival
		Sample no. 2	Before departure
	ONE MONTH*		
	Sampling Day 2	Sample no. 3	On arrival (before medication taken)
		Sample no. 4	Before departure

*Approximate timeframe, depending on patients' ability to visit clinic.

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Hospitalised patients

For the purpose of the study a total of seven (7) blood samples (4 ml per sample) will be taken from each inpatient over his/her full stay in hospital. The sampling schedule for hospitalised patients would be as follows: 0hr, 0.5hr, 1hr, 2hr, 4hr, 8hr and 12hr post dose samples.

Sample size

It is envisaged to enrol 100 patients (50 pts ARVs + FLC; 50 pts ARVs only)

Site

Blood sampling will be performed at three centres; Tygerberg Hospital, Karl Bremer Hospital and the T.C. Newman Clinic.

Screening

Patients will be recruited when meeting the inclusion criteria. When a patient who fulfils the inclusion criteria is recruited from the out-patient clinics at either Tygerberg Hospital, Karl Bremer Hospital or T.C. Newman Clinic, the research nurse will be informed who will then inform the patient about the study. If the patient agrees, written informed consent will be obtained.

Methods

The determination of plasma levels will be performed by High Pressure Liquid Chromatography (HPLC) and Gas-Chromatography (GC) as described by Langmann *et al*. (2002, 2006). The fluconazole assay will be developed by the research group in Wuerzburg, Germany and determination of fluconazole plasma levels would then be performed according the method developed.

Data recording and management

Each patient will be allocated a study specific number and the following data will be recorded for each patient on a case report form (CRF1) at the time of admittance to the study and on the occasion of each blood sampling:

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- Current HAART regimen
- Current fluconazole treatment
- Reason for fluconazole use
- Concurrent drug therapy, including over-the-counter and herbal preparations
- Estimated times of the last three ARV doses according to patient recall
- Estimated times of the last three fluconazole doses according to patient recall
- Date of birth
- Gender
- Body weight
- Height
- Nutritional classification

The following information will also be obtained from the patient's clinical file:

- Latest viral load and CD4/CD8 count (with date and time)
- Latest serum creatinine (with date)
- Serum albumin (with date)
- Serum ALT (with date)
- Significant past medical history and laboratory parameters related to toxicity e.g. liver function tests

At the time of each pharmacokinetic study (taking of a blood sample at admittance and the second visit) the following data will be recorded for each patient on a patient specific case record form (CRF2):

- Patient randomization number
- Current HAART regimen as well as the brand names and batch numbers of the drugs that the patient is taking.
- Current fluconazole regimen as well as brand names and batch numbers of the drugs the patient is taking.
- ARV dosing schedule.
- Fluconazole dosing schedule.
- Exact time of the last dose of ARV.
- Exact time of the last dose of fluconazole.
- Exact time of venepuncture.

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In this study no specific drug or medical device will be investigated or tested on patients. Any potential study-related adverse events will be reported in the CRF by the Principal Investigator or a sub-investigator.

Data monitoring

Monitoring for data integrity will be performed quarterly and at the end of the study by the Division of Pharmacology (Stellenbosch University).

Patient confidentiality and anonymity

To protect patient confidentiality, especially the patients' HIV status, a study number will be assigned to each patient. Only the study number will be used as a reference in the database to protect patient confidentiality. The study number and name will be kept in a separate database. The electronic database will be password-protected and access restricted to the study investigators. The blood samples will be allocated a sample barcode and linked to the patient through the individual subject barcode. Personal information will only be found on the consent form, which will be stored separately from all other anonymous patient information, including the CRF. As the proposed project is a co-operation between Stellenbosch University, University of Würzburg medical centre and the University of Florida, anonymized data will also be transmitted to these universities under strict obedience of data protection. The reporting on patients from this study in any type of manuscript will be done anonymously.

Laboratory method

The plasma concentration of nevirapine, efavirenz, lopinavir, ritonavir and fluconazole will be performed by the Division of Infectious Diseases, University of Würzburg Medical Centre (Germany). A material transfer agreement will be obtained for the transfer of blood samples.

The population PK evaluation will be performed by the Division of Pharmacology (Stellenbosch University) in consultation with the Department of Pharmaceutics, University of Florida.

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Data analysis and Statistical method

The data will be captured using MS Excel whereafter a Nonlinear Mixed Effects Modelling (NONMEM) software program will be used to analyse drug pharmacokinetics in the study population through pooling data. The specific population pharmacokinetic method as described by Barrett et al. (2005) and Sheiner and Ludden (1992) will be used to perform the pharmacokinetic (PK) evaluation. This statistical method will generate population pharmacokinetic data using a sparse sampling scheme taken from a large number of individuals from the same population. This will be done by the Department of Pharmaceutics (University of Florida) and the Division of Pharmacology (Stellenbosch University).

A one-compartment model will be used to assess the following parameters in both the treatment and control group: absorption rate constant (k_a), oral clearance (CL) and the area under the concentration-time curve (AUC). After constructing a model for each of these patient populations, comparisons can be made with regards to abovementioned parameters.

Serum concentration and all other data will also be analyzed by descriptive statistics and graphically presented.

ETHICAL CONSIDERATIONS

Written informed consent will be obtained from each patient when submitted to hospital. The patient information and consent forms will be available in English, Afrikaans and Xhosa.

Patients taking part in this study will not receive feedback about their drug plasma levels, since the plasma samples will be stored for a few weeks to a few months before being analyzed. If, however, the results of the drug plasma concentration of specific patients show non-compliance to their ART or antifungal therapy or virus resistance is established, the patients will be informed and necessary support will be given to such a patient.

This study will deliver benefit to future patients on the studied ARV's and fluconazole, since the pharmacokinetic data and covariates that influence combined drug therapy will serve as guidance for safe and efficacious ART and antifungal treatment in the future.

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This study will be conducted according to internationally accepted ethical standards and guidelines.

FUNDING AND TIMELINE

An MSc student fellowship has been provided by the International Research Training Group (IRTG). Additional funding requests will be made. A detailed budget is attached (Appendix 1).

It is estimated that the study will take approximately 2 years to complete. We plan to start collecting specimens in 2010 as soon as ethics approval is granted.

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PROTOCOL SIGNATURE PAGE

Title: Pharmacokinetic study to assess potential drug-drug interactions between antiretroviral therapy and fluconazole in patients with HIV/AIDS diagnosed with cryptococcal meningitis and/or oropharyngeal candidiasis.

Principle Investigator: Prof Bernd Rosenkranz

....

Signature Date

Student: Ms Desiré Fouché

....

Signature Date

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Pat-No			

PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM - ARVs

TITLE OF THE RESEARCH PROJECT:

Pharmacokinetic study to assess potential drug-drug interactions between antiretroviral therapy and fluconazole in patients with HIV/AIDS diagnosed with cryptococcal meningitis and/or oropharyngeal candidiasis.

REFERENCE NUMBER: N10/06/212

PRINCIPAL INVESTIGATOR:
Prof. Bernd Rosenkranz

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Invitation Paragraph

You are being invited to take part in a research project that possibly involves the long-term storage of blood. Please take some time to read the information presented here, which will explain the details of this project. Please ask the study staff or doctor any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how you could be involved. Also, your participation is **entirely voluntary** and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do agree to take part.

This study has been approved by the **Health Research Ethics Committee at Stellenbosch University** and will be conducted according to the ethical guidelines and principles of the international declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical research Council (MRC) Ethical Guidelines for Research.

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Thank you for reading this.

Pat-No			

Why is this study being done and why have you been invited to participate?

You have been diagnosed to be HIV positive. Nowadays, it is possible to treat the HIV-infection with antiretroviral (ARV) medication to help keep you healthy for a long time. To achieve successful antiretroviral therapy, we have to be sure that the patient's blood always contains a sufficient concentration of medication. This will avoid virus replication and disease progression. It is therefore essential that the patient takes his/her medication regularly over a long period of time.

The level of medication in the blood, however, differs from patient to patient. This can be due to interaction with other drugs, food or other factors that affect the breakdown or absorption of the ARVs. If the drug level in a patient's blood is too high there is a greater risk of developing side effects. On the other hand, patients may not have any clinical benefit from the medication or the virus might develop resistance against the medication if the drug concentration in the blood is too low.

The purpose of this study is to investigate the medication level in your blood. With this knowledge we can see if anything is interfering with the amount of medicine in your blood and then determine whether the dosage of your treatment is sufficient and not too low or too high.

How many people will take part in the study?

We are planning to recruit 100 patients.

Who is conducting the study?

The study is a cooperative project between Stellenbosch University (South Africa), University of Wuerzburg Medical Centre (Germany) and the University of Florida (USA).

What will happen if I take part in this research study?

For the purpose of this study 4 blood samples will be taken from you during your routine visits to the clinic. If possible, these blood samples will be taken at the time of routine clinical sampling to minimize needle pricks. All other normal investigations will be done as usual.

If the results of the study show that the concentration of ARVs in your blood is too low, an adjustment of the drug dose or a change in the dosing schedule may have to occur, so that you will get the best treatment available. The results will not be available immediately though since it will take time to measure the samples.

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Pat-No			

What will be investigated in the blood sample?

We will investigate:

- Drug concentration

The results of the investigations will be analyzed and published anonymously by the universities and co-workers.

You may choose if you wish to get information about you personal results.

Can I stop being in the study?

Yes. You can decide to stop at any time. Tell the study nurse if you are thinking about stopping or have decided to stop. Your data will then not be evaluated.

What risks can I expect from being in the study?

There are no risks because there are only 2 additional blood tubes taken per visit when blood is routinely drawn for clinical reasons. You may experience minor pain or bruising at the site where blood is taken during the routine clinical sampling. Other risks of drawing blood such as fainting or feeling light-headed and excessive bleeding rarely occur. There is no special treatment or any change of the treatment.

If your blood is to be stored is there a chance that it will be used for other research?

Your blood will only be used for research that is directly related to the investigation of the medication level in your blood. If the researchers wish to use your stored blood for **additional research in this field** they will be required to apply for permission to do so from the Human Research Ethics Committee at Stellenbosch University.

If you do not wish your blood specimen to be stored after this research study is completed, you will have the opportunity to request that it should be discarded when you sign the consent form.

Are there benefits to taking part in the study?

Taking part in this study can help us determine if the ARV concentration in your blood is at the correct level so as to be effective in treatment and not cause unwanted side effects. There is no direct benefit to you at this point in the study, but this information will help us to treat HIV positive patients better and could therefore also help future patients.

You will not receive feedback about your drug plasma levels, since the plasma samples will be stored for a few weeks to a few months before being analyzed. If however, we have found that the drug concentration of your medication is too low or too high, we will let you know. You may choose if you want to get this information or not.

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Pat-No			

Will my medical information and me taking part in this study be kept confidential?

All information which is collected about you during the course of the research will be kept strictly confidential. Any information about you which leaves the hospital will have your name and address removed so that you cannot be recognized by a third party.

Will you be paid to take part in this study and are there any costs involved?

You will not be paid for taking part in this study. You will not need to pay for any laboratory tests and examinations which are study specific procedures.

What are my rights if I take part in this study?

Taking part in this study is your choice. You may choose either to take part or not to take part in the study. If you decide to take part in the study, you may leave the study at any time. No matter what decision you make, there will be no penalty to you and you will not lose any of your regular benefits. Leaving the study will not affect your medical care. You can still get your medical care from our institution.

We shall tell you about new information or changes in the study that may affect your health or your willingness to continue in the study.

Who can answer my questions about the study?

You may talk to Dr. Jantjie J. Taljaard (021 938 9645) or Dr. M. Zeier (021 938 5230) (Tygerberg Hospital), Dr. Z. Joubert (021 979 5463) (Karl Bremer Hospital) and Dr. D. Hagemeister (021 860 2621) (T.C. Newman Clinic) the study doctors, about any questions or concerns you have about this study.

You can contact the Health Research Ethics Committee at 021-938 9207 if you have any concerns or complaints that have not been adequately addressed by your study doctor.

You will receive a copy of this information and consent form for your own records.

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Pat-No			

Declaration by participant

By signing below, I .... agree to take part in a research study entitled *(insert title of study)*.

I declare that:

- I have read or had read to me this information and consent form and it is written in a language in which I am fluent and comfortable.
- I have had a chance to ask questions and all my questions have been adequately answered.
- I understand that taking part in this study is **voluntary** and I have not been pressurised to take part.
- I may choose to leave the study at any time and will not be penalised or prejudiced in any way.
- I may ask to leave the study before it has finished, if the study doctor or researcher feels it is in my best interests, or if I do not follow the study plan, as agreed to.

☐ I agree that my blood or tissue sample can be stored, but I can choose to request at any time that my stored sample be destroyed. I have the right to receive confirmation that my request has been carried out.

OR

☐ Please destroy my blood sample as soon as the current research project has been completed. **(Tick the chosen option)**

Signed at *(place)* .... on *(date)* .... 2010.

....

Signature of participant

....

Signature of witness

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Pat-No			

Declaration by investigator

I *(name)* .... declare that:

- I explained the information in this document to ....
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research, as discussed above.
- I did/did not use an interpreter. *(If an interpreter is used then the interpreter must sign the declaration below.)*

Signed at *(place)* .... on *(date)* .... 2010.

....

Signature of investigator **Signature of witness**

Declaration by interpreter

I *(name)* .... declare that:

- I assisted the investigator *(name)* .... to explain the information in this document to *(name of participant)* .... using the language medium of English/Afrikaans/Xhosa.
- We encouraged him/her to ask questions and took adequate time to answer them.
- I conveyed a factually correct version of what was related to me.

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- I am satisfied that the participant fully understands the content of this informed consent document and has had all his/her questions satisfactorily answered.

Signed at *(place)* .... on *(date)* ....2010.

....

Signature of interpreter **Signature of witness**

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Pat-No			

PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM – ARVs + FCZ

TITLE OF THE RESEARCH PROJECT:

Pharmacokinetic study to assess potential drug-drug interactions between antiretroviral therapy and fluconazole in patients with HIV/AIDS diagnosed with cryptococcal meningitis and/or oropharyngeal candidiasis.

REFERENCE NUMBER: N10/06/212

PRINCIPAL INVESTIGATOR:
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Thank you for reading this.

Stellenbosch University http://scholar.sun.ac.za

Pat-No			

Why is this study being done and why have you been invited to participate?

You have been diagnosed to be HIV positive. Nowadays, it is possible to treat the HIV-infection with antiretroviral (ARV) medication to help keep you healthy for a long time. To achieve successful antiretroviral therapy, we have to be sure that the patient's blood always contains a sufficient concentration of medication. This will avoid virus replication and disease progression. It is therefore essential that the patient takes his/her medication regularly over a long period of time.

Your immune system is compromised and therefore you are susceptible to a variety of other infections. You have also been diagnosed with cryptococcal meningitis (fungal infection of the brain) and/or oropharyngeal candidiasis (fungal infection of the mouth and throat) which is treated with the antifungal, fluconazole. Successful therapy also requires a sufficient amount of medication in the blood.

The level of medication in the blood, however, differs from patient to patient. This can be due to interference in the metabolism of either medication. If the drug level in a patient's blood is too high there is a greater risk of developing side effects. On the other hand, patients may not have any clinical benefit from the medication or the virus might develop resistance against the medication if the drug concentration in the blood is too low.

The purpose of this study is to investigate the medication level in your blood. With this knowledge we can see if any interactions are occurring between your medications and then determine whether the dosage of your treatment is sufficient and not too low or too high.

How many people will take part in the study?

We are planning to recruit 100 patients.

Who is conducting the study?

The study is a cooperative project between Stellenbosch University (South Africa), University of Wuerzburg Medical Centre (Germany) and the University of Florida (USA).

What will happen if I take part in this research study?

For the purpose of this study 4 blood samples will be taken from you during your routine visits to the clinic. If possible, these blood samples will be taken at the time of routine clinical sampling to minimize needle pricks. All other normal investigations will be done as usual.

If the results of the study show that the concentration of either drug (ARV or antifungal) in your blood is too low due to an interaction between the drugs, an adjustment of the drug dose or a change in the dosing schedule may have to occur, so that you will get the best treatment available. The results will not be available immediately though since it will take time to measure the samples.

What will be investigated in the blood sample?

We will investigate:

- Drug concentration

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Pat-No			

The results of the investigations will be analyzed and published anonymously by the universities and co-workers.
You may choose if you wish to get information about you personal results.

Can I stop being in the study?

Yes. You can decide to stop at any time. Tell the study nurse if you are thinking about stopping or have decided to stop. Your data will then not be evaluated.

What risks can I expect from being in the study?

There are no risks because there are only 2 additional blood tubes taken per visit when blood is routinely drawn for clinical reasons. You may experience minor pain or bruising at the site where blood is taken during the routine clinical sampling. Other risks of drawing blood such as fainting or feeling light-headed and excessive bleeding rarely occur. There is no special treatment or any change of the treatment.

If your blood is to be stored is there a chance that it will be used for other research?

Your blood will only be used for research that is directly related to the investigation of the medication level in your blood. If the researchers wish to use your stored blood for **additional research in this field** they will be required to apply for permission to do so from the Human Research Ethics Committee at Stellenbosch University.

If you do not wish your blood specimen to be stored after this research study is completed, you will have the opportunity to request that it should be discarded when you sign the consent form.

Are there benefits to taking part in the study?

Taking part in this study can help us determine if the ARV and antifungal medication are interfering with one another resulting in a change in the concentration of the medication in your blood. There is no direct benefit to you at this point in the study, but this information will help us to treat HIV positive patients using the antifungal medication better and could therefore also help future patients.

You will not receive feedback about your drug plasma levels, since the plasma samples will be stored for a few weeks to a few months before being analyzed. If however, we have found that the drug concentration of either medication is too low or too high due to an interaction, we will let you know. You may choose if you want to get this information or not.

Will my medical information and me taking part in this study be kept confidential?

All information which is collected about you during the course of the research will be kept strictly confidential. Any information about you which leaves the hospital will have your name and address removed so that you cannot be recognized by a third party.

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Pat-No			

Will you be paid to take part in this study and are there any costs involved?

You will not be paid for taking part in this study. You will not need to pay for any laboratory tests and examinations which are study specific procedures.

What are my rights if I take part in this study?

Taking part in this study is your choice. You may choose either to take part or not to take part in the study. If you decide to take part in the study, you may leave the study at any time. No matter what decision you make, there will be no penalty to you and you will not lose any of your regular benefits. Leaving the study will not affect your medical care. You can still get your medical care from our institution.

We shall tell you about new information or changes in the study that may affect your health or your willingness to continue in the study.

Who can answer my questions about the study?

You may talk to Dr. Jantjie J. Taljaard (021 938 9645) or Dr. M. Zeier (021 938 5230) (Tygerberg Hospital), Dr. Z. Joubert (021 979 5463) (Karl Bremer Hospital) and Dr. D. Hagemeister (021 860 2621) (T.C. Newman Clinic) the study doctors, about any questions or concerns you have about this study.

You can contact the Health Research Ethics Committee at 021-938 9207 if you have any concerns or complaints that have not been adequately addressed by your study doctor.

You will receive a copy of this information and consent form for your own records.

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Pat-No			

Declaration by participant

By signing below, I ………………………………………………………………… agree to take part in a research study entitled *(insert title of study)*.

I declare that:

- I have read or had read to me this information and consent form and it is written in a language in which I am fluent and comfortable.
- I have had a chance to ask questions and all my questions have been adequately answered.
- I understand that taking part in this study is **voluntary** and I have not been pressurised to take part.
- I may choose to leave the study at any time and will not be penalised or prejudiced in any way.
- I may ask to leave the study before it has finished, if the study doctor or researcher feels it is in my best interests, or if I do not follow the study plan, as agreed to.

☐ I agree that my blood or tissue sample can be stored, but I can choose to request at any time that my stored sample be destroyed. I have the right to receive confirmation that my request has been carried out.

OR

☐ Please destroy my blood sample as soon as the current research project has been completed. **(Tick the chosen option)**

Signed at *(place)* …………………………………………………… on *(date)* ……………………………… 2010.

……………………………………………………………… ……………………………………………………………

Signature of participant **Signature of witness**

Stellenbosch University http://scholar.sun.ac.za

Pat-No			

Declaration by investigator

I *(name)* .... declare that:

- I explained the information in this document to ....
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research, as discussed above.
- I did/did not use an interpreter. *(If an interpreter is used then the interpreter must sign the declaration below.)*

Signed at *(place)* .... on *(date)* .... 2010.

.... **Signature of investigator**

.... **Signature of witness**

Declaration by interpreter

I *(name)* .... declare that:

- I assisted the investigator *(name)* .... to explain the information in this document to *(name of participant)* .... using the language medium of English/Afrikaans/Xhosa.
- We encouraged him/her to ask questions and took adequate time to answer them.
- I conveyed a factually correct version of what was related to me.

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- I am satisfied that the participant fully understands the content of this informed consent document and has had all his/her questions satisfactorily answered.

Signed at *(place)* .... on *(date)* ....2010.

....

Signature of interpreter **Signature of witness**

Stellenbosch University http://scholar.sun.ac.za

Pat-No			

PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM – ARVs + FCZ – Serial blood draw

TITLE OF THE RESEARCH PROJECT:
Pharmacokinetic study to assess potential drug-drug interactions between antiretroviral therapy and fluconazole in patients with HIV/AIDS diagnosed with cryptococcal meningitis and/or oropharyngeal candidiasis.

REFERENCE NUMBER: N10/06/212

PRINCIPAL INVESTIGATOR:
Prof. Bernd Rosenkranz

ADDRESS:
Division of Pharmacology
Department of Medicine
University of Stellenbosch
PO Box 19063
Tygerberg, Cape Town, 7505
South Africa

CONTACT NUMBER:
Phone: +27-21-938 9331
Mobile: +27-82-955 0017
Fax: +27- 21-932 6958

Invitation Paragraph

You are being invited to take part in a research project that possibly involves the long-term storage of blood. Please take some time to read the information presented here, which will explain the details of this project. Please ask the study staff or doctor any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how you could be involved. Also, your participation is **entirely voluntary** and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do agree to take part.

This study has been approved by the **Health Research Ethics Committee at Stellenbosch University** and will be conducted according to the ethical guidelines and principles of the international declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical research Council (MRC) Ethical Guidelines for Research.

Thank you for reading this.

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Pat-No			

Why is this study being done and why have you been invited to participate?

You have been diagnosed to be HIV positive. Nowadays, it is possible to treat the HIV-infection with antiretroviral (ARV) medication to help keep you healthy for a long time. To achieve successful antiretroviral therapy, we have to be sure that the patient's blood always contains a sufficient concentration of medication. This will avoid virus replication and disease progression. It is therefore essential that the patient takes his/her medication regularly over a long period of time.

Your immune system is compromised and therefore you are susceptible to a variety of other infections. You have also been diagnosed with cryptococcal meningitis (fungal infection of the brain) and/or oropharyngeal candidiasis (fungal infection of the mouth and throat) which is treated with the antifungal, fluconazole. Successful therapy also requires a sufficient amount of medication in the blood.

The level of medication in the blood, however, differs from patient to patient. This can be due to interference in the metabolism of either medication. If the drug level in a patient's blood is too high there is a greater risk of developing side effects. On the other hand, patients may not have any clinical benefit from the medication or the virus might develop resistance against the medication if the drug concentration in the blood is too low.

The purpose of this study is to investigate the medication level in your blood. With this knowledge we can see if any interactions are occurring between your medications and then determine whether the dosage of your treatment is sufficient and not too low or too high.

How many people will take part in the study?

We are planning to recruit 12 patients.

Who is conducting the study?

The study is a cooperative project between Stellenbosch University (South Africa), University of Wuerzburg Medical Centre (Germany) and the University of Florida (USA).

What will happen if I take part in this research study?

For the purpose of this study 8 blood samples will be taken from you during an 8 hour period at the clinic.

What will be investigated in the blood sample?

We will investigate:

- Drug concentration

Can I stop being in the study?

Yes. You can decide to stop at any time. Tell the study nurse if you are thinking about stopping or have decided to stop. Your data will then not be evaluated.

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Pat-No			

What risks can I expect from being in the study?

You may experience minor pain or bruising at the site where blood is taken during the routine clinical sampling. Other risks of drawing blood such as fainting or feeling light-headed and excessive bleeding rarely occur. There is no special treatment or any change of the treatment.

Are there benefits to taking part in the study?

Taking part in this study can help us determine if the ARV and antifungal medication are interfering with one another resulting in a change in the concentration of the medication in your blood. There is no direct benefit to you at this point in the study, but this information will help us to treat HIV positive patients using the antifungal medication better and could therefore also help future patients.

You will not receive feedback about your drug plasma levels, since the plasma samples will be stored for a few weeks to a few months before being analyzed. If however, we have found that the drug concentration of either medication is too low or too high due to an interaction, we will let you know. You may choose if you want to get this information or not.

Will my medical information and me taking part in this study be kept confidential?

All information which is collected about you during the course of the research will be kept strictly confidential. Any information about you which leaves the hospital will have your name and address removed so that you cannot be recognized by a third party.

Will you be paid to take part in this study and are there any costs involved?

You **will** be paid for taking part in this study. You **will not** need to pay for any laboratory tests and examinations which are study specific procedures.

What are my rights if I take part in this study?

Taking part in this study is your choice. You may choose either to take part or not to take part in the study. If you decide to take part in the study, you may leave the study at any time. No matter what decision you make, there will be no penalty to you and you will not lose any of your regular benefits. Leaving the study will not affect your medical care. You can still get your medical care from our institution.

We shall tell you about new information or changes in the study that may affect your health or your willingness to continue in the study.

Stellenbosch University http://scholar.sun.ac.za

Pat-No			

Who can answer my questions about the study?

You may talk to Dr. Jantjie J. Taljaard (021 938 9645) or Dr. M. Zeier (021 938 5230) (Tygerberg Hospital), Dr. Z. Joubert (021 979 5463) (Karl Bremer Hospital) and Dr. D. Hagemeister (021 860 2621) (T.C. Newman Clinic) the study doctors, about any questions or concerns you have about this study.

You can contact the Health Research Ethics Committee at 021-938 9207 if you have any concerns or complaints that have not been adequately addressed by your study doctor.

You will receive a copy of this information and consent form for your own records.

Stellenbosch University http://scholar.sun.ac.za

Pat-No			

Declaration by participant

By signing below, I .... agree to take part in a research study entitled *(insert title of study)*.

I declare that:

- I have read or had read to me this information and consent form and it is written in a language in which I am fluent and comfortable.
- I have had a chance to ask questions and all my questions have been adequately answered.
- I understand that taking part in this study is **voluntary** and I have not been pressurised to take part.
- I may choose to leave the study at any time and will not be penalised or prejudiced in any way.
- I may ask to leave the study before it has finished, if the study doctor or researcher feels it is in my best interests, or if I do not follow the study plan, as agreed to.

☐ I agree that my blood or tissue sample can be stored, but I can choose to request at any time that my stored sample be destroyed. I have the right to receive confirmation that my request has been carried out.

OR

☐ Please destroy my blood sample as soon as the current research project has been completed. **(Tick the chosen option)**

Signed at *(place)* .... on *(date)* .... 2010.

....

Stellenbosch University http://scholar.sun.ac.za

Signature of participant **Signature of witness**

Pat-No			

Declaration by investigator

I *(name)* .... declare that:

- I explained the information in this document to ....
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research, as discussed above.
- I did/did not use an interpreter. *(If an interpreter is used then the interpreter must sign the declaration below.)*

Signed at *(place)* .... on *(date)* .... 2010.

....
Signature of investigator **Signature of witness**

Declaration by interpreter

I *(name)* .... declare that:

- I assisted the investigator *(name)* .... to explain the information in this document to *(name of participant)* .... using the language medium of English/Afrikaans/Xhosa.
- We encouraged him/her to ask questions and took adequate time to answer them.
- I conveyed a factually correct version of what was related to me.
- I am satisfied that the participant fully understands the content of this informed consent document and has had all his/her questions satisfactorily answered.

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Signed at *(place)* .... on *(date)* ....2010.

....

Signature of interpreter **Signature of witness**

Stellenbosch University http://scholar.sun.ac.za

Protocol No. 1002 CRF I Version 2.0 6 June 2010

Pat. –No.			

DATE ☐☐☐☐☐☐ (D D M M Y Y)

PATIENT CHARACTERISTICS

Date of birth ☐☐☐☐☐☐ (D D M M Y Y)

Sex

male	
female	

Date of informed consent ☐☐☐☐☐☐ (D D M M Y Y)

Ethnic group

Black	
Coloured	
Indian/Asian	
White	
Other	

Smoke

yes	
no	

PHYSICAL EXAMINATION

Weight ☐☐☐☐ kg

Height ☐☐☐ cm

BODY WEIGHT CLASSIFICATION (ACCORDING TO BMI)

	Underweight (< 18.5)	**Normal (18.5-24.99)**	**Overweight (≥ 25.0)**	**Obese (≥ 30.0)**
YES				
NO				

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Pat. –No.			

HIV DIAGNOSIS

Date of first diagnosis

D	D	M	M	Y	Y

STAGING (according to WHO: 1,2,3,4) Stage: ☐

CONCOMITANT DISEASES (significant) None ☐

	Disease	Date onset DDMMYY	On going
1			
2			
3			
4			
5			
6			
7			

CONCOMITANT MEDICATION None ☐

	Medication	Route	Dosage	Date onset DDMMYY	On going
1					
2					
3					
4					
5					
6					
7					

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Pat. –No.			

Clinical Tests		**Standard Unit**	**Other unit** (if applicable)	**Value**
CD4 Cells	D D M M Y Y h h m m	Cells/µl		
Creatinine	D D M M Y Y	mg/dl		
Viral load	D D M M Y Y	Copies/ml		
Serum albumin	D D M M Y Y	mg/l		
Serum ALT	D D M M Y Y	Units/l		

COMMENTS Yes ☐ No ☐

____

____

CASE REPORT FORM REVIEW

I confirm that all Information reported for this patient is accurate and complete.

D D M M Y Y ________________________________

Investigator Signature

Pat. – No.			

DATE

D	D	M	M	Y	Y

HIV MEDICATION

Medication	Formulation (T, C, S)	Dosage	Date of onset	Time of medication intake *prior* to blood sampling	
			D D M M Y Y	h h m m	D D M M Y Y
			D D M M Y Y	h h m m	D D M M Y Y
			D D M M Y Y	h h m m	D D M M Y Y
			D D M M Y Y	h h m m	D D M M Y Y

MISSING LAST 7 DAYS

None ☐

Number of days ☐

Pat. – No. | | |

FLUCONAZOLE MEDICATION **N/A** ☐

Medication	Formulation	Dosage	Date of onset	Time of medication intake *prior* to blood sampling
			D D M M Y Y	h h m m D D M M Y Y h h m m D D M M Y Y

REASON FOR USE OF FLUCONAZOLE

Cryptococcal meningitis ☐ Oropharyngeal candidiasis ☐ Other (please specify) ☐ ____________________

MEAL PRIOR TO ARVs

Heavy meal (eg. a full plate of food) ☐ Medium meal (eg. a sandwich) ☐

Light meal (eg. a biscuit, cracker or fruit) ☐ Time of meal h h m m

No meal ☐

Pat. – No.			

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Pat. –No.			

LABORATORY – BLOOD SAMPLING

1. First blood sample

Collection time

h	h	m	m

Collection date

D	D	M	M	Y	Y

2. Second blood sample (if applicable)

Collection time

h	h	m	m

COMMENTS Yes ☐ No ☐

CASE REPORT FORM REVIEW

I confirm that all information reported for this patient is accurate and complete

D	D	M	M	Y	Y

____
Investigator Signature

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Appendix I

Interday precision of fluconazole

Date	Day	0.5 µg/ml	4 µg/ml	10 µg/ml	20 µg/ml
19-07-2011	1	0.47	4.3	10.9	20.1
20-07-2011	2	0.43	4.4	11	20
26-07-2011	3	0.42	3.8	12.3	19.8
27-07-2011	4	0.55	3.8	12	20
28-07-2011	5	0.54	3.8	9.3	18.3