ASSESSMENT OF DRUG INTERACTIONS BETWEEN ANTIRETROVIRAL AND ANTHELMINTHIC DRUGS

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy

By

Gabriel Kimutai Kigen

June, 2009

ORIGINAL COPY TIGHTLY BOUND

CONTENTS

ABSTRACT

Background

Drug interactions are amongst the commonest cause of medication error and yet frequently unrecognised; especially in Sub-Saharan Africa where there is high prevalence of HIV and accompanying co-infections. Amongst these co-infections are systemic helminthic infections, lymphatic filariasis and schistosomiasis which together afflict over 250 million people annually people and over 1.5 billion at risk.

Aims

The main aims of the research was to assess the prevalence of drug-drug interactions between antiretrovirals and co-administered drugs in a large outpatient programme in Eldoret, Kenya and to obtain data for the *in vitro* assessment of clinically significant drug-drug interactions between antiretrovirals and anthelminthic drugs; praziquantel (PZQ) and ivermectin (IVM).

Methods

Retrospective audit of prescriptions for 1040 consecutive HIV positive patients enrolled into the programme between 4/01/2006 and 19/04/07 was carried out. Records of age, gender, body weight, baseline CD4 counts, WHO staging, antiretrovirals and concomitant drugs were taken and the screening for interactions carried out using the Liverpool website (www.hiv-druginteractions.org). For the *in vitro* studies drug transporter mediated drug-drug interactions between antiretrovirals saquinavir (SQV), lopinavir (LPV), nevirapine (NVP) and efavirenz and PZQ/IVM were determined by investigating transport through Caco-2 cell monolayers and by accumulation experiments in cells that overexpress P-glycoprotein (P-gp), an efflux transporter. A high pressure liquid chromatography method for simultaneous determination of PZQ and SQV was validated and the substrate specificity of PZQ in relation to P-gp determined. The ability of the anthelminthics (PZQ/IVM) to induce ABCB1, CYP2B6 and CYP3A4 genes, and the effect of nanodispersion on the transport of SQV along the Caco-2 cell monolayers were also assessed.

Results

Clinically significant drug-drug interactions were common (affecting over 1:3 patients), and 32% of these interactions could have resulted in lowering of plasma antiretroviral concentrations. NVP inhibited the uptake transport of IVM, LPV increased the uptake transport of IVM, IVM inhibited the efflux transport of SQV by a threefold margin and IVM significantly facilitated the uptake of LPV (eightfold margin). PZQ was neither a substrate nor inhibitor of P-gp and both anthelminthics did not induce the genes under study. Nanodispersion improved the intracellular accumulation of SQV.

Conclusions

Clinically significant drug-drug interactions involving antiretrovirals are prevalent in Africa and are likely to impact on the therapy of HIV/AIDS. Urgent measures are required to address the problem so as not to compromise on the efficacy of antiretrovirals. IVM interacted with antiretrovirals and *in vivo* studies should be carried out to investigate clinical implications. Nanodispersion appears to circumvent the drug transporter efflux of SQV and thereby facilitating entry into the cells. Further research is required to determine *in vivo* effects.

PREFACE

Chapter 1: General Introduction

This chapter introduces the thesis with an overview of Human immunodeficiency virus (HIV) infection and Acquired immunodeficiency syndrome (AIDS), helminthic infections, the current therapies and their modes of action. The prevalence of the two conditions and co-infections is also reviewed. The Pharmacokinetics and potential for interactions between the two classes of drugs are described, with a focus on ivermectin (IVM) and praziquantel (PZQ) for the antihelminthics. *In vitro* methods for studying interactions, P-glycoprotein (P-gp) and expression and function are also described. Nanotechnology and its application to medicine is also reviewed.

Chapter 2: Potential for drug-drug interactions between antiretroviral and co-administered drugs in Kenya

The prevalence of clinically significant drug-drug interactions (DDIs) between antiretroviral (ARV) and co-administered drugs in a large ARV- programme in Kenya is assessed. Data from a retrospective survey and audit of follow up prescriptions of HIV positive patients, identification and characterisation of the severity of the DDIs are presented. The effects of the interactions on the ARV concentrations are also reviewed.

Chapter 3: Investigations of potential interactions between praziquantel and saquinavir in CEM and CEM$_{VBL}$ cells

The chapter outlines the development and validation of a high pressure liquid chromatography (HPLC) method for simultaneous quantification of PZQ and saquinavir (SQV). Details of the characterisation of PZQ as a substrate or inhibitor

of the efflux transporter P-gp, by accumulation in CEM and CEMVBL cells using SQV as positive control are discussed.

Chapter 4: Investigation of the interactions between antiretroviral and antihelminthic drugs in Caco-2 cell monolayers

The evaluation of the potential interactions between ARVs and antihelminthic drugs by determining their transport along the Caco-2 cell monolayers (CCM) is discussed. The effects of IVM on the transport of SQV, nevirapine (NVP), lopinavir (LPV) and efavirenz (EFV) along the Caco-2 cell monolayers (CCM) is outlined. Conversely, the impact of SQV, NVP, LPV and EFV on the transport of IVM is examined. Effects of SQV, NVP and EFV on PZQ transport, and PZQ on the transport of SQV is also assessed.

Chapter 5: Induction of ABCB1, CYP2B6 and CYP3A4 genes in Caco-2 cell culture by the anthelminthics, praziquantel and ivermectin

The potential for PZQ and IVM to induce the transporter gene ABCB1 and genes for metabolic enzymes CYP2B6 and CYP3A4 is assessed. Expression of the genes in Caco-2 cells in the presence of the anthelminthic drugs is evaluated.

Chapter 6: Effect of nanodispersion on the transport of SQV along the Caco-2 cell monolayers

In this chapter the ability of nanoengineering technology to improve the bioavailability of SQV by circumventing the P-gp mediated efflux is examined.

Details of the transport of the nanoengineered SQV along the CCM are compared to that of normal SQV are presented and evaluated. The accumulation of SQV and nanodispersed SQV in CEM and CEMVBL cells to determine whether the nanodispersed SQV or excipients circumvent the P-gp is also investigated.

Chapter 7: Concluding discussion

The chapter concludes the thesis by stressing the importance of the DDIs between ARVs and co-administered drugs in the clinical management of HIV infection, with special emphasis of interactions between IVM and LPV, SQV and NVP. The prevalence of DDIs and potential for nanodispersion in the improvement of therapy using Highly Active Antiretroviral therapy (HAART) is also discussed.

ACKNOWLEDGEMENTS

The experimental work described in the thesis was carried out in the Department of Pharmacology and Therapeutics, University of Liverpool. The data collection was carried out at the Moi Teaching and referral hospital (MTRH), Eldoret - Kenya at the AMPATH (Academic Model for Prevention and Treatment of HIV/AIDS) project.

I would like to thank Dr Geoffrey Edwards, Dr Andrew Owen and Dr Saye Khoo for their supervision, guidance and support throughout my study period. I am particularly grateful to Dr Andrew Owen for his immense contribution. I would also like to sincerely express my gratitude to Prof David Back for his tremendous support.

Many thanks also go to the AMPATH team led by Dr Sylvester Kimaiyo and the IREC team of the MTRH. A huge thank you is also extended to Dr John Tjia and Sarah Gibbons for their interest in my work.

My special thanks to my mum and sister for their emotional support and encouragement and finally, The Government of Kenya for sponsoring my education. In particular I would extend my appreciation to the Education attaché, at the Kenyan Embassy in London.

Thank you all.

This thesis is dedicated to my family.

ABBREVIATIONS

ABC	Abacavir
ABZ	Albendazole
ACN	Acetonitrile
ACTs	Arthemeter combination Therapies
AIDS	Acquired immunodeficiency syndrome
AMPATH	Academic Model for the Prevention and Treatment of HIV/AIDS
AP	Apical
APV	Amprenavir
ARVs	Antiretroviral drugs
ATP	Adenosine triphosphate
AUC	Area under the curve
BHIVA	British HIV association
BL	Baseline
cDNA	Complimentary Deoxyribonucleic acid
^{14}C	Carbon isotope
°C	Degrees Celcius
CAR	Cellular accumulation ratio
C_{max}	Maximum plasma concentration
CCM	Caco-2 cell monolayers
CCR5	Chemokine receptor 5
CD	Cluster differentiation (A glycoprotein predominantly found on the surface of helper T cells, dendritic cells, monocytes and macrophages)
CD4+	T cells expressing CD4 (A measure of the number of helper T cells p cubic millimeter of blood)
CDC	Centres for Disease Control
CLZ	Clozapine
CNS	Central nervous system
Conc.	Concentration
CV	Coefficient of variation
CXCR4	α-CXC Chemokine receptor 4
CYP	Cytochrome P450
ddC	Zalcitabine

ABBREVIATIONS

ABC	Abacavir
ABZ	Albendazole
ACN	Acetonitrile
ACTs	Arthemeter combination Therapies
AIDS	Acquired immunodeficiency syndrome
AMPATH	Academic Model for the Prevention and Treatment of HIV/AIDS
AP	Apical
APV	Amprenavir
ARVs	Antiretroviral drugs
ATP	Adenosine triphosphate
AUC	Area under the curve
BHIVA	British HIV association
BL	Baseline
cDNA	Complimentary Deoxyribonucleic acid
^{14}C	Carbon isotope
°C	Degrees Celcius
CAR	Cellular accumulation ratio
C_{max}	Maximum plasma concentration
CCM	Caco-2 cell monolayers
CCR5	Chemokine receptor 5
CD	Cluster differentiation (A glycoprotein predominantly found on the surface of helper T cells, dendritic cells, monocytes and macrophages)
CD4+	T cells expressing CD4 (A measure of the number of helper T cells p cubic millimeter of blood)
CDC	Centres for Disease Control
CLZ	Clozapine
CNS	Central nervous system
Conc.	Concentration
CV	Coefficient of variation
CXCR4	α-CXC Chemokine receptor 4
CYP	Cytochrome P450
ddC	Zalcitabine

d4T	Stavudine
ddi	Didanosine
DDIs	Drug-drug interactions
DEC	Diethylcarbamazine
DLV	Delavirdine
DMSO	Dimethylsulphoxide
DMEM	Dulbecco's modified Eagles medium
DNA	Deoxyrebonucleic acid
EDTA	Ethylamine-diaminetetra-acetic acid
EFV	Efavirenz
eMC	Electronic medicine compedium
FBS	Foetal Bovine Syrum
FDCs	Fixed dose combinations
Fig	Figure
FTC	Emtricabine
GIT	Gastro intestinal tract
h	Hour
^{3}H	Tritium
HAART	Highly active antiretroviral therapy
HBSS	Hanks Balanced Salt Solution
HIV	Human immunodeficiency virus
HPLC	High performance liquid chromatography
HQC	High quality control
IDV	Indinavir
IVM	Ivermectin
IREC	Institutional Research and Ethics Committee
kDa	Kilodalton
L	Litre
LHPG	Liverpool HIV Pharmacology group
LLD	Lower limit of detection
LLQ	Lower limit of quantification
LOD	Limit of detection
LPV	Lopinavir

LPV/r	Aluvia/Kaletra
LQC	Low quality control
mg	Milligram
MeOH	Methanol
min	Minute
µC	Microcurie
µg	Microgram
µL	Microlitre
µmol	Micromole
µMml	Micromolar
Ml	Millilitre
mmol	Millimole
mM	Millimolar
MDR1	Multidrug resistance 1
MQC	Medium quality control
mRNA	Messenger Ribonucleic acid
MRP	Multidrug resistance associated protein
MTRH	Moi Teaching and referral Hospital
MTT	Methyl thiazolyl diphenyl-tetrazolium bromide
NASCOP	National AIDS and STI control Programme
NFV	Nelfinavir
No	Number
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NRTI	Nucleoside reverse transcriptase inhibitor
NVP	Nevirapine
OATP	Organic anionic transporter protein
OI	Opportunistic infections
*P*app	Apparent permeability coefficient
PCR	Polymerase chain reaction
P-gp	P-glycoprotein
PI	Protease inhibitor
PK	Pharmacokinetic
PZQ	Praziquantel

PAR	Peak area ratio
RNA	Ribonucleic acid
QC	Quality control
RPMI	Roswell Park Memorial Institute medium
RT-PCR	Reverse transcriptase polymerase chain reaction
RTV	Ritonavir
s.d	Standard deviation
SLC	Slute-like carrier
SMC	Summary of product characteristics
SPC	Summary of Product Characteristics
SQV	Saquinavir
TB	Tuberculosis
3TC	Lavimudine
TEER	Trans Epithelial Electrical Resistance
TDM	Therapeutic drug monitoring
TDF	Tenofovir
TPV	Tipranavir
VBL	Vinblastine
Vol	Volume
V/v	Volume to volume ratio
WHO	World Health Organisation
XR	Tariquidar
ZDV/AZT	Zidovudine

PUBLICATIONS

Abstract

Kigen G, Kimaiyo S, Owen A, Edwards G, Back D, Gibbons S, Sang E, Khoo S. Prevalence of drug interactions between antiretroviral and co-administered drugs at the Moi teaching and Referral Hospital (AMPATH), ELDORET-Kenya. *9th International Congress on Drug Therapy in HIV Infection, Glasgow, November 2008, abstract O122 (O121 in abstract book/CD).*

Chapter 1

General introduction

Chapter 1

General introduction

1.1 Aetiology of HIV

1.2 Helminthic infections and HIV

1.2.1 Schistosomiasis

1.2.2 Lymphatic filariasis

1.2.3 Schistosomiasis and HIV

1.3 Structure of HIV

1.4 Life cycle of HIV

1.5 Pathogenesis of HIV

1.5.1 Course of infection

1.5.2 Progression to AIDS

1.5.3 Classification of HIV disease

1.6 Antiretroviral drugs

1.6.1 Classification

1.6.2 Reverse transcriptase inhibitors

1.6.2.1 NRTI

1.6.2.2 NNRTI

1.6.3 Protease inhibitors

1.6.4 Entry inhibitors

1.6.5 Integrase inhibitors

1.6.6 Maturation inhibitors

1.7 Combination therapy

1.7.1 Standardized Kenyan national antiretroviral drug regimens

1.7.1.1 First line ARV drug regimens for adult and adolescents in Kenya

1. Introduction

1.1 Aetiology of HIV

The term 'acquired immunodeficiency syndrome' or AIDS was first used by the Centres for Disease Control (CDC) in 1982 to describe 'a disease, at least moderately predictive of deficit in cell-mediated immunity, occurring in a person with no known cause for diminished resistance to that disease. Such diseases include KS (Kaposi's sarcoma), PCP (Pneumocystis carinii pneumonia), and serious OOI ["opportunistic" infections]' (CDC, 1982). Later in 1983 a retrovirus [later named the human immunodeficiency virus (HIV)] was isolated from a patient with AIDS (Barre-Sinoussi *et al.*, 1983). Since then, more than 65 million persons have been infected with HIV, and more than 25 million have died of AIDS. Most of these infections and deaths have occurred in developing countries, with Sub-Saharan Africa being home to almost 68 percent of the estimated 33.2 million persons living with HIV infection (UNAIDS, 2006; UNAIDS/WHO, 2008). AIDS is now one of the leading causes of premature death among people 15 to 59 years of age and HIV incidence is now reported to be on the rise in China, and India and Eastern Europe (Hamers *et al.*, 2003; Kaufman *et al.*, 2002; Merson, 2006).

1.2 Helminthic infections and HIV

Parasitic diseases are a common problem in the tropics. There is a geographical overlap of neglected tropical diseases, malaria, and HIV/AIDS, and therefore frequent occurrence of co-infections with these disorders. Studies have contributed additional evidence identifying parasitic helminth infections in Africa as important factors affecting the transmission of HIV and its pathogenesis (Gallagher *et al.*, 2005; Kallestrup *et al.*, 2005; Kjetland *et al.*, 2006; Nielsen *et al.*, 2006). Among

the parasitic diseases, systemic helminthic infections (schistosomiasis, lymphatic filariasis and onchocerciasis) are only second to malaria as a global burden [Table 1.1] (WHO, 2004).

1.2.1 Schistosomiasis

Schistosomiasis, also known as bilharzia, is a chronic disease caused by water-borne parasitic trematode flatworms of the genus Schistosoma. Infection with *Schistosoma mansoni, S. haematobium,* and *S. japonicum* causes illness in humans. It is endemic in 74 developing countries, infecting more than 200 million people in rural and peri-urban areas. Of these, 120 million have symptoms of the disease of which 20 million have severe consequences with 280,000 deaths annually (Savioli *et al.*, 2004b; Steinmann *et al.*, 2006; van der Werf *et al.*, 2003). An estimated 650 million people worldwide live in endemic areas, mainly in Africa (*S. mansoni* and *S. haematobium*), Latin America and the Caribbean (*S. mansoni*) as well as South-East Asia [*S. Japonicum*] (Hotez *et al.*, 2007; WHO, 2007b).

This disease is second only to malaria in human impact among tropical diseases and is the third most prevalent parasitic disease in the world. The prevalence is greatest in teenage years with higher rates among boys than girls (Elliott, 1996; Gryseels *et al.*, 2006). Its economic impact is reportedly second only to malaria in reducing productivity at work and in limiting children's ability to learn (King *et al.*, 2005). Praziquantel, a cheap and effective drug is the mainstay in the treatment of schistosomiasis for both individual and mass treatment recommended by World Health Organisation [WHO] (Fenwick *et al.*, 2006; Ferrari *et al.*, 2003). Artemether has been reported to treat schistosomiasis (Utzinger *et al.*, 2004), and oxadiazoles

and cysteine protease inhibitors have shown activity against schistosome infection (Abdulla *et al.*, 2007; Sayed *et al.*, 2008). Oxaminquine is no longer in use largely due to its narrow therapeutic index as well as resistance (Cioli *et al.*, 1993).

1.2.2 Lymphatic filariasis

Lymphatic filariasis afflicts over 120 million in more than 80 countries and over 1 billion are at risk (WHO, 2000a). The causative agents are the filarial worms *Wuchereria bacrofti* and *Brugia Malayi.* Ivermectin is the drug used for its mass control and treatment (Molyneux *et al.*, 2003).

1.2.3 Schistosomiasis and HIV

Co-infections between HIV/AIDS and schistosomiasis are not uncommon as both conditions are endemic in similar geographical areas [Table 1.1] (Lloyd-Smith *et al.*, 2008; Secor, 2006). A recent study concluded that the seroprevalence of schistosomiasis in HIV-infected patients of African origin in the UK is high (Smith *et al.*, 2008). Helminthic infections have been hypothesised to be due to an increase in hosts' susceptibility to infection owing to immunodeficiency (Maggi *et al.*, 1994; Pantaleo *et al.*, 1993), and a study in rural Zimbabwe has linked women with genital schistosomiasis to a threefold higher risk of contracting HIV (Kjetland *et al.*, 2006). Similarly, studies in western Kenya indicate that HIV-1 co-infection leads to increased susceptibility to re-infection with schistosomiasis, owing to breakdown of protective mechanisms as the CD4+ (cluster differentiation 4) cell count decreases (Secor, 2006; Secor *et al.*, 2004). A recent study has provided direct evidence that acute schistosomiasis significantly increases the risk of *de novo* HIV virus acquisition (Chenine *et al.*, 2008) .

Table 1.1 Burden of AIDS and parasitic diseases in DALYs and deaths by cause and sex in WHO Regions, estimates for 2002

Cause	Disease burdens (DALYs)			Deaths(thousands)		
	Both sexes	Males	Females	Both sexes	Males	Females
	(×1000)	*(×1000)*	*(×1000)*	*(×1000)*	*(×1000)*	*(×1000)*
HIV/AIDS	84 458	42 663	41 795	2 777	1 447	1 330
Malaria	46 486	22 243	24 242	1 272	607	665
Tropical diseases	12 245	8 273	3 973	129	79	50
Trypanosomiasis	1 525	966	559	48	31	17
Chagas disease	667	343	324	14	8	7
Schistosomiasis	1 702	1 020	681	15	10	5
Leishmaniasis	2 090	1 249	840	51	30	21
Lymphatic filariasis	5 777	4 413	1 364	0	0	0
Onchocerciasis	484	280	204	0	0	0
Leprosy	199	117	82	6	4	2
Dengue	616	279	337	19	8	10
Japanese encephalitis	709	338	371	14	7	7
Trachoma	2 329	597	1 732	0	0	0
Intestinal nematode infections	2 951	1 490	1 461	12	6	6
Ascariasis	1 817	910	907	3	1	2
Trichuriasis	1 006	519	488	3	2	1
Hookworm disease	59	31	27	3	2	1

DALYs - Disability Adjusted Life years(number of healthy years lost due to premature death or disability)

1.3 Structure of HIV

HIV belongs to a subgroup of retroviruses known as lentiviruses, or 'slow' viruses, which are members of the RNA retrovirus family (Haase, 1986b). Lentiviruses are characterised by a long period of persistence and replication before any onset of serious symptoms of disease (Haase, 1986a). Structurally, HIV is spherical in shape. The viral envelope is formed by glycoprotein knobs made up of gp120 and gp41 (encoded by the gene *env*), which penetrate a lipid bilayer membrane that surrounds a protein capsomere consisting of p17 and p24 proteins [encoded by the *gag* gene] (Fig 1.1) (Muesing *et al.*, 1985). The gp120 protein binds to the host cell via a CD4 receptor, whereas the gp41 induces fusion of the virus to the host cell membrane as well as anchor gp120. They are expressed as trimers to make up the viral envelope (Lee *et al.*, 1998). The capsomere contains two copies of a single stranded RNA genome and the viral enzymes reverse transcriptase, protease and integrase (encoded by *pol*).

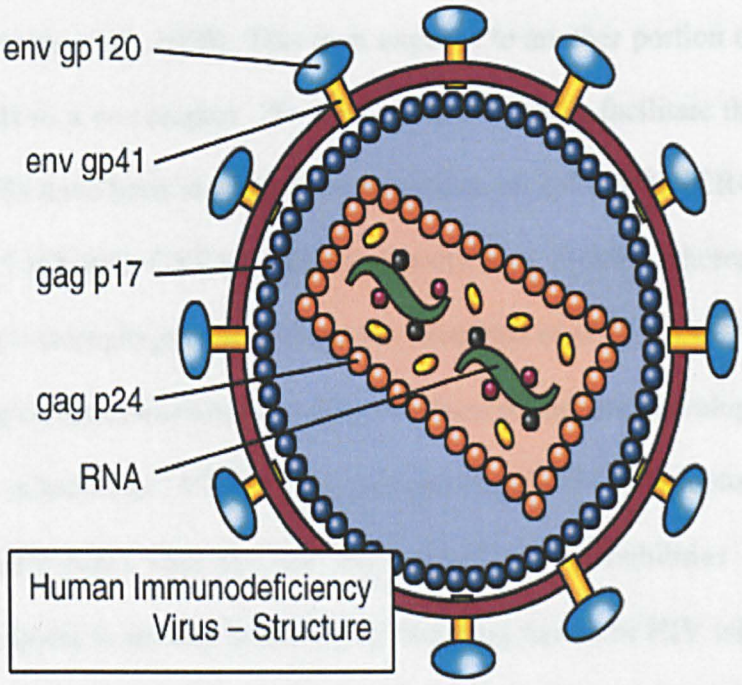


Fig.1.1 HIV Virion Structure: This above cartoon shows the HIV virion. The central nucleocapsid core contains two copies of the viral genome (ssRNA), plus core proteins and reverse transcriptase. Surrounding this is the viral envelope, which is comprised of cellular lipids interspersed with viral envelope proteins. Adapted from Avert.org (www.avert.org).

1.4 Life cycle of HIV

The stages of viral infection of a human CD4 T lymphocyte are shown in fig 1.2. HIV begins its infection of a susceptible host cell by binding of the virion envelope gp120 molecule to the CD4 receptor, expressed on the surface of the host cell (Dalgleish *et al.*, 1984; Kwong *et al.*, 1998). This then exposes to another portion of the trimer which then binds to a co-receptor. Two co-receptors which facilitate the entry of the virus to the cells have been identified, α-chemokine receptor 4 (CXCR4) and Chemokine receptor 5 (CCR5). CXCR4 facilitates entry into T-cells, whereas CCR5 facilitates entry into macrophages and monocytes (Scarlatti *et al.*, 1997). The gp120 can then bind to one of the co-receptors leading to fusion of the viral envelope to the host cell membrane (Chan *et al.*, 1998). The recognition of HIV co-receptors and understanding how HIV fuses with the cell has opened new possibilities of antiviral drugs that are designed to prevent infection by blocking fusion of HIV with its host cell [fusion inhibitors and chemokine antagonists] (Berger *et al.*, 1999; Starr-Spires *et al.*, 2002).

Following the fusion of the virus with host cell, there is uncoating of the viral envelope leading to the release of the viral genetic material (viral RNA) into the cell (Fig 1.2). The reverse transcriptase enzyme in HIV then catalyses the transcription or copying of the RNA to DNA, which is incorporated into the chromosomal DNA of the host cell by the viral integrase enzyme (Miller *et al.*, 1997). Once the viral DNA is integrated into the genetic material of the host (provirus), it is possible that the HIV may persist in latent state (virion) for hours to many years until cell activation through transcription [copying of DNA into RNA] (Adams *et al.*, 1994). The ability of HIV to persist in certain latently infected cells is the major barrier to eradication or

cure of HIV, and for this reason patients must remain on anti-viral therapy for life (Fauci, 1996).

The activation of the host cells results in the transcription of viral DNA into messenger RNA (mRNA), which is then translated to viral proteins [the new viral RNA forms the genetic material of the next generation of viruses] (Zimmerman *et al.*, 2002). These are then cleaved by the viral protease enzyme into mature, functional forms. This enzyme also mediates further modification and assembly of the proteins into an infectious virion which is then released from the host cell by budding from the cell membrane to infect another cell (Barre-Sinoussi *et al.*, 2004; Ho *et al.*, 1987). After infection, the cell may remain in a dormant state after incorporation of viral DNA into its genome or act as a reservoir for the formation and storage of more virions for subsequent release (Bukrinsky *et al.*, 1991; Zack *et al.*, 1990). There may also be cell death after the release of the virions. Unless the HIV lifecycle is interrupted by treatment, the virus spreads throughout the body and results in the destruction of the body's immune system (Ho *et al.*, 1987).

1.5 Pathogenesis of HIV

HIV infection is characterized by an insidious deterioration of the cellular immune system (Vergis *et al.*, 2000). The pathogenesis of HIV infection is a function several factors including the quality of viruses in the viruses in the infected individual, host cellular environment, and the virus life cycle. The level of virulence of an individual strain of virus, and co-infection with other microbes may influence the rate and severity of disease progression (Claydon *et al.*, 1991; Coffin, 1995; Fauci, 1996; Fauci, 1993). The infection begins with the binding of the virus to the host cells T cells. CD4 receptors at surface of the target cells are the primary receptors (Kwong *et al.*, 1998). The cells become infected and viral replication begins within them. This is followed by release virions by surface budding or the infected cells can undergo lysis to release new virions, which can then infect additional cells. Some HIV virions are carried from the site of infection to the regional lymph nodes where other immune system cells become infected (Clark *et al.*, 1991; Fauci, 1993). Large amounts of virus can become trapped here in networks of specialized cells with long, tentacle-like extensions referred to as follicular dendritic cells (FDCs) and are susceptible to infection but will survive for a long time. $CD4^+$ T cells, the primary targets of HIV, may become infected as they encounter HIV trapped on FDCs. Active replication of HIV occurs at all stages of the infection (Baroni *et al.*, 1988; Biberfeld *et al.*, 1986; Spiegel *et al.*, 1992).

Over a period of years, even when little virus is detectable in the blood, significant amounts of virus accumulate both within infected cells and bound to FDCs. HIV trapped on FDC's remains infectious, even when coated with antibodies. Thus, FDCs are a significant reservoir of infectious HIV and may explain in part how the

momentum of HIV infection is maintained (Spiegel *et al.*, 1992). Both the quantity and proportion of plasma CD4+ T-cells decrease steadily over a period of years to decades, and this progressive loss of CD4+ T-cells is associated with the development of acquired immunodeficiency syndrome (AIDS) in infected individuals (Hazenberg *et al.*, 2000). The degree of immunodeficiency associated with HIV-1 infection, as defined by the onset of opportunistic diseases, correlates closely with plasma CD4+ T-cell counts (Cohen, 1993; Ho *et al.*, 1995; Mellors *et al.*, 1997; Phillips, 1992; Picker *et al.*, 2004).

1.5.1 Course of Infection

Primary HIV infection is defined as the period from initial infection with HIV to the time of antibody response that is detectable. It is characterised by a burst of viremia in which virus is easily detected in peripheral blood in mononuclear cells and plasma. The number of CD4+ T cells in the bloodstream may decline gradually, occasionally to levels that allow opportunistic infections (OIs) to develop (Gupta, 1993; Pedersen *et al.*, 1990; Picker *et al.*, 2004; Vento *et al.*, 1993). Two to four weeks after exposure to the virus, most of HIV-infected persons suffer flu-like symptoms related to the acute infection (Cooper *et al.*, 1985; Schacker, 1996). The most common symptom is fever seen in over 75% of the patients. Others include headache, lymphadenopathy, rash and fatigue (Vanhems *et al.*, 1999). The burst of viremia does not persist. The patient's immune system fights back to dramatically reduce HIV levels with killer T cells (CD8+ T cells). They attack and kill infected cells producing the virus and B-cell-produced antibodies. This leads to viral replication at a lower level, and a patient's CD4+ T cell count may rebound to 80 to

90 percent of its original level (Daar *et al.*, 1991; Roos *et al.*, 1994; Vento *et al.*, 1993).

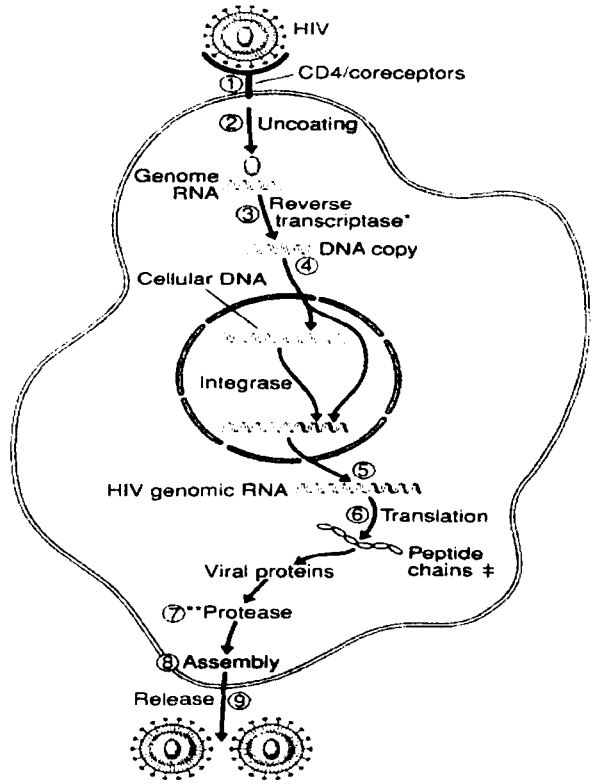
The initial reduction in viremia varies between individuals and correlates with the rate of progression of HIV disease (Borrow *et al.*, 1994; Koup *et al.*, 1994; Pantaleo *et al.*, 1997). A person then may remain free of HIV-related symptoms, often for years. This is despite the latent ongoing immune system destruction from a smouldering low-level replication of HIV in the lymphoid organs. Equilibrium between viral replication and host immune system is achieved and there are no manifestations of HIV infection. During this latency period, enough of the immune system remains sufficiently intact to provide immune surveillance and to prevent most infections (Ho *et al.*, 1995). The host cells may be infectious even if there are detectable HIV antibodies or if no virus can be measured in the patient's serum. This is because the fact that the HIV provirus becomes part of the infected host's cellular DNA (Busch *et al.*, 1995; Rosenberg *et al.*, 1997). The final phase of HIV infection occurs when a significant number of CD4+ lymphocytes have been destroyed and when production of new ones cannot match destruction (Hazenberg *et al.*, 2000). The failure of the immune system leads to appearance of clinical AIDS (Moss *et al.*, 1989; Moss *et al.*, 1988; Rosenberg *et al.*, 1997; Vanhems *et al.*, 1999).

1.5.2 Progression to AIDS

Even without treatment, the time taken for an HIV-infected person to develop clinical symptoms of AIDS averages between 8 to 10 years after initial infection. The rate of progression may vary considerably (Bacchetti *et al.*, 1989; O'Brien *et al.*, 1996; van den Berg *et al.*, 1994). Most AIDS-defining conditions are marked by a

CD4+ T count of less than 200 cells per μL of blood and the appearance of one or more of the typical OIs or cancers, including Kaposi's sarcoma, Pneumocystis carinii pneumonia, and Mycobacterium avium complex (Longini *et al.*, 1991; Osmond *et al.*, 1994). OIs are caused by microbes that usually do not cause illness in healthy people; infections are often severe and sometimes fatal because the immune system is so ravaged by HIV that the body cannot combat them. As a result of the rapid disappearance of these important immune cells the virus and infectious microbes take over (Mellors *et al.*, 1995; Mellors *et al.*, 1997; Picker *et al.*, 2004).

A normal CD4 count of between 600-1200 cells/μL indicates that the immune system has not undergone sufficient damage to put the patient at risk of OIs. They are therefore unlikely to require treatment. Counts that are less than 350 cells/μL indicate impairment of immunity and prompt considerartion of antiretroviral therapy. CD4 counts of less than 200 cells cells/μL indicate imminent risk of serious OIs or other complications of HIV and therefore immediate treatment (Dybul *et al.*, 2002).



1-Entry: Virus binds to a CD4 molecule and one type of "coreceptor" (either CCR5 or CXCR4). Then the virus fuses with the cell.
2-Uncoating: virus empties its contents into cell.
3-Reverse Transcription: single strands of viral RNA are used by the reverse transcriptase enzyme to create double-stranded DNA. ***Reverse transcriptase enzyme** required to convert viral RNA to DNA.
4-The newly-formed HIV DNA enters the nucleus of the CD4 cell
5-Viral RNA leaves the nucleus
6-Translation: When the infected cell divides, the viral DNA is "read" and long chains of proteins are made.
7-HIV Protease enzyme**- cuts up newly produced viral proteins.
8-Assembly: sets of viral proteins chains come together
9-Budding and release: The newly-assembled virus pushes ("buds") out of the original CD4 cell. This new virus is now able to target and infect other CD4 cells.

Fig 1.2. The life cycle of the HIV. The cartoon shows the various processes including binding of the virus to the CD4 receptors, transcription, budding and release. Adapted from www.hopkins.edu.

1.5.3 Classification of HIV disease

The clinical progression of AIDS may be monitored by the tracking of the CD4+ counts, which provides critical information on the HIV disease stage and clinical management. There are two major classification systems:

i. Centres for Disease Control and Prevention (CDC) classification system (CDC, 1992)
ii. World Health Organization (WHO) Clinical Staging and Disease Classification System (WHO, 2005).

The CDC disease staging system assesses the severity of HIV disease by CD4 cell counts and by the presence of specific HIV-related conditions and the definition of AIDS includes all HIV-infected individuals with CD4 counts of less than 200 cells/μL as well as those with certain HIV-related conditions and symptoms. The CDC categorization of HIV/AIDS is based on the lowest documented CD4 cell count (Table 1.2) and on previously diagnosed HIV-related conditions (categories A, B and C). For example, if a patient had a condition that once met the criteria for Category B but now is asymptomatic, the patient would remain in Category B. Additionally, categorization is based on specific conditions, as indicated (Table 1.2). Patients in categories A3, B3, and C1-C3 are considered to have AIDS (CDC, 1993).

The WHO clinical staging and disease classification system classifies HIV disease on the basis of clinical manifestations that guide the diagnosis, evaluation, and management of HIV/AIDS, and does not require a CD4 cell count (WHO, 2005). This staging system is used to determine eligibility for antiretroviral therapy. Clinical stages are categorized as 1 through 4, progressing from primary HIV

infection to advanced HIV/AIDS (Table 1.3 and 1.4). These stages are defined by specific clinical conditions or symptoms. For the purpose of the WHO staging system, adolescents and adults are defined as individuals aged ≥15 years (WHO, 2007a).

Table 1.2: CDC Classification System for HIV-Infected Adults and Adolescents

	Clinical categories		
CD4 Cell count (cells/mm^3)	**A**	**B**	**C**
(1) ≥500 cells/μL	A1	B1	C1
(2) 200-499 cells/μL	A2	B2	C2
(3) <200 cells/μL	A3	B3	C3

Clinical category A

Asymptomatic infections,

Acute HIV,

Persistent generalized lymphadenopathy.

Clinical category B

Category B symptomatic conditions are defined as symptomatic conditions occurring in an HIV-infected adolescent or adults that meet at least one of the following criteria:

a) They are attributed to HIV infection or indicate a defect in cell-mediated immunity.

b) They are considered to have a clinical course or management that is complicated by HIV infection, e.g. Persistent or resistant vulvovaginal candidiasis or oropharyngeal candidiasis (thrush).

Clinical category C

AIDS-Indicator Conditions e.g., oesophageal candidiasis, *Mycobacterium* tuberculosis, *Pneumocystis carinii* pneumonia (PCP), wasting syndrome due to HIV (involuntary weight loss >10% of baseline body weight) associated with either chronic diarrhoea or chronic weakness and documented fever ≥1 month.

Table 1.3: WHO Clinical Staging of HIV/AIDS for Adults and Adolescents (Interim Definitions)

Primary HIV Infection
Asymptomatic
Acute retroviral syndrome
Clinical Stage 1
Asymptomatic
Persistent generalized lymphadenopathy
Clinical Stage 2
Moderate unexplained weight loss (<10% of presumed or measured body weight)
Recurrent respiratory infections (respiratory tract infections, upper respiratory infections, sinusitis, bronchitis, otitis media, pharyngitis)
Herpes zoster
Minor mucocutaneous manifestations (angular cheilitis, recurrent oral ulcerations, seborrheic dermatitis, prurigo, papular pruritic eruptions, fungal fingernail infections
Clinical Stage 3
Conditions for which a presumptive diagnosis can be made on the basis of clinical signs or simple investigations
Severe weight loss (>10% of presumed or measured body weight)
Unexplained chronic diarrhoea for >1 month
Unexplained persistent fever for >1 month (intermittent or constant)
Oral candidiasis (thrush)
Oral hairy leukoplakia
Pulmonary tuberculosis within the last 2 years
Severe presumed bacterial infections (e.g., pneumonia, empyema, pyomyositis, bone or joint infection, meningitis, bacteremia)
Acute necrotizing ulcerative stomatitis, gingivitis or periodontitis
Conditions for which confirmatory diagnostic testing is necessary
Unexplained anaemia (haemoglobin <8 g/dL)
Neutropenia (neutrophils <500 cells/μL)
Thrombocytopenia (platelets <50,000 cells/μL)

Table 1.4: WHO Clinical Staging : Clinical Stage 4

Conditions for which a presumptive diagnosis can be made on the basis of clinical signs or simple investigations
HIV wasting syndrome, as defined by the CDC (see Table 2 .1)
Pneumocystis carinii pneumonia
Recurrent severe or radiologic bacterial pneumonia
Chronic herpes simplex infection (oral or genital, or anorectal site) for >1 month
Oesophageal candidiasis
Extrapulmonary tuberculosis
Kaposi sarcoma
Central nervous system toxoplasmosis
HIV encephalopathy
Conditions for which a confirmatory diagnostic testing is necessary
Cryptococcosis, extrapulmonary
Disseminated nontuberculosis *Mycobacteria* infection
Progressive multifocal leukoencephalopathy
Candida of the trachea, bronchi, or lungs
Cryptosporidiosis
Isosporiasis
Visceral herpes simplex infection, cytomegalovirus infection (retinitis or organ other than liver, spleen, or lymph node)
Any disseminated mycosis (e.g., histoplasmosis, coccidioidomycosis, penicilliosis)
Recurrent nontyphoidal Salmonella septicaemia
Lymphoma (cerebral or B-cell non-Hodgkin)
Invasive cervical carcinoma
Visceral leishmaniasis

1.6 Antiretroviral Drugs

1.6.1 Classification

The introduction of antiretroviral drugs has remarkably improved the treatment of HIV/AIDS with a decrease in morbidity and mortality (Berrey *et al.*, 2001; Palella *et al.*, 1998; Vittinghoff *et al.*, 1999). The drugs are usually used in combination to treat HIV, and for this reason are referred to as 'highly active antiretroviral therapy' or HAART. The drugs currently approved for the treatment of HIV infection are broadly classified into four main categories according to their mechanisms of action: Nucleoside Reverse Transcriptase Inhibitors (NRTI), Non - Nucleoside Reverse Transcriptase Inhibitors (NNRTI), Protease inhibitors (PI) and Fusion Inhibitors, Chemokine antagonists and Intergrase inhibitors (Raffanti *et al.*, 2001) [Table 1.5].

1.6.2 Reverse transcriptase inhibitors

Reverse transcriptase inhibitors target reverse transcriptase enzyme, an RNA-dependent DNA polymerase.

1.6.2.1 NRTIs

These were the first class of compounds discovered to be inhibitors of viral replication (Mitsuya *et al.*, 1986). By inhibition of the viral reverse transcriptase enzyme, they decrease the turnover of viral RNA to double stranded DNA. NRTIs are structurally similar to endogenous host nucleosides required for the DNA synthesis. They are prodrugs that must undergo phosphorylation once inside the cells to produce active metabolites (Stein *et al.*, 2001). Following entry to the infected host cell, the host kinase enzymes phosphorylate the drugs from their parent to the active triphosphate form [5'-triphosphates]. This inhibits HIV replication by

competing with endogenous nucleotides for incorporation into the growing viral DNA strands; and terminating the formation of the chain once incorporated (Hoggard *et al.*, 2000; Mitsuya *et al.*, 1986).

Table 1.5: Classification of antiretroviral drugs

Reverse transcriptase inhibitors		**Protease inhibitors**	**Entry inhibitors**		**Integrase inhibitors**	**Maturation inhibitors**
(NRTIs)	**(NNRTIs)**		**Fusion inhibitor**	**CCR5 inhibitor**		
Zidovudine (AZT/ZDV) Zalcitabine (ddC) Lavimudine (3TC) Didanosine (ddI) Stavudine (d4T) Emtricabine (FTC) Abacavir (ABC) Tenovofir* (TDF)	Efavirenz (EFV) Nevirapine (NVP) Delarvidine (DLV) Etravirine	Saquinavir (SQV) Ritonavir (RTV) Indinavir (IDV) Nelfinavir (NFV) Amprenavir (APV) Lopinavir (LPV) Tipranavir (TPV) Duranavir Atazanavir Fosamprenavir	Enfurvitide	Maraviroc	Raltegravir Elvitegravir	Bevirimat Vivecon

CCR5 - Chemokine receptor 5

NRTI - Nucleoside reverse transcriptase inhibitors

NNRTI - Non-nucleoside reverse transcriptase inhibitors

*Nucleotide reverse transcriptase inhibitor

NRTIs with the exception of didanosine are well absorbed. The plasma elimination half-lives of NRTIs range between 2-6 hrs, whereas those of the active intracellular metabolites are generally longer, and correlate moderately to poorly with the plasma concentrations of the parent drug. Tenovofir, didanosine, and lavimudine are excreted largely unchanged in the kidney, whereas zidovudine is excreted via the liver, mainly through glucoronidation (Beach, 1998; Stein *et al.*, 2001). NRTIs are not metabolised by the cytochrome P450 (CYP 450) system and their exact route of metabolism has yet to be fully elucidated. Many are glucuronidated, and some are recovered without being metabolized in the urine and faeces (Ray *et al.*, 2004; Zapor *et al.*, 2004). They have been reported to inhibit drug transporters ABCC1, ABCC2 and ABCC3; and AZT to induce ABCB1 expression in tumour cells (Signoretti *et al.*, 1997; Weiss *et al.*, 2007a; Weiss *et al.*, 2007b).

As a class they are generally safe and well tolerated, though there are several life threatening toxicities including mitochondrial toxicity. NRTIs are competitive inhibitors of human mitochondrial DNA polymerase, the depletion of which disrupts phosphorylation leading to toxic accumulation of fatty acids, dicarboxylic acids and free radicals (Fromenty *et al.*, 1997). The major toxicities of NRTI include rash, lactic acidosis and mitochondrial dysfunction (through inhibition of DNA polymerase). Others include neuropathy (didanosine, stavudine, lavimudine, and zalcitabine), pancreatitis (didanosine), anaemia/neutropenia (zidovudine) and myositis [zidovudine] (Brinkman *et al.*, 1998b; Carr *et al.*, 2000; Kakuda, 2000; Simpson *et al.*, 1995).

Tenofovir disoproxil fumarate (TDF) is a prodrug of tenofovir and is the first nucleotide reverse transcriptase inhibitor approved for treatment of HIV infection. It inhibits HIV reverse transcriptase by competing with endogenous deoxyadenosine 5`-diphoaphate for incorporation in viral DNA (Chapman *et al.*, 2003; Fung *et al.*, 2002). Unlike the NRTIs which must undergo 3 phosphorylation steps for activation; it only needs to be converted to diphosphate which is the active form of the drug, since it already contains phosphonate group attached to the adenine base (Robbins *et al.*, 1998). As a result, it is active in both dividing (activated) and non-dividing (resting) cells (Grim *et al.*, 2003).

1.6.2.2 NNRTIs

NNRTIs are a structurally unrelated group of drugs and require no activation to exert their antiviral effect. Their inhibition is caused by binding to a hydrophobic pocket close to the active site and is non-competitive and allosteric (Jacobo-Molina *et al.*, 1993). They are well absorbed and are metabolised by Cytochrome P450s (CYP3A4 and CYP2B6), which is important when considering ethnic variability in pharmacokinetics because of genetic heterogenicity (Mouly *et al.*, 2002). Efavirenz (EFV) and nevirapine (NVP) are both substrates and inducers of CYP3A4, CYP2B6 and have the potential to inhibit CYP2C19 (Adkins *et al.*, 1998; Erickson *et al.*, 1999; von Moltke *et al.*, 2001). The relationship between NNRTIs and drug transporters is sparse and inconclusive. Experiments on calcein uptake and flow cytometric rhodamine123 efflux assay suggest that they are inhibitors of P-gp (Storch *et al.*, 2007; Stormer *et al.*, 2002). A recent study concluded that EFV and delavirdine (DLV) inhibited ABCC1, ABCC2, and ABCC3 transporters (Weiss *et al.*, 2007a; Weiss *et al.*, 2007b).

Both EFV and NVP have long elimination half-lives (30-35hrs) compared to DLV (6hrs), which is rarely used. NNRTIs are excreted via the liver and drug transporters in the kidney and biliary tract play an important role in the elimination of antiretroviral drugs. Nevirapine and efavirenz may cause hepatotoxicity and therefore pre-existing liver dysfunction (such as chronic viral hepatits) is an important risk factor for drug-induced hepatotoxicity (Balzarini, 2004; van Leth *et al.*, 2004). These agents should only be used in combination regimens as monotherapy is associated with rapid resistance (Richman *et al.*, 1994). The most frequently reported side effects are mild rash (Bardsley-Elliot *et al.*, 2000; Scott *et al.*, 2000), asymptomatic elevation of liver enzymes (Dieterich *et al.*, 2004) and fat redistribution (Adkins *et al.*, 1998).

1.6.3 Protease inhibitors (PIs)

PIs target the viral protease enzyme. This enzyme is essential for the formation of intact infectious virions (Deeks *et al.*, 1997; McDonald *et al.*, 1997; Meek *et al.*, 1990) and cleaves the polyprotein products of the *gag* and *gag-pol* genes into functional core viral proteins and enzymes (Kohl *et al.*, 1988; Kramer *et al.*, 1986). PIs inhibit the catalysis of this cleavage process with the resultant virions produced being immature and uninfective (Craig *et al.*, 1991). The absorption of the PIs is improved with food, especially for nelfinavir where the drug exposure is almost twice that when taken fasting. It is also limited by metabolic degradation by cytochrome enzymes within the gut (mainly CYP3A4 isoform) as well as drug efflux transporters since PIs are substrates and inhibitors of drug transporters (Kim *et al.*, 1998a; Lee *et al.*, 1998; Srinivas *et al.*, 1998). In a study comparing the inhibitory influence of PIs on P-gp using calcein assay and flow cytometric rhodamine123

efflux assay, the ranking order of inhibition was: nelfinafir (NFV) > ritonavir (RTV) > tipranavir (TPV) > lopinavir (LPV) > saquinavir (SQV) > amprenavir (APV) > atazanavir (Storch *et al.*, 2007); while the order of inhibition of CYP3A4 enzyme is RTV > indinavir (IDV) > NFV > APV > LPV and SQV (Hsu *et al.*, 1998a). PIs may therefore influence the pharmacokinetics of concomitantly administered drugs in several ways, leading to increased or decreased bioavailability (Barry *et al.*, 1999; de Maat *et al.*, 2003). The interactions may lead to beneficial effects such as the use RTV to boost the bioavailability of other PIs such as SQV or LPV (van Heeswijk *et al.*, 2001).

The excretion of all the PIs is mainly via the liver. PIs are extensively metabolised by CYP enzymes, mostly by the CYP3A4 isoform. Nelfinavir is also metabolised by the CYP2C19 isoform, resulting in an active M8 metabolite. PIs [with the exception of indinavir (IDV)] have short plasma elimination half-lives (generally less than 8 hrs), even when boosted by RTV. RTV boosting reduces the hepatic clearance of IDV, APV, fosamprenavir and atazanavir by inhibiting hepatic metabolism, thus increasing the plasma concentrations of these drugs. PI drugs are associated with gastrointestinal (GIT) disturbance, hepatotoxicity, lipodystrophy, hypersensitivity, elevated lipids, and glucose intolerance. Other recognised toxicities include osteopenia, osteoporosis and vascular necrosis (Montessori *et al.*, 2004).

1.6.4 Entry inhibitors

Entry inhibitors interfere with binding, fusion and entry of HIV-1 to the host cell by blocking one of several targets. Maraviroc and enfuvirtide are the two drugs that have been approved in this class and are used in combination therapy for the treatment of HIV (Biswas *et al.*, 2007). Maraviroc binds to CCR5 (chemokine receptor 5), preventing an interaction with gp120 (Pugach *et al.*, 2008) whereas enfuvirtide binds to gp41 leading to the disruption of the HIV-1 molecular machinery at the final stage of fusion with the target cell thus preventing uninfected cells from becoming infected (Lalezari *et al.*, 2003). All are available for oral treatment of HIV infection except enfuvirtide, which is administered parenterally in combination with other antiretrovirals when all other treatments have failed.

1.6.5 Integrase inhibitors

Integrase inhibitors inhibit the integrase enzyme, which is responsible for integration of viral DNA into the DNA of the infected cell and raltegravir has recently been approved for the treatment of HIV (Sayana *et al.*, 2008).

1.6.6 Maturation inhibitors

Maturation inhibitors (bevirimat and vivecon) are still undergoing clinical trials. They act by interfering with the processing of the newly translated HIV polyprotein precursor, gag thus preventing conversion of the polyprotein into the mature capsid protein (p24). Unlike the protease inhibitors which bind to protease. The nascent virus particles are then rendered incapable of infecting other cells (Salzwedel *et al.*, 2007).

1.7 Combination therapy

Anti-HIV treatment using antiretrovirals is sophisticated and complex and would require a cocktail of 3 to 4 drugs for routine treatment in addition to other drugs used in the management of opportunistic infections (Ledergerber *et al.*, 1999; Temesgen *et al.*, 1997; Volberding *et al.*, 1998). The specific choice of the drugs depends on a number of factors, such as drug resistance, tolerability, drug interactions and effectiveness of the treatment (Dresser *et al.*, 2000; Flexner, 2000; Piscitelli *et al.*, 2001).

HAART does not completely eradicate HIV infection from an infected person because of the presence of latently infected CD4+ T cells harbouring replication competent HIV-1. HIV replicates within the cells and therefore the drug that targets its replication must penetrate into infected cells and anatomical compartments such as the central nervous system (CNS) at sufficiently high concentrations to exert effects and avoid the establishment of sanctuary sites. Low-level replication may also still continue because of the sub-optimal infiltration of drugs into anatomical sanctuary sites e.g. CNS, macrophages and lymphocytes (Jones *et al.*, 2001b). The distribution of antiretroviral agents within the cell also depends on physicochemical properties such as lipophilicity as well as active systems that are mediated by transporters (influx and efflux), and sequestration because of intracellular protein binding or ion trapping (Owen *et al.*, 2004c). The inadequate drug penetration results in viral replication in the presence of low drug concentrations thus facilitating viral mutations and precipitating complete drug failure (Acosta *et al.*, 2000; Hoggard *et al.*, 2003). Combinations of antiretroviral drugs are therefore used to create multiple obstacles to HIV replication to keep the number of offspring low and reduce the

possibility of a resistance mutation. If a mutation arises that conveys resistance to one of the drugs being taken, the other drugs continue to suppress reproduction of that mutation. Section 1.7.1.1 and 1.7.1.2 outline the common combinations used in Kenya.

HAART consist of a combination of three or more drugs. Several factors are involved in the choice of the ARV combination. The most important is the clinical stage of the disease, age, underlying medical conditions, adverse effects and other factors such as cost and availability of the ARVs. The most common combination given to those beginning treatment consists of two NRTIs combined with either an NNRTI or a "boosted" protease inhibitor. Ritonavir (in small doses) is the drug most commonly used to boost a protease inhibitor (Gallant, 2004). An example of a common combination is the two NRTIs zidovudine and lamivudine combined with the NNRTI efavirenz (www.avert.org). Fixed dose combinations (FDCs) of ARV are multiple antiretroviral drugs combined into a single pill, which helps reduce pill burden and therefore encourage compliance. FDCs may combine different classes of ARVs or contain only a single class (Table 1.6).

Table 1.6 Fixed dose ARV combinations

Brand Name	Antiretroviral Drugs
Combivir	zidovudine + lamivudine
Trizivir	abacavir + zidovudine + lamivudine
Kaletra	lopinavir + ritonavir
Kivexa	abacavir + lamivudine
Truvada	emtricitabine + tenofovir
Atripla	efavirenz + emtricitabine + tenofovir

1.7.1 Standardized Kenyan national antiretroviral drug regimens

Like many other sub-Saharan countries Kenya still has a high HIV prevalence with the latest survey indicating a rate of 5.1% in 2007, having decreased from 14% in the mid 1990s (MOH-Kenya, 2005; NACC-Kenya, 2007; UNAIDS/WHO, 2008). The decline in the prevalence has been linked to behaviour change, with the percentage of men with multiple partners decreasing, and the use of condoms. In addition, the ministry of Health has rolled out several programmes to provide ARVs to afflicted population in several health outlets (NASCOP, 2005; UNAIDS/WHO, 2008).

1.7.1.1 First line ARV drug regimens for adult and adolescents in Kenya

Stavudine (d4T) or Zidovudine (AZT)

+

Lavimudine (3TC)

+

Nevirapine (NVP) or Efavirenz (EFV)

As outlined above, d4T and 3TC is the preferred NRTI combination owing to the cost and efficacy. However, due to toxicity, it may be substituted by AZT or TDF unless there is evidence of anaemia which is a common presentation owing to nutritional deficiencies. As for the choice of the NNRTI, NVP has the cost advantage and can be used during pregnancy. Efavirenz is preferred in patients with HIV/TB co-infection who are on rifampicin (NASCOP, 2005).

1.7.1.2 Second line ARV drug regimens for adults and adolescents in Kenya

Didanosine (ddi) + Abacavir (ABC) + Lopinavir/ritonavir [LPV/r](Kaletra)	or	Tenovovir (TDF) + Abacavir + Lopinavir/ritonavir (Kaletra)

Second line regimens are outlined above. These drugs are used once failure of the first line regimen is diagnosed, either clinically or using immunological criteria (viral load testing). The factors that would influence the decision to change the ARV drug regimens include the following:

- Patient treatment history
- Adherence history
- Clinical, virological and immunological indicators (CD4 counts)
- Evidence of toxicity

3TC/FTC and d4T/AZT should not be used in subsequent treatments owing to cross resistance between the pairs and patients who develop ABC hypersensitivity can be treated with LPV/r + TDF/DDI or without AZT (NASCOP, 2005).

1.8 Antihelmintic drugs

The broad-spectrum anthelminthic drugs currently in use include praziquantel (PZQ), ivermectin (IVM) and albendazole (ABZ). ABZ is mainly indicated in the management of nematodes and trematodes whereas IVM and PZQ have a larger spectrum and may also be used in the management of filariasis and cestodes. The two drugs in addition to diethylcarbamazine (DEC) are used for mass treatment of lymphatic filariasis (Ottesen *et al.*, 2008). In addition, PZQ is the most widely used drug in the management of schistosomiasis (Cioli *et al.*, 2003; Fenwick *et al.*, 2006). It is also effective against hydatid disease (due to *Echinococcus spp*) whereby it is administered with ABZ (Cobo *et al.*, 1998).

1.8.1 Praziquantel (PZQ)

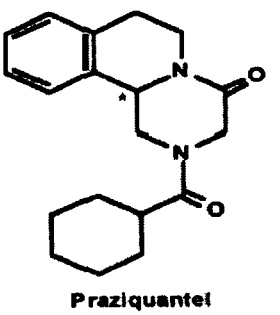


Fig 1.3 Chemical structure. Adapted from Pubchem (www.ncbi.nlm.nih.gov)

PZQ is 2-(cylohexylcarbonyl)-1, 2, 3, 6, 7, 11b-hexahydro-4H-pyrazino [2, 1-a] isoquinoline-4-one. The commercial preparation of PZQ is a racemate composed of 'levo' R (-) and 'dextro' S (+) isomers of which only the (-) enantiomer has antischistosomal activity, both *in vivo* and *in vitro* (Wu *et al.*, 1991). The two isomers however have similar toxicity. The detailed mechanism of action has not yet been elucidated. The exposure of the schistosomes to the drug either *in vitro* or *in*

vivo results in spastic paralysis of the worm musculature. The contraction is accompanied, and probably caused by rapid Ca^{2+} influx into the schistosome (Doenhoff *et al.*, 2008; Greenberg, 2005b). Vacuolisation at the base of the tegumental syncytium and blebbing at the surface also occurs (Cioli *et al.*, 2003; Greenberg, 2005a; Mehlhorn *et al.*, 1981). These morphological alterations are accompanied by an increased exposure of schistosome antigens at the parasite surface. Some of the antigens have been identified and appear to be connected with the host immune response that is required for the complete activity of PZQ (Brindley *et al.*, 1989). The schistosome calcium channels are the possible molecular targets of PZQ (Kohn *et al.*, 2001). A recent hypothesis suggests that it is an adenosine antagonist (Angelucci *et al.*, 2007).

Orally administered PZQ is rapidly absorbed, measurable amounts appearing in the blood as early as 15 minutes after dosing with peak levels occurring after 1-2hrs. The maximum plasma concentrations after standard dose of 40mg/Kg shows wide inter individual variations in the range of 200-2000ng/ml. PZQ undergoes a pronounced liver first pass metabolism, with rapid disappearance from the circulation, the plasma half-life ranging between 1-3 hrs. Elimination occurs through urine and faeces and is more than 80% complete after 24 hrs (Giorgi *et al.*, 2001). The main metabolites are represented by mono-, di-, and tri-hydroxylated compounds that are produced in the liver by microsomal cytochrome P450, particularly by the isoforms 2B1 and 3A, which are experimentally inducible by phenobarbitone (Giorgi *et al.*, 2001; Ridtitid *et al.*, 2002). The most abundant metabolite is the 4-hydroxycyclohexyl-carbonyl analogue, which represents 60% of the metabolites. The bioavailability of PZQ is increased by simultaneous administration of substances that inhibit cytochrome P450

activities e.g. cimetidine causes a 100% increase (Jung *et al.*, 1997; Metwally *et al.*, 1995). For this reason, cimetidine has been used in combination with PZQ, especially in the treatment of neurocystercosis, where high concentrations are required. Diphenhydramine and 17 alpha-ethinylestradiol have the same effect, and the concomitant administration with food also increases its bioavailability (Masimirembwa *et al.*, 1994a). Epileptic drugs, especially carbamazepine and phenytoin, as well as corticosteroids reduce its bioavailability (Masimirembwa *et al.*, 1994a). Chloroquine similarly decreases its bioavailability to a significant extent (Masimirembwa *et al.*, 1994b). Ketoconazole, a CYP-450 inhibitor has been reported to double the plasma concentration of PZQ in humans, while rifampicin an inducer has been reported to dramatically reduce its concentration, and dose adjustment upon co-administration has been recommended (Ridtitid *et al.*, 2007; Ridtitid *et al.*, 2002).

To date PZQ has not been conclusively characterised in relation to drug transporters. In a study involving permeability through Caco-2 cells, PZQ did not show potential for interacting with cellular efflux pumps at a concentration range of 10-100µg/ml despite being highly permeable (Gonzalez-Esquivel *et al.*, 2005). A related study concluded that PZQ among other anthelminthics was an inhibitor of P-gp without being its substrate based on transport along Caco-2 monolayers (Hayeshi *et al.*, 2006). P-gp has also been postulated to play a role in the resistance of helminths to PZQ. Increased levels of SMDR2 RNA, a P-gp homologue from *S.Mansoni* following exposure to sublethal concentrations of PZQ has been reported (Messerli *et al.*, 2009).

In general, the toxicity in animal studies has been found to be low. Side effects are observed after administration of PZQ in about 30-60% of the patients, but are usually mild and transient and disappear usually within 24 hrs. The common side effects include headache, nausea, anorexia, vomiting, epigastric pain and diarrhoea (with or without blood and/or mucus), fever, sleepiness and occasionally skin rash and oedema. The severity of the side effects has been correlated with the intensity of infection, probably due to the dying schistosomes and the release of their products. No genotoxic or carcinogenic risks have been reported, but more studies should be carried out since there may be some genetic polymorphisms leading to accumulation of mutagenic metabolites (Dayan, 2003; Stelma *et al.*, 1995).

1.8.2 Ivermectin (IVM)

IVM is a potent antiparasitic drug from the macrocyclic lactone (MLs) family, the most powerful agents against a broad spectrum of both ecto- and endoparasites. It was widely used in veterinary medicine due to its efficacy and wide margin of safety until 1987 when it was introduced for human use for treatment of onchocerciasis (Lindley, 1987; Steel, 1993). Since then, it has been used in combination with ABZ and diethylcarbamazine (DEC) for the treatment and control of onchocerciasis and lymphatic filariasis (Molyneux *et al.*, 2003). It is thought to act by opening the glutamate-gated chloride (Cl-) channels leading to paralysis of body wall and the pharynx of the nematodes. The basis of selective toxicity is the absence of the ligand-gated channels in vertebrates (Cully *et al.*, 1994; Yates *et al.*, 2003).

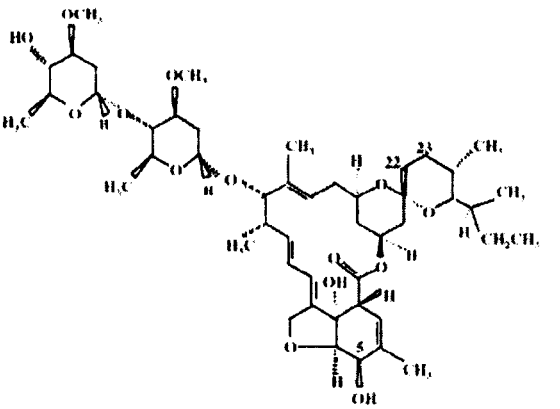


Fig.1.4 Ivermectin chemical structure. Adapted from PubChem (*www.ncbi.nlm.nih.gov*)

IVM and other MLs are highly lipophilic molecules characterized by the ML ring as a special feature (Fig 1.4), and therefore widely distributed in the body (Krishna *et al.*, 1993; Scott *et al.*, 1992). The antiparasitic activities of IVM and other MLs are related to the presence of effective concentrations for a suitable length of time in the systemic circulation and in target tissues (Lanusse *et al.*, 1997; McKellar *et al.*, 1996).

Studies regarding metabolism of IVM in humans is scarce (Gonzalez Canga *et al.*, 2008). IVM is extensively metabolised by human cytochrome P450. The predominant isoform responsible is CYP3A4 converting the drug to at least 10 metabolites, most of them hydroxylated and demethylated derivatives (Zeng *et al.*, 1998). Radioactive metabolites were reported after oral administration of IVM to healthy volunteers (Fink, 1989). IVM is both a substrate and inhibitor of P-gp, and has been demonstrated to inhibit Pgp, ABCC1, ABCC2, and ABCC3 activities after stimulation by their respective activators (Lespine *et al.*, 2006; Pouliot *et al.*, 1997),

and studies have indicated that it is a potential inducer of several cytochrome P450 isozymes including CYP1A, CYP2B and CYP3A subfamilies (Skalova *et al.*, 2001).

1.8.3 Albendazole (ABZ)

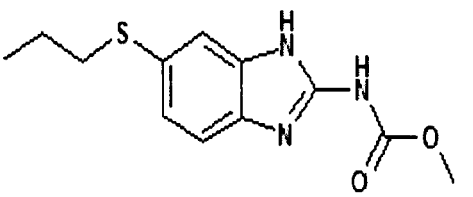


Fig.1.5 ABZ chemical structure. Adapted from Pubchem (*www.ncbi.nlm.nih.gov*)

ABZ is a broad-spectrum anthelminthic agent (Bennett *et al.*, 2000; Cox, 2000), which is also used to treat microsporidial infections, an emerging disease of relevance, particularly among those infected with human immunodeficiency virus (Costa *et al.*, 2000). ABZ therapy is hampered by its low solubility and poor absorption from the gastrointestinal tract, resulting in low bioavailability and reduced efficacy. It is relatively insoluble in water and most organic solvents, properties that influence its absorption behaviour in the body. As it undergoes very rapid first pass metabolism, the unchanged drug has not been reliably detected in plasma. A fatty meal enhances the absorption up to 5-fold (Dollery, 1999). It is converted *in vivo* into albendazole sulphoxide (ABZSO), the therapeutically active form and ABZ sulfone ($ABZSO_2$) in sequential sulfoxidation. CYP450 and flavin monooxygenase systems have been suggested to be responsible for the sequential sulfoxidation. Both systems are involved in the sulfoxidation , whereas CYP450 is the main determinant

of sulfonation (Rawden *et al.*, 2000; Souhaili-El Amri *et al.*, 1988) [ABZO has a chiral centre and it appears that the formation of ABZ (-) sulphoxide depends on P450 enzymes, whereas that of ABZ (+) sulphoxide depends on flavin enzyme and subsequent oxidation to ALB sulfone is dependent on P450 enzymes] (el Amri *et al.*, 1987; Souhaili-El Amri *et al.*, 1988). ABZ, though a substrate of CYP3A4, is neither a substrate nor an inhibitor of P-glycoprotein or breast cancer resistant protein, BCRP/ABCG2. Accordingly, the interactions between albendazole and P-gp substrates or inhibitors are unlikely to be clinically important (Merino *et al.*, 2002; Merino *et al.*, 2005). However, one cannot rule out that ABZ may be a substrate/inhibitor of other, yet uncharacterised transport systems.

1.9 Drug interactions between ARVs and comedicated agents

Whereas the combination therapy of antiretrovirals substantially improves the clinical prognosis for patients infected with HIV, it concurrently increases the risk for drug-drug interactions (de Maat *et al.*, 2003; Piscitelli *et al.*, 2001). Drug interactions with antiretroviral agents are also of extreme importance as they are routinely co-administered with other agents used to treat HIV associated infections, malignancies and other common co-existing conditions (Yeni *et al.*, 2002; Young, 2005). Most of the drugs used in treatment of HIV infection can alter drug metabolising enzymes and thereby induce the metabolism of concomitantly administered drugs. This may result in a reduction in efficacy, or augmentation toxicity. Drug interactions between antiretroviral and other drugs may occur during metabolism or via modulation of drug transporters (Hsu *et al.*, 1998a; Pontali, 2007).

1.9.1 Interactions involving cytochrome P450 enzymes

Cytochrome P450 system (CYP450) is the most versatile enzyme system responsible for the metabolism of a wide variety of compounds including ARVs and several other drugs that they are likely to be co-administered with including antibiotics, cardiovascular drugs, azoles and cholesterol-lowering drugs (Anzenbacher *et al.*, 2001; Guengerich *et al.*, 1991). Drug interactions, can be very complex when the combination therapy involves drugs that interact differently with the enzyme system, as inducers, inhibitors or simply substrates (competitition). As discussed earlier in section 1.6, PIs and NNRTIs are lipophilic drugs that undergo biotransformation by the CYP450 group of enzymes, thus making them prone to clinically significant drug interactions when combined with other drugs. In addition, they interact with other medications acting as either inducers or inhibitors of drug metabolising enzymes (Erickson *et al.*, 1999; Flexner, 1998; Smith *et al.*, 2001). Indeed, PIs (especially RTV) are amongst the most potent inhibitors of CYP450 enzymes, which are also responsible for the metabolism of structurally related drugs (Kumar *et al.*, 1996). Of all the CYP450s involved in drug metabolism, CYP3A4 is the most prominent (Anzenbacher *et al.*, 2001). The enzyme is present in the gut wall (enterocytes) and the liver (hepatocytes), metabolizing an array of therapeutic drugs, almost 50% of all drugs in clinical use. The other CYP isoforms demonstrated to be important for drug metabolism include CYP2D6, 2C9, 2C19, 1A2, 2B6, 2E1 and 2A6 (Paine *et al.*, 2006; Shen *et al.*, 1997). Although hepatic biotransformation is the main contributor to systemic drug elimination, the combination with intestinal drug metabolism may cause presystemic or first-pass drug loss (Kolars *et al.*, 1992).

The interactions may either be beneficial or unfavourable. An example of a beneficial interaction is the co-administration of RTV with other PIs. Being a potent inhibitor of CYP3A4, it increases their bioavailability by decreasing its rate of breakdown (Kumar *et al.*, 1996). This enables administration of lower doses at reduced dosing frequency, thus decreasing the pill burden. On the contrary, rifampicin is a potent inducer of CYP3A4, and its co-administration with PIs will increase their metabolism, thus decreasing their bioavailability and efficacy, and potential for the emergence of resistant strains (Grub *et al.*, 2001; Polk *et al.*, 2001). Co-administration of these drugs would therefore require dose adjustment.

1.9.2 Drug transporters

A wide variety of transporters are found in the intestine, and are involved in the membrane transport of daily nutrients as well as drugs. These intestinal transporters are located in the brush border membrane as well as the basolateral membrane. Each transporter exhibits its own substrate specificity, and some have broader specificities than others (Inui, 1991; Kim, 2003; Murer *et al.*, 1989; Okano *et al.*, 1986). Most of the drug transporters belong to two super-families, adenosine triphosphate (ATP) - binding cassette and solute-linked carrier (SLC); the latter include both cellular uptake and influx transporters (Ayrton *et al.*, 2001). Several drugs have been characterised as either substrates, inhibitors or inducers of these transporters (Dresser *et al.*, 2001). There are two main classes of drug transporters, uptake and efflux transporters.

1.9.2.1 Uptake transporters

Uptake transporters act by facilitating the translocation of drugs into cells. They include:

i. Organic anion transporting polypeptide (OATP, SLCO) family,

ii. Organic anion transporter (OAT, SLC22A) family,

iii. Organic cation transporting polypeptide (OCT, SLC22A) family,

iv. Peptide transporter (PEPT, SLC15A) family (Ho *et al.*, 2005).

1.9.2.2 Efflux transporters

Efflux transporters function to export drugs from the intracellular milieu, often against concentration gradients. Most efflux transporters are members of the ATP - binding cassette superfamily of transmembrane proteins. They utilize energy derived from ATP hydrolysis in order to mediate substrate translocation across biological membranes (Ho *et al.*, 2005). They include:

i. P-glycoprotein (ABCB) family e.g. multidrug resistant protein 1 (MDR1) and bile salt export pump (BSEP),

ii. Multidrug resistance associated (MRP, ABCC) protein family (BCRP, ABCG).

Some of the transporters have been shown to exhibit both influx as well as efflux properties e.g. OATPs which are primarily uptake transporters that mediate transport via sodium-taurocholate co-transporting polypeptide. This mechanism appears to be due to anion exchange (Satlin *et al.*, 1997).

The localization of the transporters on the apical or basolateral membranes may impede or facilitate the dynamic interplay between the uptake and efflux transporters within any given epithelial cells. More importantly, the extent and direction of drug movement across organs such as liver, kidney and brain is dependent on the combined and often complimentary actions of transporters expressed within specific membrane domains of epithelial cells (Ho *et al.*, 2005).

1.9.3 P-glycoprotein

P-glycoprotein (P-gp), the encoded product of the ABCB1 gene was first extensively studied for its role in mediating multiple drug resistance phenotype associated with some cancers (Ueda *et al.*, 1987). However, the constitutive expression of this transporter in normal tissues has been shown to play an important role in drug disposition and response as it influences the passage of drugs through membranes (Marzolini *et al.*, 2004; Schinkel, 1997). P-gp is a phosphorylated and glycosilated transmembrane protein (170kDa) that is 1280 amino acids long. It is composed of 2 homologous and symmetric sequences, each of which contains 6 transmembrane domains and an ATP binding motif (Endicott *et al.*, 1989). P-gp is a transporter that functions as a transmembrane efflux pump, moving drugs from the intracellular to the extracellular domain (van Helvoort *et al.*, 1996). ATP hydrolysis provides the energy for active drug transport, enabling the transporter to function against steep concentration gradients (Higgins *et al.*, 1992) [Fig1.6]. The "vacuum cleaner" metaphor has often been used to illustrate its mechanism of action (Marzolini *et al.*, 2004).

P-gp is expressed in many normal tissues, suggestive of its physiologic action. It is present in the canalicular surface of hepatocytes, apical surface of proximal tubular cells in kidneys, brush border surface of enterocytes as well as the luminar surface of blood capillaries of the brain [blood-brain barrier] (Cordon-Cardo *et al.*, 1989; Thiebaut *et al.*, 1987). In addition, P-gp is also present in other tissues known to have blood-tissue barriers such as placenta, ovaries and testes as well as in the hematopoietic stem cells, peripheral blood mononuclear cells (PBMCs), macrophages, antigen-presenting dentritic cells, and T and B lymphocytes (Klimecki *et al.*, 1994).

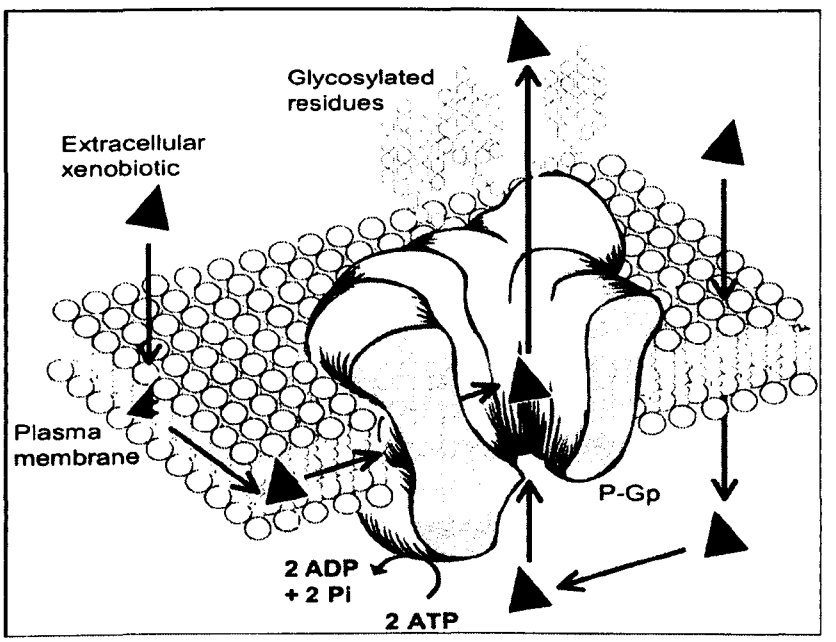


Fig.1.6. Model of P-glycoprotein (P-gp) function. The model shows that P-gp mediated efflux transport of drug substrates can occur at the level of the plasma membrane or from the intracellular compartment Adapted from Marzolini *et al.* (Marzolini *et al.*, 2004).

ATP-Adenosine triphosphate

ADP-Adenosine diphosphate

Pi-inorganic phosphate

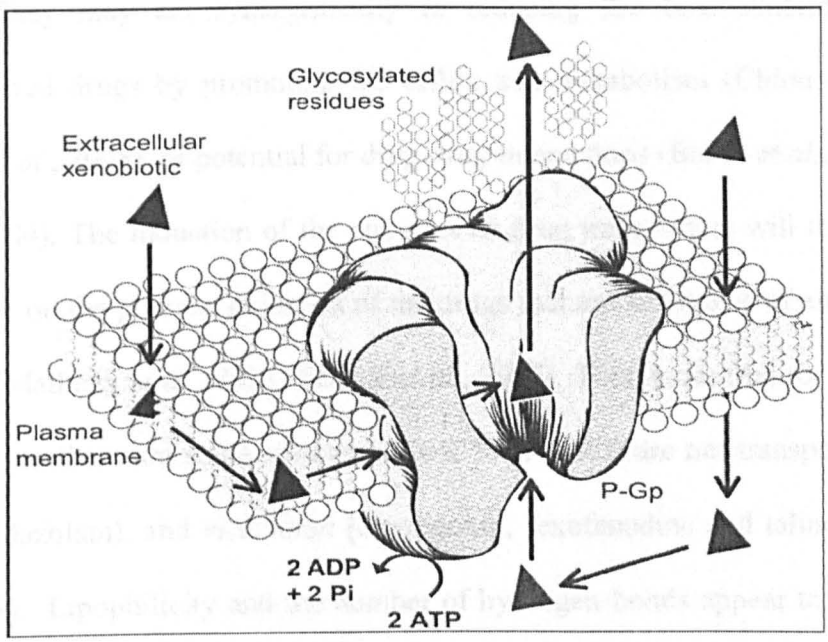


Fig.1.6. Model of P-glycoprotein (P-gp) function. The model shows that P-gp mediated efflux transport of drug substrates can occur at the level of the plasma membrane or from the intracellular compartment Adapted from Marzolini *et al.* (Marzolini *et al.*, 2004).

ATP-Adenosine triphosphate

ADP-Adenosine diphosphate

Pi-inorganic phosphate

1.9.4 Substrates for P-gp

A wide range of drugs are substrates fot P-gp including those that are substrates of drug metabolic enzymes, particularly CYP3A4. There is co-localization of the transporters and the enzymes as well as substrate overlap (Benet *et al.*, 2004; Kim *et al.*, 1999; Kolars *et al.*, 1992; Wacher *et al.*, 1995; Watkins *et al.*, 1987; Zhang *et al.*, 1998). They may act synergistically in reducing the bioavailability of orally administered drugs by promoting the efflux and metabolism (Chiou *et al.*, 2000; Perloff *et al.*, 2005), or potential for drug-drug interactions (Baron *et al.*, 2001; Benet *et al.*, 2004). The induction of the enzymes or drug transporters will therefore have an impact on the pharmacokinetics of the drugs that are substrates of either proteins, or both (Matheny *et al.*, 2001; Perloff *et al.*, 2005). This is not true for all the drugs however, as there are some substrates for CYP3A4 that are not transported by P-gp (e.g. mildazolam), and *vice versa* [e.g digoxin, fexofenadine and talinolol] (Kim *et al.*, 1999). Lipophilicity and the number of hydrogen bonds appear to be important parameters in structural activity relationship for P-gp substrates as both have been proportionally correlated to the affinity of compounds for P-gp (Ecker *et al.*, 1999).

1.9.5 Interactions involving drug transporters

As discussed earlier (section 1.9.4), the interplay between the drug transporters and CYP450 enzymes may have tremendous impact on the disposition of drugs, which may increase the possibility of drug interactions (Baron *et al.*, 2001; Benet *et al.*, 2004). Most drugs are substrates of drug transporters and metabolic enzymes. As a result there are chances of interaction during absorption or metabolism since these are expressed in the gastrointestinal tract (GIT) and the liver (Kim *et al.*, 1999; Okamura *et al.*, 1993; Patel *et al.*, 2001). These drug interactions may lead to drug failure and other adverse effects. Because of the overlap of CYP enzymes and

transporter expression the outcome may be a complex pharmacokinetic profile (Pal *et al.*, 2006). An example of such interactions is the PIs which are substrates for both P-gp and CYP3A4, and therefore can interact with each other and with other P-gp and CYP3A4 substrates such as rifampicin (la Porte *et al.*, 2004), and omeprazole (Fang *et al.*, 2008). Examples are RTV boosting by LPV (van Heeswijk *et al.*, 2001), or the boosting of SQV by RTV (Alsenz *et al.*, 1998).

1.9.6 Potential for pharmacokinetic drug interactions between antiretroviral and anthelminthic drugs

As discussed earlier in section 1.2, concomitant administration of both antiretroviral and anthelminthic drugs is not uncommon, and therefore there is potential for their interactions. These interactions could be at the level of drug transporters or metabolic enzymes. Most ARVs have been characterised with regards to their relationship to CYP enzymes and drug transporters. Conversely not many studies have been conducted on the anthelminthic drugs. Table 1.7 and 1.8 below summarizes available data for common ARVs and anthelminthic drugs. Most ARVs are substrates of both drug transporters and CYP450 enzymes, especially PIs which are substrates and inhibitors for both. NRTIs and NNRTIs have been reported to be substrates of CYP enzymes and inhibitors of drug transporters (Weiss *et al.*, 2007b; Weiss *et al.*, 2008). IVM is a known substrate and inhibitor of drug transporters, while ABZ is a substrate of CYP enzymes. There is to date no available data about the specificity of PZQ to modulate drug transporters and metabolic enzymes. Any potential pharmacokinetic interactions upon co-administration of ARVs and anthelminthics are therefore most likely to involve PIs or NNRTIs and IVM. Interactions involving NRTIs and ABZ may be pharmacodynamic e.g. by additive or synergistic effects.

IVM interacts with P-gp modulators (Alvinerie *et al.*, 2008; Ballent *et al.*, 2006) and recently the inhibition of P-gp has been described as a strategy to counter the emerging resistance to ivermectin (Lespine *et al.*, 2008). In a study, IVM resistant strains of nematodes that was associated with increased expression of nematode multidrug resistant proteins (MRP) and P-gp was reversed by co-administration of MRP, and P-gp inhibitors (James *et al.*, 2008). It is therefore more likely, that any interactions involving IVM occur via drug transporters other than CYP enzymes. Interactions relating to ABZ and its metabolites with ARVs if any are likely to be through metabolism by the CYP450 enzymes system.

Table 1.7 Anthelminthic and antiretroviral substrates/inhibitors for drug transporters

	Substrate	**Inhibitor**	**Reference**
Antiretroviral drugs			
NRTIs	Unknown	Yes	(Storch *et al.*, 2007; Weiss *et al.*, 2007a; Weiss *et al.*, 2007b)
NNRTIs	Unknown	Yes	(Storch *et al.*, 2007; Weiss *et al.*, 2007a; Weiss *et al.*, 2007b)
PIs	Yes	Yes	(Kim *et al.*, 1998a; Lee *et al.*, 1998; Profit *et al.*, 1999; Srinivas *et al.*, 1998; Storch *et al.*, 2007)
Antihelmintic drugs			
Albendazole	No	No	(Merino *et al.*, 2002; Merino *et al.*, 2005)
Ivermectin	Yes	Yes	(Alvinerie *et al.*, 2008; Ballent *et al.*, 2006; Lespine *et al.*, 2008; Lespine *et al.*, 2006; Pouliot *et al.*, 1997)
Praziquantel	Unknown	Unknown	

Table 1.7 Anthelminthic and antiretroviral substrates/inhibitors for CYP450 enzymes

	Substrate	Inhibitor	Reference
Antiretroviral drugs			
NRTIs	Yes	Unknown	(Back *et al.*, 2003)
NNRTIs	Yes	Yes	(Smith *et al.*, 2001; von Moltke *et al.*, 2001)
PIs	Yes	Yes	(Eagling *et al.*, 1997; Ernest *et al.*, 2005; Hsu *et al.*, 1998a; Ikezoe *et al.*, 2004)
Antihelmintic drugs			
Albendazole	Yes	Unknown	(Merino *et al.*, 2002; Rawden *et al.*, 2000)
Ivermectin	Unknown	Unknown	
Praziquantel	Unknown	Unknown	

1.9.7 Induction of P-gp and metabolic enzymes

Drugs may induce transport proteins and/or CYP enzymes, and need not necessarily be their substrates in order to induce them. The induction of P-gp would alter the absorption and distribution of co-administered drugs that are substrates of these proteins (Collett *et al.*, 2004b; de Maat *et al.*, 2003; Perloff *et al.*, 2005). Caco-2 cell monolayers (CCM) express P-gp and CYP enzymes (Anderle *et al.*, 1998; Peters *et al.*, 1989). CCM may therefore be used to evaluate the induction of the transporters and the enzymes (Collett *et al.*, 2004b; Huang *et al.*, 2001; Vermeir *et al.*, 2005). Presently there is scant information from the literature on the abilities of PZQ and IVM to induce transporters and CYP enzymes. IVM has been reported to induce CYP enzymes in mouflon sheep including CYP1A, 2B and 3A sub-families (Skalova *et al.*, 2001).

1.10 Nanodispersion and drug disposition

Nanodispersion is a technology that has been used to improve the efficacy of hydrophobic drugs by either improving solubility by formation of nanoparticles [nanodispersion] (Tadros *et al.*, 2004). The technology involves the manipulation of the drug into sub-micron particles suspended in a liquid, in most cases water. This is achieved through various techniques including milling, high pressure homogenisation and controlled precipitation (Horn *et al.*, 2001; Kesisoglou *et al.*, 2007). A recent non-attrition method of drug-nanodispersions has been developed by a collaboration between the University of Liverpool and IOTA Nanosolutions Limited (IOTA) that involves the manipulation of emulsions to form template porous structures. Hydrophobic drugs are dissolved into volatile water-immiscible organic solvent, followed by dissolution of water-soluble materials into water and mixing the solutions to form an oil-in-water emulsion. The volatile oil/aqueous phase is then removed by freeze-drying to produce a dry highly porous material comprising the drug dispersed throughout the water-soluble material (Zhang *et al.*, 2008a).

This technology has been employed to improve the bioavailability and efficacy of poorly- water soluble drugs in the development of drug delivery systems (Merisko-Liversidge *et al.*, 2003). It can therefore be used as a drug delivery system for most ARV drugs which have poor solubility e.g. 4% for SQV, whose oral bioavailability has been shown to be improved in oil- in water nanoemulsions (Shahiwala *et al.*, 2007; Vyas *et al.*, 2008). As discussed earlier (section 1.7), one of the major contributing factors to the ARV treatment failure relates to inadequate penetration within anatomical and cellular viral sanctuary sites (Acosta *et al.*, 2000; Hoggard *et al.*, 2003). Whereas nanodispersed forms of the ARV drugs are now available, there

is no available data to date on the influence of nanodispersion on the bioavailability of the drugs. This may be assessed by comparing their transport across the CCM to that of the transport of non-dispersed drugs (Hidalgo *et al.*, 1989; Hilgers *et al.*, 1990). An increase in permeability across the CCM correlates with increased *in vivo* absorption and therefore overall efficacy of the ARVs.

1.11 Aims of thesis

From our discussions, co-infection between HIV and helminthic infections is not uncommon (section 1.2). In addition there are several programmes for mass treatment of filariasis and leishmaniasis in Africa and other tropical areas that are ravaged by HIV/AIDS, mainly involving IVM and PZQ (Alleman *et al.*, 2006; Fenwick *et al.*, 2006; Molyneux *et al.*, 2003). Up to 1.9 billion doses of anthelminthics (ABZ, IVM and DEC) have been administered to at least 570 million people living with lymphatic filariasis over the last 8 years in 48 countries (Ottesen *et al.*, 2008). There is therefore probability of co-administration of IVM or PZQ and ARVs in these regions and potential for their interactions and resistance (Collett *et al.*, 2004b; Lespine *et al.*, 2008). Despite the widespread use of these anthelminthic drugs, limited studies have been undertaken to evaluate their potential for drug interaction.

The primary aim of this thesis was to investigate the potential interactions between the drugs used in both infections in order to determine whether interactions are likely *in vivo*. It involved the development of methods for simultaneous determination of PZQ and SQV subsequent characterisation of PZQ as a P-gp substrate and/or inhibitor. The impact of both IVM and PZQ on the transport of ARVs along the

Caco-2 cells and *vice versa* was then investigated to assess the potential for transporter mediated interactions. The prevalence for interactions between ARVs and co-administered drugs in Kenya was assessed by an audit of prescriptions from a large data base of ARV patients from a leading hospital in Kenya. The transport of nanodispersed SQV along the caco-2 cell monolayers was also assessed in order to establish whether nanodispersion improves the bioavailability of ARVs.

Chapter 2

Potential for drug-drug interactions between antiretroviral and co-administered drugs in Kenya

Chapter 2

Potential for drug-drug interactions between antiretroviral and co-administered drugs in Kenya

2.1 Introduction

2.2 Methods

2.2.1 Study design

2.2.1.1 Patients

2.2.1.2 Case definition

2.2.2 Statistical analysis

2.3 Results

2.4 Discussion

2.1 Introduction

The introduction of highly active antiretroviral drugs (HAART) has remarkably improved the treatment of HIV/AIDS (acquired immunodeficiency syndrome) with a decrease in morbidity and mortality (Berrey *et al.*, 2001; Vittinghoff *et al.*, 1999). Of the five main classes of antiretrovirals: nucleoside reverse transcriptase Inhibitor (NRTIs), non - nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), entry inhibitors and integrase inhibitors; only the first three are available for treatment of HIV in Africa. Currently, the management of HIV involves concomitant administration of at least three antiretrovirals, in combination with other agents for the management of opportunistic infections and accompanying disorders (Yeni *et al.*, 2002; Young, 2005). HIV-infected patients are frequently confronted with opportunistic infections and malignancies as a result of impaired immune response. Often, this is accompanied by co-morbidity such as neurologic manifestations of HIV-disease, hepatic and drug dependence and numerous other conditions. As a result the patients undergoing treatment of HIV infection are often on several drug combinations with a high potential for drug-drug interactions (DDIs) that may lead to toxicity or treatment failure (de Maat *et al.*, 2003; Pontali, 2007; Robertson *et al.*, 2007; Young, 2005).

DDIs may either be pharmacokinetic or pharmacodynamic or a combination of both. Pharmacokinetic drug interactions involve the alterations in absorption, transport, distribution, metabolism or excretion of a given drug, leading to an increase or reduced exposure and in turn reduced efficacy or toxicity. The pharmacodynamic drug interactions result in direct alteration in the response to a drug leading to potentiation of the effects in an additive (synergism) or antagonistic effects.

Currently all antiretroviral drugs except enfuvirtide are administered orally and would therefore require absorption through the gastrointestinal tract (GIT), and an alteration in the absorption directly affects the plasma concentrations. For example, delarvidine (DLV) and indinavir (IDV) require normal gastric pH for absorption and concomitant administration with antacids with DLV impairs its absorption by causing a reduction in 44% of its area under the plasma concentration curve [AUC] (Rescriptor, 2006). A change in the GIT motility can also influence the absorption of the ARV, an example being methadone which causes a 25% reduction in the AUC of stavudine (d4T) by decreasing the GIT motility (Rainey *et al.*, 2000).

The metabolism of most drugs occurs in the liver by phase I reactions (oxidation, reduction and hydrolysis) and phase II reactions (conjugation) to produce inactive water soluble metabolites that are easily excreted by the kidneys. Phase I reactions are mainly facilitated by cytochrome P450 (CYP) enzymes, such as CYP3A (Anzenbacher *et al.*, 2001; Kolars *et al.*, 1992; Shimada *et al.*, 1994; Watkins *et al.*, 1987). These enzymes may be induced, leading to decreased effect of a drug or decreased effect of a pro-drug; or inhibited thereby causing an opposite effect (Perloff *et al.*, 2005). In addition, CYP3A4 is located in the small intestine and the liver thereby its involvement in presystemic or first-pass metabolism (Dresser *et al.*, 2000). As discussed earlier in section 1.9.3, P-glycoprotein which is expressed in the epithelial cells of the GIT, liver, kidney, blood-brain-barrier and CD4+ lymphocytes acts as an efflux pump that exports xenobiotics outside the cells; and thus plays an important role in the pharmacokinetics and subsequent DDIs (Kim *et al.*, 1998b; Malingre *et al.*, 2001). NNRTIs and PIs are both substrates for CYP 450 enzymes (Kumar *et al.*, 1996; Smith *et al.*, 2001; Sommadossi, 1999), and PIs are in addition

substrates and/or inhibitors for drug transporters, and are therefore involved in these type of interactions (Huang *et al.*, 2001; Huisman *et al.*, 2000; Smith *et al.*, 2001). The co-localization of CYP enzymes with drug transporters and the overlap of substrate specificity of drugs may lead to synergistic action in the bioavailability of the drugs, metabolism and potential for DDIs (Baron *et al.*, 2001; Benet *et al.*, 2004; Perloff *et al.*, 2005).

Herbal remedies have also been associated with clinically important DDIs with ARVs via the induction of CYP enzymes and P-glycoprotein. In a study, garlic supplements administered twice daily for 20 days led to 51% decrease in the AUC of saquinavir [SQV] (Piscitelli *et al.*, 2002), while grapefruit juice reduced the AUC of IDV by 26% and increased the SQV AUC by 39-121% depending on the dose of the grapefruit juice (Fuhr, 1998; Kupferschmidt *et al.*, 1998). Herbal preparations therefore have potential for significant DDIs and since their extent has not been elucidated, it is therefore important to be always aware of these interactions especially in Africa where there is still widespread use of herbal medications. Like most drugs, PIs are partly protein-bound, mainly bound to α_1-acid glycoprotein (AAG) whereas NNRTIs are predominantly bound to albumin (Smith *et al.*, 2001; Sommadossi, 1999). Only the unbound drug is pharmacologically active and the levels of the AAG/albumin and displacement by a co-administered drug may alter ARV plasma concentration. Drug interactions may also occur due to the alterations in the renal elimination via competition for the active transport excretory system in the kidney tubules. The NRTIs are also predominantly excreted by the renal system and this may equally give rise to DDIs (Hoetelmans *et al.*, 1997). Probenecid, a known inhibitor of tubular secretion have been demonstrated to increase the AUC of

zidovudine (ZDV) by 80-115% upon co-administration (Hedaya *et al.*, 1990; Somogyi, 1996), while aminoglycosides decreased the renal clearance of zalcitabine (HIVID, 2000).

The combination of didanosine (ddI) with hydroxyurea provides an example of a synergistic pharmacodynamic interaction. The addition of hydroxyurea leads to a reduction in the levels of deoxyadenosine triphosphate (dATP), favouring the incorporation of dideoxyadenosine triphosphate (ddATP) into proviral DNA and therefore simultaneous inhibition of cellular protein and viral protein by ddI (since ddI is a precursor of ddATP) and suppression of HIV-1, though the increased efficacy is accompanied by a higher rate of toxicity (Havlir *et al.*, 2001; Lori *et al.*, 1997; Zala *et al.*, 2002). The combination of ZDV and gangciclovir increases the toxic effect of bone marrow suppression (Cimoch *et al.*, 1998; Retrovir, 2007). stavudine (d4T), ddI, and ddC are associated with peripheral neuropathy (HIVID, 2000; Videx, 2008; Zerit, 2007) and lamivudine (3TC) and ddI are associated with pancreatitis (Epivir, 2008; Videx, 2008). Patients who are on combinations of these drugs are therefore at risk of these toxicities, and would require close monitoring for the adverse effects.

Managing these interactions therefore is one of the major challenges in the optimisation of HIV therapy (de Maat *et al.*, 2003; Pontali, 2007; Robertson *et al.*, 2007; Young, 2005). Whereas studies in developed countries have shown that DDIs involving antiretroviral therapy are frequent and under-recognized (Alvarez-Requejo *et al.*, 1998; Williams *et al.*, 1999), no similar work has been carried out in developing countries, where monitoring is less intense and use of fixed dose

combinations (FDCs) limits the scope for dose modification. The primary aim of the study described in this chapter is to assess the potential pharmacokinetic for DDIs between ARVs (antiretroviral drugs) and co-administered drugs in HIV+ adults enrolled into an ARV programme in Eldoret, Kenya. The secondary aims are to characterise the severity of identified DDIs, identify particular interactions that are common and to characterise the potential effect of DDIs on ARV concentrations.

Academic Model Providing Access to Healthcare (AMPATH) is a partnership between Indiana University School of Medicine and Moi University School of Medicine (Kenya) and is Kenya's most comprehensive initiative to combat HIV. It is based at the Moi Teaching and Referral Hospital (MTRH), Eldoret-Kenya. AMPATH is a working model of urban and rural HIV preventive and treatment services in the public sector. AMPATH cares for more than 55,000 HIV infected adults and children, with nearly one-half of all patients on anti-retroviral drugs, and enrolment into the programme rising by 2,000 patients per month (medicine.iupui.edu/kenya/index.html). In the study, we investigated the frequency of DDIs in follow up prescriptions for 1040 consecutive patients enrolled into the AMPATH programme.

2.2 Methods

2.2.1 Study design

2.2.1.1 Patients

The study was approved by the institutional research and ethics committee (IREC) of MTRH. Inclusion criteria were patients on ARVs, those currently under active follow up at MTRH/AMPATH project and of adult age. Children were excluded. Patients who had ARV treatment interrupted or discontinued during the course of follow up were included for the duration of time they were on ARVs and thereafter censored from analysis. Retrospective study was carried out using the follow up prescriptions for 996 consecutive HIV positive patients on ARVs from the database of MTRH which included the patients seen over a 22 month period between 4/01/2006 and 19/11/2007. Details of age, gender and body weight, baseline CD4 counts, World Health Organisation (WHO) staging at the onset of ARV therapy, together with all ARVs and concomitant medications were recorded. Under Kenyan National AIDS and STI (sexually transmitted infection) Control Programme guidelines (NASCOP), first line drugs were defined as stavudine (d4T) or zidovudine (ZDV) plus lavimudine (3TC) plus nevirapine (NVP) or efavirenz (EFV), and substitution with tenofovir (TDF) or abacavir (ABC) was allowed for toxicity. Second line include any of these agents in combination with a protease inhibitor: indinavir (IDV), lopinavir/ritonavir (LPVr), or nelfinavir (NFV) (NASCOP, 2005). The guidelines for entry into the ARV programme were as follows:

i) WHO stage 1 or 2 HIV disease if CD4 count is less than 200 cells/mm^3

ii) WHO stage 3 disease if CD4<350

iii) WHO stage 4 disease, irrespective of the CD4 cell count (NASCOP, 2005).

The records of the prescribed drugs for each patient in every visit over the entire period were then listed and an audit was then carried out to identify the co-prescribed drug pairs that would potentially lead to DDIs using the Liverpool HIV Pharmacology Group (LHPG) Website (www.hiv-druginteractions.org), accessed between January to August 2008, as well as other sources that include electronic Medicines Compendium (*e*MC), clinical options options (CCO) and PubMed. Details of the patient dermographics and the interactions are summarised in Table 2.8 [supplementary tables 1a-6a]).

2.2.1.2 Case definition

There is no universally accepted classification for severity of interactions. Many publications have utilized a classification system by Tatro (Tatro(ed), 2008) or equivalent, which grades severity according to illness or laboratory abnormalities caused by that interaction , and the potential consequences of that toxicity [e.g. hospitalisation] (Table 2.1). We took the view that such a classification was not appropriate to our study setting, since:

i) potentially serious adverse effects may be sub clinical, delayed or indirect with HIV therapy (such as low plasma concentrations leading to rebound, resistance and loss of future therapeutic options in a setting where access to second or third line regiment is limited),

ii) the threshold for admitting patients into hospital is considerably different in Africa compared with developed countries, and

iii) clinical and laboratory monitoring is limited in resource poor settings.

We therefore utilised a modified version of Tatro's (Tatro(ed), 2008) classification and classified the significant rating of the interactions as major moderate and minor as outlined in Table 2.2. Interactions relating solely to overlapping toxicities, between ARVs, topical applications and PI boosting with ritonavir were excluded.

2.2.2 Statistical analysis

Patients were grouped according to whether they had a major or moderate interaction; versus minor or no interactions. Differences between these two groups were assessed as follows: Age, baseline (BL) CD4 and weight were assessed by Mann-Whitney U test, and antiretroviral use, differences in gender and WHO stages assessed by Chi square test with Yates correction and a p value of < 0.05 was considered to be statistically significant. Analysis was performed using Stats Direct version 2.6.2 (Stats Direct Ltd, Cheshire, UK). The analysis of the correlation between CD4 count and major/moderate interactions was done by the use of Prism™. CD4 counts were grouped into various deciles containing a range of the counts, and the odds ratio for the DDIs assessed by the Chi square test (2 by 2 with the decile highest strata as reference).

Table 2.1 Tatro's Classification of drug interactions

Category	Definition
Major effects	Drug-drug interactions involving drugs that should not be co administered unless the benefits outweigh the risks
Moderate effects	Interactions which require regular monitoring as the effects may be exacerbated especially on prolonged use and dose adjustment may be required
Minor effects	Mild effects that may not significantly alter the therapeutic outcome

Table 2.2 Classification of drug interactions

Category	Definition
Major effects	Drug-drug interactions involving drugs that fall into the following category: Either, i. potentially life-threatening, or capable of causing permanent damage; or ii. associated with 'significant' clinical toxicity (i.e. hospitalisation, extended hospital admission, additional treatment); or iii. 'contraindicated', 'avoid' or 'not recommended' in the summary of the product characteristics (SPC); accessed from the electronic medicine compendium, emc.medicines.org.uk between January to August 2008); or iv. requiring dose modification of at least one of the drugs required in all/majority of the patients.
Moderate effects	Drug-drug interactions involving drugs that are not a major interaction but fall into any of the following categories: Either, i. causes deterioration in clinical status, or dose modification of at least one of the drugs may be considered for some patients, or ii. having recommendation 'caution, or requires close monitoring for toxicity of therapeutic failure in SPC.'
Minor or insignificant effects	Drug-drug interactions involving drugs that are not a major or moderate but fall into any of the following categories: Either, i. having usually mild consequences which may be bothersome but should not significantly affect the therapeutic outcome without requiring additional treatment, or ii. theoretical interaction where large scale clinical studies have demonstrated little or no clinical relevance.

2.3 Results

We screened 1040 patients out of which 996 were eligible. 4 patients had ARVs discontinued or interrupted during acute opportunistic infections and were excluded from further analysis. A further 40 patients were excluded as they were of paediatric age. The study population consisted of 346 (35%) male and 650 (65%) female patients aged between 21 and 68 years, their mean body weight was 58.1 kg and they were followed up for 1-22 months, median 15 months with a total of 15,060 patient-months of follow up. Baseline CD4 counts were available for 965 patients with a median count of 135 (0-1137) cells/mm^3 whereas the records for WHO staging of 986 patients were registered (Table 2.3). There were of 67 drugs recorded from all the prescriptions, 15 ARVs and 52 co-prescribed drugs (Supplementary tables 2a and 3a). A total of 880 patients (88.4%) were on first-line ARV (d4T, 3TC, NVP), and 116 patients (11.6%) were on second line treatment (Table 2.4). Of the 996 patients, the use of individual drugs was as follows: d4T 709 (71.2%), 3TC 938 (94.2%), ZDV 248 (24.9%), NVP 639 (64.2%), EFV 241 (24.2%), TDF 7 (0.7%), LPV/r 70 (7.0%), NFV 43 (4.3%), ddi 42 (4.2%) and ABC 48 (4.8%). All ARVs were prescribed at standard doses, regardless of whether a drug interaction was present or not.

Interactions were identified in 362 patients (36.3%) with major interactions being observed in 147 patients (14.8%), predominantly involving rifampicin (124 patients; 12.4%) and azoles (27 patients; 2.7%) [Tables 2.5 & 2.6]. Moderate interactions were identified in 230 patients (23.1%), involving azoles in 129 patients (13%), steroids in 106 patients (10.6%) and antimalarials in 29 patients (2.9%) whereas minor interactions were recorded for 60 patients (6%). A total of 334 patients

(33.5%) had either a major or moderate interaction, while minor or no drug interactions were recorded in 662 patients (66.5%). Of the patients with major/moderate interactions, 251 patients had one interaction recorded while 83 patients had more than one, with 70 patients having two interactions, 11 patients had three interactions and two patients having four interactions. The following observed major/moderate interactions were likely to result in a decrease in concentrations of the co-prescribed ARV drug: lopinavir/ritonavir (LPV/r) and rifampicin, nevirapine and rifampicin, nelfinavir and omeprazole, nelfinavir and lansoprazole, zidovudine and rifampicin, and efavirenz and rifampicin; whereas the following led to potentially increased concentrations of ARVs: nevirapine and fluconazole, zidovudine and fluconazole, and nevirapine and ketoconazole. Of the 432 major/moderate DDIs in 334 patients, 137 interactions in 120 patients (32 %) could have resulted in decreased ARV concentrations.

Within each drug class (NRTI, NNRTIs or PIs), there was significant difference in frequency of major/moderate associated with the PIs [p = 0.0008] (Table 2.4). The use of 1st line regimens (n = 880) were as follows: d4T/3TC/NVP in 504 patients (50.6%), d4T/3TC/EFV in 194 patients (19.5%), 3TC/ZDV/EFV in 44 patients (4.4%), ZDV/3TC/NVP in 128 patients (12.9%), ABC/3TC/NVP in 3 patients (0.3%), TDF/3TC/EFV in 3 patients (0.3%) and TDF/3TC/NVP in 4 patients (0.4%). Use of 2nd line treatments (n = 116) were as follows: 3TC/ZDV/NFV in 34 patients (3.4%), ABC/ddi/LPV/r in 28 patients (2.8%), ABC/ZDV/LPV/r in 16 patients (1.6%), d4T/3TC/LPV/r in 2 patients (0.2%), NFV/3TC/d4T in 9 patients (0.9%), ZDV/3TC/LPV/r in 12 patients (1.2%), ZDV/ ddi /LPV/r in 14 patients (1.4%) and ABC/3TC/LPV/r in 1 patient (0.1%). There was statistically significant difference in

frequency of major/moderate DDIs between 1st and 2nd line regimens ($p = 0.02$) and within the second line drug regimen ($p = 0.01$) [Table 2.4]. Risk of DDI was associated with weight ($p < 0.0001$), but was not associated with gender and age (Table 2.3).

The median BL CD4 count was 110 cells / mm^3 for patients with major / moderate interactions and 146 cells/mm^3 for patients with minor or no interactions, and there was statistically significant difference in the DDI between the two groups ($p < 0.0001$). A total of 363 patients were in WHO stage 1, 156 in stage 2, 385 in stage 3 while 82 were in stage 4 at the initiation of therapy. Out of these, 330 patients had major/moderate interactions with 86 patients (23.7%) on WHO stage 1, 47 patients on stage 2 (30.1%), 161 patients on stage 3 (41.8%) and 36 patients (43.9%) on stage 4 ($p < 0.0001$). The significant gradual increase in the proportion of major/moderate interactions to the WHO staging could be attributed to the use of rifampicin and azoles in the management of the opportunistic infections associated with the progression of the HIV disease (Table 2.3). In addition patients in higher WHO staging tend to have lower weight and BL CD4 counts. The odds ratio for the deciles of the strata of less than a CD4 count of less than 79 were three times higher in comparison with that of the highest strata (reference). This suggested that patients with CD4 counts of between 0-79 had a statistically threefold increase in the risk of major or moderate interactions (Fig 2.2 & Table 2.7).

Table 2.3 Patient demographics				
		Minor or no interactions (n=662)	**Major or moderate interactions(n=334)**	***p* values**
Age(years)		38(22-68)	39(21-67)	0.16 (CI =0-2, MD=1)
BL CD4(cells/mm^3)		146(1-1137)[n=641]	110(0-994)[n=324]	<0.0001(CI =16-46, MD=31)
Weight(kg)		58(20-99)	55(25-88)	<0.0001 (CI =2-4.5, MD=3)
Gender	Male	239	107	0.23‡ (CI=0.6-1.1)
	Female	423	227	
WHO staging	1	277	86(23.7%)	<0.0001‡ All stages
	2	109	47(30.1%)	
	3	224	161(41.8%)	
	4	46	36(43.9%)	
	n/a	10		

‡ Chi square test

CI Confidence interval

MD Median difference

Table 2.4 Interactions by antiretroviral drugs

Antiretroviral drug/s	Total no. of patients	Minor or no interactions	Major or moderate interactions	*p* values
NRTIs				
Abacavir (ABC)	48	34(70.8%)	14(29.2%)	0.54 all NRTIs ‡
Didanosine (ddi)	42	24(57.1%)	18(42.9%)	
Lavimudine (3TC)	938	625(66.6%)	313(33.4%)	
Stavudine (d4T)	709	470(66.3%)	239(33.7%)	
Zidovudine (ZDV)	248	168(67.8%)	80(32.3%)	
Tenofovir (TDF)	7	3(42.9%)	4(57.1%)	
NNRTIs				
Efavirenz (EFV)	241	153(63.5%)	88(36.5%)	0.59 EFV vs. NVP‡
Nevirapine (NVP)	639	420(65.7%)	219(34.3%)	
PIs				
Lopinavir(LPV/r)	73	32(68.5%)	23(31.5%)	0.0008 LPV vs. NFV ‡
Nelfinavir (NFV)	43	39(90.7%)	4(9.3%)	
1st Line regimen	880	573(65.1%)	307(34.9%)	0.02‡
2nd line regimen	116	89(76.8%)	27(23.3%)	
OI	4			
1st Line regimen				0.39‡
D4T/3TC/NVP	504	333(66.1%)	171(33.9%)	
D4T/3TC/EFV	194	126(64.9%)	68(35.1%)	
3TC/ZDV/EFV	44	27(61.4%)	17(38.6%)	
ZDV/3TC/NVP	128	82(64.1%)	46(35.9%)	
ABC/3TC/NVP	3	2(66.7%)	1(33.3%)	
TDF/3TC/EFV	3	0	3(100%)	
TDF/3TC/NVP	4	3(75%)	1(33.3%)	
2nd line regimen				0.01‡
3TC/ZDV/NFV	34	30(88.2%)	4(11.8%)	
ABC/ddi/LPV/r	28	18(64.3)	10(35.7%)	
ABC/ZDV/LPV/r	16	13(81.3%)	3(18.8%)	
ABC/3TC/LPV/r	1	1(100%)	0	
D4T/3TC/LPV/r	2	2(100%)	0	
NFV/3TC/D4T	9	9(100%)	0	
ZDV/3TC/LPV/r	12	10(83.3%)	2(16.7%)	
ZDV/ddi/LPV/r	14	6(42.9%)	8(57.1%)	

‡ Chi square test

OI Opportunistic infections (HAART stopped)

Table 2.5 Classification of interactions

Type of interaction	No of patients
Total number of patients on ARVs	996
Patients showing any form of interactions	362(36.3%)
Patients without any form of interactions	634(63.7%)
Patients with major or moderate interactions	334(33.5%)
Patients with minor or no interactions	662(66.5%)
Patients with major interactions	147(14.8%)
Patients with moderate interactions	230(23.1%)
Patients with minor interactions	60(6%)
Patients with both major and moderate interactions	43(4.3%)
Patients with both major and minor interactions	22(2.2%)
Patients with both moderate and minor interactions	18(1.8%)
Patients with major, moderate and minor interactions	8(0.8%)

Fig 2.1 Distribution of the various interactions

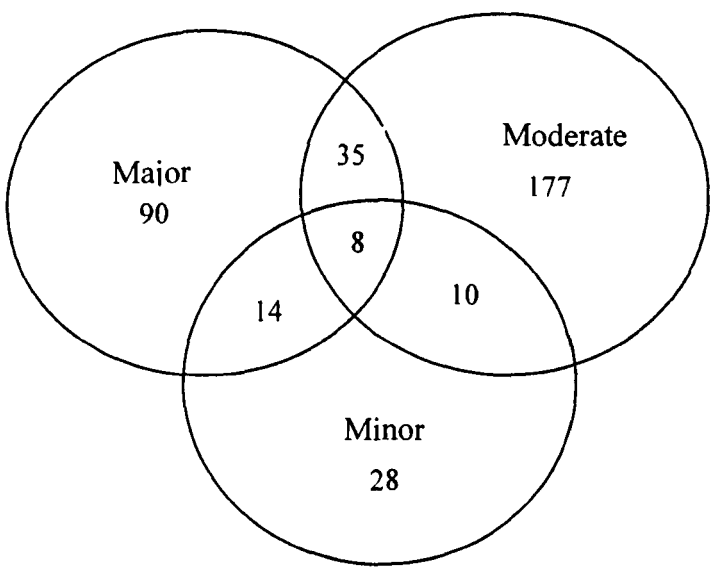


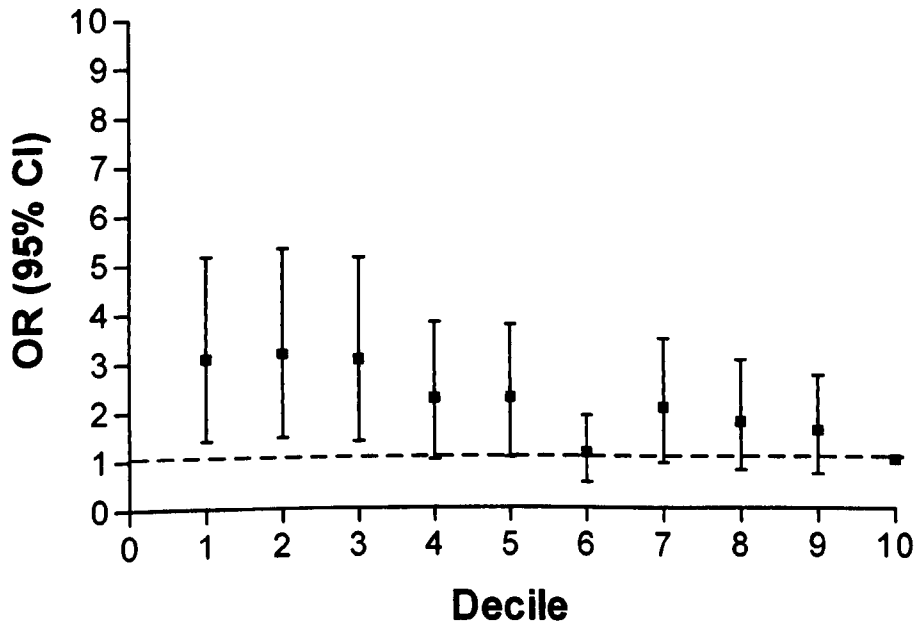
Table 2.6 Frequency and rate of interactions

	No of interactions
Coprescribed drug pairs	**No. %**
Major interactions (162 interactions in 147 patients)	
LPV/r + artemether/lumefantrine	1 (0.1)
LPV/r + fluoxetine	2 (0.2)
LPV/r + rifampicin	3 (0.3)
Efavirenz + rifampicin	76 (7.6)
Nelfinavir + lansoprazole	4 (0.4)
Nelfinavir + omeprazole	4 (0.4)
Nevirapine + ketoconazole	27 (2.7)
Nevirapine + rifampicin	45 (4.5)
Moderate interactions (270 interctions in 230 patients)	
Aluvia + ketoconazole	2 (0.2)
Efavirenz + ketoconazole	10 (1.0)
Efavirenz + artemether/lumefantrine	5 (0.5)
Nevirapine + fluconazole	97 (9.7)
Nevirapine + artemether/lumefantrine	24 (2.4)
Nevirapine + prednisone	106 (10.6)
Zidovudine + dapsone	1 (0.1)
Zidovudine + fluconazole	20 (2.0)
Zidovudine + rifampicin	5 (0.5)
Minor interactions (72 interctions in 61 patients)	
Abacavir + rifampicin	2 (0.2)
LPV/r + sulfadoxine/pyrimethamine	9 (0.9)
LPV/r + prednisone	6 (0.5)
Didanosine + sulfadoxine/pyrimethamine	6 (0.6)
Efavirenz + prednisone	49 (4.9)

Table 2.7 Correlation between CD4 counts and major/moderate interactions

Decile	Strata	No	DDI	Odds Ratio	95%Confidence Interval
1	0-20	94	40	2.69	1.40-5.16
2	21-49	97	42	2.77	1.45-5.3
3	50-79	90	38	2.65	1.37-5.12
4	80-100	89	31	1.94	0.99-3.79
5	101-134	111	39	1.97	1.04-3.73
6	135-164	110	30	0.99	0.52-1.89
7	165-199	100	33	1.79	0.92-3.45
8	200-259	88	26	1.52	0.77-3.02
9	260-399	98	27	1.38	0.70-2.71
10	400-1137	88	19	1.00	

Fig 2.2 Odds ratio for major/moderate DDIs by CD4 deciles



2.4 Discussion

The maintenance of maximally suppressive drug concentrations during complete dosage intervals is pivotal in antiretroviral therapy as well as prevention of emergence of resistance (Moyle *et al.*, 2001). Drug interactions are amongst the commonest cause of medication error in developed countries. Studies in the Netherlands and New York suggest prevalence of 20-25% patients on ARVs (de Maat *et al.*, 2004; Shah *et al.*, 2007). A related study conducted still in New York had a prevalence of 41.3% (Miller *et al.*, 2007), whereas two recent studies conducted in Liverpool and Switzerland (using the LHPG website) reported prevalence of 26.3% and 61% respectively (Cottle *et al.*, 2009; Marzolini *et al.*, 2009). There has been to date no such studies in Africa despite the resource-limited settings that include: i) less laboratory monitoring, ii) high background of illness – adverse effects may be missed, iii) lack of affordable alternative treatments, iv) use of Fixed dose Combinations (FDCs) that offer less flexibility for managing interactions, v) lack of data, vi) higher 'cost' of failure and vii) fewer safeguards in place. The purpose of the study was to investigate the potential for DDIs between ARVs and co-administered drugs in a large outpatient cohort in Kenya. In Africa, the coexisting epidemics of HIV, tuberculosis (TB) and malaria to a large degree make significant DDI's unavoidable. We observed that clinically significant DDIs were not uncommon in the patients studied, affecting one in three patients (34%). Of particular concern was that 32% of these interactions could have resulted in the lowering of the plasma concentrations of the antiretroviral drugs, thereby compromising their efficacy.

There are several limitations to our study including the following: i) first, we were not able to identify the actual adverse consequences of the interactions, but rather only the potential for DDIs. It is likely that the majority of the patients may not have suffered adverse consequences from the co-administration of the drugs, ii) the current WHO guidance and national policy in Kenya recommends the use of EFV (without weight-based dose modifications) when using rifampicin for TB co-infection. Thus, these drugs were prescribed in accordance with existing best practice even though they would have appeared as major interactions in our series, iii) adverse clinical events may have been missed. Lack of routine laboratory monitoring and the presence of overlapping syndromes such as fever may confound the correct identification of adverse events resulting from DDIs. We did not also actively seek information on the use of herbal or traditional medicines, and the use of oral/injectable contraceptives may have been incompletely recorded. In addition, interactions between co-administered ARVs, topical applications and PI boosting with ritonavir were excluded, and we did not ascertain whether the patients were receiving treatment for other ailments elsewhere other than the HIV clinic. Our findings are therefore likely to represent an underestimation of the true incidence of significant drug interactions in this cohort of patients.

Most of the major interactions involved interactions between ARVs and rifampicin, since most of the patients were being treated for TB. Despite the modest number of clinical trials assessing novel TB drugs, there is still no credible alternative to rifampicin-based therapy, and this remains the predominant cause of significant DDIs in Africa. Interactions involving efavirenz and rifampicin were recorded for 76 patients (7.6%), nevirapine and rifampicin in 45 patients (4.5%) and LPV/r and

rifampicin in 3 patients (0.3%). In one study, rifampicin decreased nevirapine concentrations upon co-administration [NVP AUC decreased by 58%] (Avihingsanon A, 2007; Ramachandran *et al.*, 2006) and the manufacturer's SPC recommends that it is contraindicated. In a related study rifampicin reduced efavirenz AUC by 26% (Sustiva, 2007). There is also evidence of interindividual variability when efavirenz is co-administered with rifampicin. Studies in Europe suggest that dosage should be increased from 600 mg to 800 mg once daily in patients over 60 Kg (Matteelli *et al.*, 2007) and the British HIV Association (BHIVA) treatment guidelines recommend dosage increment in patients over 50Kg (BHIVA, 2009). Rifampicin reduced lopinavir AUC by 75% upon co-administration (la Porte *et al.*, 2004) and the authors advise adjustment of dose regimens of lopinavir-ritonavir as well as therapeutic drug monitoring and monitoring of liver function.

The widespread use of azoles, (either as treatment for *Candida* infections, or prophylaxis against Cryptococcal disease) also accounted for a significant number of interactions, which increased proportionately with the advancement of HIV/AIDS. Interactions involving nevirapine and fluconazole were identified in 97 patients (9.7%), while nevirapine and ketoconazole were recorded for 27 patients (2.7%). Neverapine has been reported to decrease ketoconazole concentrations upon co-administration [ketoconazole AUC decreased by 72%] (Lamson M, 1998), and the SPC recommends that it is contraindicated. The co-administration of fluconazole and nevirapine resulted in approximately 29% increase in nevirapine exposure compared to when nevirapine was administered alone (Wakeham *et al.*, 2009).

Artemether-combination therapies (ACT'S) have replaced regimens as first line antimalarials, and have potential for clinically relevant pharmacokinetic interactions and toxicity with HIV PI's and NNRTI's. A significant interaction involved lopinavir/ritonavir and artemether/lumefantrine in one patient. The AUC of lumefantrine increased by 193% in a study when lopinavir/ritonavir was co-administered with artemether/lumefantrine, since lumefantrine is extensively metabolized by CYP3A4 and PIs inhibit CYP3A4 (German *et al.*, 2009). The manufacturer's SPC advises that it is contraindicated. Of the other interactions, proton pump inhibitors (PPIs) were important, mainly with nelfinavir; a PI no longer in widespread use in developed countries but may become an increasing problem if atazanavir is used in 2nd line regimens.

Patients with CD4 counts of less than 79 had a threefold increase in the risk of major or moderate interactions in comparison to patients with those with higher counts. There was also a proportionate increase in the major or moderate interactions with increase in WHO staging, and significant difference in weight between these patients and those with minor or no interactions. Patients with lower CD4 counts are likely to suffer from more opportunistic infections and therefore are likely to be on more drugs and higher probability of DDIs. They are also likely to suffer from weight loss with the progression of HIV/AIDS (Hare, 2006).

It should be noted that most of the information relating to the interactions is based on theoretical considerations and *in vitro* data, and that the extrapolation to *in vivo* situations would require consideration of several factors including interindividual differences in clearance and the role of metabolites (Bertz *et al.*, 1997). The

interpretation of case reports should be done with caution as they usually provide limited information and often there are outliers within a given population. In addition, pharmacokinetic studies are usually performed on healthy volunteers whereas the treatment of HIV infection is a lot more complex as multidrug regimens are utilized and the patient's body functions are often compromised. Indeed it has been demonstrated that HIV/AIDS patients with acute illness have altered drug metabolism patterns, and that CYP3A4 activity is more variable in HIV- positive patients than non-infected patients (Lee *et al.*, 1993; Slain *et al.*, 2000). There should also be special precaution in addressing the effect of a drug interaction when an enzyme-induction agent is discontinued as toxicity may occur as a result of continuation of the high dose of the drug initially required to offset the inducing effect. Drug interactions involving ARVs is therefore interplay of several factors and new information in this field is constantly emerging. However, despite the shortcomings, the awareness of the possible mechanisms of drug interactions encountered during the treatment of HIV-infected persons, clinical consequences and interventions to minimise the interactions are pivotal in the optimisation of treatment of HIV/AIDS.

Therapeutic drug monitoring (TDM) of PIs and NNRTIs has now been considered a clinical tool of value in the management of HIV in monitoring plasma concentrations as this relates to the efficacy and/or toxicity (Dieleman *et al.*, 1999; Marzolini *et al.*, 2001; Murphy *et al.*, 1999; Veldkamp *et al.*, 2001b; Yeni *et al.*, 2002). It may also be used in the management of drug interactions through regular monitoring of the most frequent toxicities. However, the use of TDM is not generally feasible as a strategy for managing DDI's in Africa owing to the element of cost. There are some

practical steps that can be instituted to reduce incidents of DDIs that include: i) instituting systems for pharmacovigilance, ii) improving on the quality of prescribing through regular training of medical and paramedical staff involved in HIV/AIDS management on the awareness of DDIs, iii) providing protocols for anti-TB and azole group of drugs and iv) establishing an integrated regional approach to monitoring of DDI's in the national ARV programmes.

In summary, one in three patients receiving ART in Kenya could potentially develop clinically significant DDIs, yet may not have been recognized. Although TB medications accounted for a significant proportion of DDIs, we identified other important interactions involving ARVs and azoles, as well as antimalarials. Given the relative lack of laboratory monitoring and widespread use of FDCs, strategies need to urgently be developed to avoid important DDIs, to identify early markers of toxicity and to manage unavoidable interactions safely in order to reduce risk of harm, and to maximize the effectiveness of mass ARV deployment in Africa.

Table 2.8 Outline of the supplementary tables

Table	Description
Table 1a	A sample of a page containing details of patient demographics and drug interactions. The details of interactions are outlined.
Table 2a	Drug interaction charts, used to identify interactions between ARVs and co-administered drugs.
Table 3a	List of abbreviations of the drugs used
Table 4a	Major interactions - Details of the major interactions including pharmacokinetic data.
Table 5a	Moderate interactions - Details of moderate interactions
Table 6a	Minor interactions - Details of moderate interactions

Patient demographics and drug interactions													
id	ref	sex	age	CD4 count	WHO stage	Date of visit	Weight	Current line	Follow up months	Potential for interactions	Major+ moderate	Minor + Insignificant	Details for interactions
319	08821mt-3	F	24	198	3	28/08/2007	59	1	2	yes	0	1	sept + D4T(C), sept + 3TC(C), metro + D4T(C)
320	08831MT-0	M	n/a	n/a	n/a	14/03/2007	86	1	2	no			
321	08835mt-3	F	38	7	1	18/09/2007	55	1	9	yes	1	0	RIHAZ-E + D4T(C), RIHAZ-E + EFV(C), sept + D4T(C), sept + 3TC(C), ethizide + D4T(C), pred + EFV(C), B6 + D4T(C)
322	08836mt-1	M	43	162	2	28/08/2007	66	1	7	yes	0	1	sept + D4T(C), sept + 3TC(C)
323	088415mt-4	M	n/a	n/a	n/a	12/11/2007	59	1	12	yes	0	1	sept + D4T(C), sept + 3TC(C)
324	08841MT-1	F	28	31	1	04/10/2007	67	1	7	yes	1	0	sept + D4T(C), sept + 3TC(C), dif + NVP(C)
325	08842mt-9	F	43	190	1	19/09/2007	82	1	9	yes	0	1	fans + 3TC(C), fans + D4T(C), INH + D4T(C), B6 + D4T(C)
326	08845MT-2	F	27	67	3	08/10/2007	53	1	11	yes	1	0	RIHAZ-E + D4T(C), RIHAZ-E + EFV(C), metro + D4T(C), ethizide + D4T(C), B6 + D4T(C)
327	08848mt-6	F	40	90	3	19/09/2007	78	1	9	yes	1	0	rif + EFV(C), sept + 3TC(C), sept + D4T(C), B6 + D4T(C)
328	08852MT-8	M	25	414	3	17/05/2007	65	2	6	yes	0	1	sept + ZDV(C), sept + D4T(C), sept + 3TC(C), ethizide + D4T(C)
329	08855MT-1	F	27	71	3	11/10/2007	72	1	11	yes	0	1	fans + 3TC(C), fans + D4T(C), metro + D4T(C)
330	08859mt-3	F	25	364	1	10/07/2007	62	1	8	yes	0	1	INH + D4T(C), metro + D4T(C)
331	08860mt-1	F	36	83	1	25/10/2007	55	1	11	yes	0	1	sept + D4T(C), sept + 3TC(C), INH + D4T(C)
332	08861MT-9	F	33	171	1	11/10/2007	60	1	11	yes	0	1	sept + D4T(C), sept + 3TC(C), fans + D4T(C), fans + 3TC(C), metro + D4T(C)
333	08875mt-9	F	27	25	3	18/10/2007	62	1	9	yes	2	0	sept + D4T(C), sept + 3TC(C), coartem + NVP(C), ethizide + D4T(C), metro + D4T(C), pred + NVP(C)
334	08884mt-1	F	36	29	3	14/08/2007	55.5	1	9	yes	1	0	sept + D4T(C), sept + 3TC(C), metro + D4T(C), pred + NVP(C)
335	08900MT-5	F	33	n/a	n/a	08/08/2007	58	1	10	yes	0	1	sept + D4T(C), sept + 3TC(C), INH + D4T(C)
336	08911MT-2	F	34	413	1	30/08/2007	53	0	6	no			

Table 1a. Sample of a page containing details of patient demographics and drug interactions

Table 2a Drug interaction charts

	ABC	APV	ATV	ddI	EFV	TVD	IDV	3TC	LPV	NVP	RTV	d4T	TDF	ZDV	NVF
ACID	None	Caution	Caution	None	None	None	Caution	None	None	None	n/a	None	None	None	None
ACYC	None	None	None	None	None	Caution	None	None	None	None	None	None	Caution	None	None
AMOX	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None
ART/LF	None	NEVER	NEVER	None	Caution	None	NEVER	None	Caution	Caution	NEVER	None	None	None	NEVER
AUG	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None
AZIT	None	None	None	None	None	None	None	None	None	None	Caution	None	None	None	None
B6	None	None	None	Caution	None	None	None	None	None	None	None	Caution	None	None	
BCO	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None
BECLO	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None
BRUF	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None
CEFA	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None
CECLOR	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None
CEF	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None
CEPHA	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None
CET	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None
CHLOR	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
CIPRO	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None
CLOT	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None
CLOX	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None
DAP	None	Caution	None	None	None	None	None	None	None	None	None	None	None	Caution	None
DOXY	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None
EMB	n/a	None	None	n/a	None	n/a	None	n/a	None	None	None	n/a	n/a	n/a	None
ENAL	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
ETHIZIDE	n/a	None	None	n/a	None	n/a	None	n/a	None	None	None	Caution	n/a	n/a	None
FESO4	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None
FOLATE	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None
FLC	None	None	None	None	None	None	None	None	None	Caution	None	None	None	Caution	None

FLX	None	Caution	Caution	None	None	None	Caution	None	Caution	None	Caution	None	None	None	None	
GRIS	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	
	ABC	**APV**	**ATV**	**ddI**	**EFV**	**TVD**	**IDV**	**3TC**	**LPV**	**NVP**	**RTV**	**d4T**	**TDF**	**ZDV**	**NVF**	
HCT	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	
HYDROCORT	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	
INH	None	None	None	None	None	None	None	None	None	None	None	Caution	None	None	None	
KETO	None	None	Caution	None	Caution	None	Caution	None	Caution	NEVER	Caution	None	None	None	None	
LANS	None	Caution	NEVER	None	None	None	None	None	None	None	Caution	None	None	None	NEVER	
LIFERON	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	
LOP	None	None	Caution	None	None	None	None	None	Caution	None	Caution	None	None	None	None	
MBZ	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	
METRO	None	None	Caution	Caution	None	None	None	None	Caution	None	Caution	Caution	None	None	None	
MVI	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	
NYSTATIN	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	
OPZ	None	Caution	NEVER	None	None	None	Caution	None	None	None	None	None	None	None	NEVER	
PARA	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	
PMZ	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	
PRED	None	Caution	Caution	None	Caution	None	Caution	None	Caution	Caution	Caution	None	None	None	Caution	
RANIT	None	Caution	Caution	None	None	None	None	None	None	None	None	None	None	None	None	
RIF	Caution	NEVER	NEVER	None	Caution	None	NEVER	None	NEVER	NEVER	Caution	None	None	Caution	NEVER	
RIHAZ-E	Caution	NEVER	NEVER	None	Caution	None	NEVER	None	NEVER	NEVER	Caution	Caution	None	Caution	NEVER	
SALB	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	
SMX/TMP	None	None	None	None	None	Caution	None	Caution	None	None	None	Caution	Caution	Caution	None	
SP	None	None	None	Caution	None	Caution	None	Caution	None	None	Caution	Caution	Caution	Caution	None	
STREP	None	None	None	None	None	Caution	None	None	None	None	None	None	Caution	None	None	

None - No interactions
Caution - Special precaution when coadministered together
Never - Coadministration is contraindicated

Table 2a Drug interaction charts

Table 3a Abbreviations

ABC = Abacavir	COMB = Combivir (3TC + ZDV)	MBZ = Mebendazole tabs(vermox)
ACID = Antacid tabs and susp	DAP = Dapsone	METRO = Metronidazole tabs(Flagyl)
ACYC = Aciclovir(Zovirax)	ddI = Didanosine(Videx)	MVI = Multivitamin tabs
ALUVIA = Lopinavir/Ritonavir(Kaletra)	DOXY = Doxycycline	NFV = Nelfinavir
AMOX = Amoxicillin	EFV = Efavirenz 600MG	NIZ = Nizoral (Ketoconazole)
APV = Amprenavir	EMB = Ethambutol	NVP = Nevirapine
ART/LF=Artemether/Lumefantrine(Coartem)	ENAL = Enalapril	NYSTATIN = Nystatin
ATV = Atazanavir	ETHIZIDE = Ethambutol/Isoniazid	OPZ = Omeprazole (Losec)
AUG = Amoxil/Clavulanate	FESO4 = $Feso_4$ tabs	PARA = Paracetamol 500 MG
AZIT = Azithromycin	FLC = dif=Fluconazole(Diflucan)	PMZ = Promethazine (Phenergan)
B6 = Pyridoxine(Vitamin B6)	FLX = Fluoxetine	PRED = Prednisolone
BCO = Vit. B Complex tabs.	FOLATE= Folic acid (Vit B9)	RANIT = Ranitidine (Zantac)
BECLO = Beclomethasone inaler	GRIS = Griseofulvin	RIF = Rifampicin
BRUF = Ibuprofen	HCT = Hydrochlorothiazide	RIHAZ-E = RiF, INH, Pyrizinamide, EMB
CEFA = Cefalcor	HYDROCORT = Hydrocortisone	RTV = Ritonavir
CECLOR = Ceclor	IDV = Indinavir	SP=fans=Sulfadoxine + Pyrimethamine(Fansidar)
CEF = Cefuroxime (Zinnat)	INH = Isoniazid	SALB = Salbutamol
Cepha = Cephalexin	INN = Metronidazole (Flagyl)	SMX/TMP = sept = Sulphamethoxazole/ trimethoprim(Septrin, Bactrim)
CET = Cetrizine 10MG	KETO = Ketoconazole (Nizoral)	STREP = Streptomycin
CHLOR = Chlorpheniramine (Piriton)	LANS = Lansoprazole	3TC = Lavimudine
CIPRO = Ciprofloxacin	LIFERON = Liferon (iron preparations)	TDF = Tenofovir
CLOT = Clotrimazole cream(Topical)	LOP = Loperamide(Imodium)	TVD = Tenofovir /Emtricitabine (TRUVADA)
CLOX = Cloxacillin	LPV = Lopinavir	ZDV = Zidovudine (Retrovir or AZT)
d4T = Stavudine (Zerit,d4T)		

Table 3a Abbreviations

Table 4a Major interactions

Interaction	Interacting drug		Mechanism of interaction and potential clinical consequence	Reference	Source
	ARV	Other			
Aluvia + ART/LF	RTV	LF	Lumefantrine is extensively metabolized by CYP3A4. Lumefantrine does not seem to prolong the QT interval and is much safer than halofantrine. Nevertheless, interactions with PIs and NNRTIs are likely and the manufacturer's SPC advises that coadministration with CYP3A4 inhibitors (e.g. PIs) is contraindicated. Given the increasing use of lumefantrine–artemether for malaria, caution is recommended when using PI/NNRTIs.	*German P, Parikh S, Lawrence J, et al. Drug interaction between antimalarial drugs and lopinavir/ritonavir. Program and abstracts of the 15th Conference on Retroviruses and Opportunistic Infections; February 3-6, 2008; Boston, Massachusetts. Abstract 132.*	**LHPG CCO**
	LPV	LF	Coadministration of lopinavir/ritonavir and artemether/lumefantrine significantly increased lumefantrine exposure (AUC increased by 193%, Cmax increased by 82% and Clast increased by 298%); data were not available for artemether. Pharmacokinetics of lopinavir were not significantly altered (no change in AUC, 4% increase in Cmax, 11% increase in Cmin) and the increase in ritonavir was not statistically significant (AUC increased by 61%, Cmax by 56% and Cmin by 29%). Lumefantrine does not seem to prolong the QT interval and is much safer than halofantrine. Nevertheless, the manufacturer's SPC advises that coadministration with CYP3A4 inhibitors (e.g. PIs) is contraindicated. Given the increasing use of lumefantrine–artemether for malaria, caution is recommended when using PI/NN		
Aluvia + Fluoxetine		FLX	Coadministration may increase fluoxetine concentrations. RTV AUC↑19% did not alter the Cmax. Cardiac and neurological events have been reported when coadministered. A decrease in fluoxetine dose may be needed. Careful monitoring of therapeutic and adverse effects is recommended..	*i)Norvir Summary of Product Characteristics, Abbott Laboratories Ltd, February 2007. ii)Ouellet D, Hsu A, et al. Antimicrob Agents Chemother, 1998, 42: 3107–12*	**LHPG**
Aluvia +	LPV	Rifampicin	Coadministration is contraindicated as lopinavir concentrations are significantly decreased by rifampicin (75% decrease in AUC, 99%	*Pharmacokinetics of adjusted-dose lopinavir-ritonavir combined with rifampin in healthy*	**LHPG**

Rifampicin			decrease in Cmin).	*volunteers. la Porte CJ, Colbers EP, Bertz R, et al. Antimicrob Agents Chemother, 2004, 48: 1553-1560*	
	RTV	Rifampicin	Coadministration of rifampicin and full dose ritonavir may lead to loss of virologic response. Based on parallel group comparison, coadministration of ritonavir (500 mg twice daily) with rifampicin (600 or 300 mg daily) decreased ritonavir AUC (35%), Cmax (25%) and Cmin (49%). Alternative antimycobacterial agents, such as rifabutin, should be considered	*Double trouble: A pharmacokinetic study of indinavir/ritonavir (800+100 mg bid) and rifampicin for patients co-infected with TB and HIV. de Gast M, Burger D, van Crevel R, et al. 2nd International Workshop on Clinical Pharmacology of HIV Therapy, Noordwijk, the Netherlands, 2001, abstract 1.10.*	**LHPG**
Aluvia + RIHAZ-E			Lopinavir (Kaletra) area under the curve decreased 75%, and Cmin decreased 99%.	*i)La Porte C, Colbers E, Bertz R, et al. Antimicrobial Agents and Chemotherapy; September 27-30, 2002; San Diego, California. Abstract A-1823 ii)Nijland, et al.. Program and abstracts of the 8th International workshop on Clinical Pharmacology of HIV Therapy; April 16-18, 2007; Budapest, Hungary. Abstract 51.*	**CCO**
EFV + Rifampicin			Coadministration of rifampicin (600 mg) with efavirenz (600 mg) decreased efavirenz Cmax (20%), AUC (26%), and Cmin (32%). The dose of efavirenz should be increased to 800 mg/day in most patients (this may not be always necessary in those with low body weights); no dose adjustment of rifampicin is recommended	*-i)Sustiva Summary of Product Characteristics, Bristol-Myers Squibb Pharmaceuticals Ltd, January 2007-ii)Ramachandran G, et al. 4th IAS Conference on HIV Pathogenesis, Treatment & Prevention, Sydney, July 2007, abstract WEPEB003.*	**LHPG**
NFV + Lansoprazole			Coadministration is not recommended due to the potentially clinically significant reduction (~40%) in nelfinavir concentrations observed when nelfinavir and omeprazole were coadministered. A similar effect could be expected with lansoprazole	*Viracept Prescribing Information, Agouron Pharmaceuticals Inc, January 2007*	**LHPG**
NFV + Omeprazole			Coadministration is not recommended due to the potentially clinically significant reduction (~40%) in nelfinavir concentrations observed when nelfinavir (1250 mg twice daily) and omeprazole (40 mg once daily) were coadministered to HIV- subjects (n=19).	*i)Viracept Summary of Product Characteristics, Roche Products Ltd, January 2007; ii)Fang A, Bamle B, Labadie R, et al. 46th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, September 2006, abstract A-384.*	**LHPG**

NFV + OPZ			Omeprazole decreased nelfinavir area under the curve by approximately 40% and 80%, respectively.	*Fang A, Damle B, Labadie R, et al. Omeprazole significantly decreases nelfinavir systemic exposure in healthy subjects. Program and abstracts of the 46th Interscience Conference on Antimicrobial Agents and Chemotherapy; September 27-30, 2006; San Francisco, California. Abstract A-384.*	**CCO**
NVP + Ketoconazole			Nevirapine and ketoconazole should not be administered concomitantly because decreased ketoconazole concentrations may reduce its efficacy. Coadministration of nevirapine (200 mg twice daily) with ketoconazole (400 mg once daily) decreased ketoconazole AUC (72%) and Cmax (44%) and Cmin was below the limit of detection for the assay. The effect on nevirapine pharmacokinetics was not significant (15-28% increase in exposure compared to historical data).	*Lamson M, Robinson P, Lamson M et al. 12th World AIDS Conference, 1998, abstract 12218.*	**LHPG**
NVP + Rifampicin			Coadministration is contraindicated due to decreased nevirapine concentrations. Coadministration of nevirapine (200 mg twice daily) with rifampicin (600 mg once daily) increased rifampicin AUC (11%) and had no effect on Cmax. Compared to historical controls, there were clinically significant decreases in nevirapine AUC (58%), Cmax (50%) and Cmin (68%). Consider using rifabutin instead or switching to a triple NRTI combination for a variable period of time, depending on the TB treatment regimen. Preliminary data suggest that adequate nevirapine concentrations may be attained in patients with low body weight. Dose escalation should not be used when starting nevirapine and a dose increase may be necessary.	*i)Avihingsanon A, et al. 14th Conference on Retroviruses and Opportunistic Infections, Los Angeles, February 2007, abstract 576; ii)Ramachandran G, Hemanthkumar AK, Rajasekaran S, et al. J Acquir Immune Defic Syndr, 2006, 42(1): 36-41;iii)Pujari et al. 13th Conference on Retroviruses and Opportunistic Infections, Denver, February, 2006, abstract 574.*	**LHPG**
NVP + RIHAZ-E			Nevirapine (Viramune) Cmin decreased 37%-68%. Nevirapine (Viramune) area under the curve decreased 37%-58%. Rifampin (Rifadin) area under the curve increased 11% (not significant).	*Centers for Disease Control and Prevention (CDC). Updated guidelines for the use of rifamycins for the treatment of tuberculosis among HIV-infected patients taking protease inhibitors or non nucleoside reverse transcriptase inhibitors. MMWR Morb Mortal Wkly Rep. 2004;53:37.*	**CCO**

Table 4a Major interactions

Table 5a Moderate interactions

Interaction	Interacting drug		Mechanism of interaction and potential clinical consequence	Reference	Source
	ARV	Other			
Aluvia + Ketoconazole	LPV		LPV Cmax ↓11%, AUC ↓13% and Cmin ↓ 25% Ketoconazole Cmax ↑ 13% and there was a 3-fold increase in AUC.	*i)Kaletra Prescribing Information, Abbott Laboratories, March 2007*	**LHPG**
	RTV		3.4-fold ↑ ketoconazole AUC and a 1.6-fold ↑ in Cmax. $t_{½}$ ↑ from 2.7 to 13.2 h. 18% ↑ RTV AUC and a 10% ↑ in Cmax in the presence of ketoconazole Due to an increased incidence of GIT and hepatic A/E, a dose reduction of ketoconazole should be considered. High doses of ketoconazole (>200 mg/day) are not recommended.	*ii)Bertz R, Wong C, Carothers L, et al. Clin Pharmacol Ther, 1998, 63:230 (abstract PIII-94).*	
Aluvia + KETO	RTV		RTV AUC ↑ 29% by Keto	*Khaliq Y, et. Al .Clinic Pharmacol Ther. 2000:68:637*	**Tatro**
EFV+keto-conazole			Interactions studies with efavirenz and ketoconazole have not been performed. However, efavirenz has the potential to decrease ketoconazole concentrations.	*Sustiva Summary of Product Characteristics, Bristol-Myers Squibb Pharmaceuticals Ltd, January 2007*	**LHPG**
EFV + ART/LF		Artemisin	Coadministration may increase plasma levels of artemisinins. Close monitoring for artemisinin toxicity required. Artemisinin and derivatives are rapidly metabolized via CYP3A4 to an active metabolite, dihydroartemisinin which has greater potency than the parent drugs. Inhibition of CYP3A4 would reduce dihydroartemisinin, but increase concentrations of the parent drug. The effects of PIs and NNRTIs are unclear.		**LHPG**
		Lumefantrine	Lumefantrine is extensively metabolized by CYP3A4. Lumefantrine does not seem to prolong the QT interval and is much safer than halofantrine. Nevertheless, interactions with PIs and NNRTIs are likely and the manufacturer's SPC advises that		**LHPG**

			coadministration with CYP3A4 inhibitors (e.g. PIs) is contraindicated. Given the increasing use of lumefantrine–artemether for malaria, caution is recommended when using PI/NNRTIs.		
NVP + ART/LF		ART	Coadministration may increase plasma levels of artemisinins. Close monitoring for artemisinin toxicity required. Artemisinin and derivatives are rapidly metabolized via CYP3A4 to an active metabolite, dihydroartemisinin which has greater potency than the parent drugs. Inhibition of CYP3A4 would reduce dihydroartemisinin, but increase concentrations of the parent drug. The effects of PIs and NNRTIs are unclear.		**LHPG**
		LF	Lumefantrine is extensively metabolized by CYP3A4. Lumefantrine does not seem to prolong the QT interval and is much safer than halofantrine. Nevertheless, interactions with PIs and NNRTIs are likely and the manufacturer's SPC advises that coadministration with CYP3A4 inhibitors (e.g. PIs) is contraindicated. Given the increasing use of lumefantrine–artemether for malaria, caution is recommended when using PI/NNRTIs.		
NVP + fluconazole			Co-administration of fluconazole and nevirapine resulted in approximately 100% increase in nevirapine exposure compared with historical data where nevirapine was administered alone. Because of the risk of increased exposure to nevirapine, caution should be exercised if the medicinal products are given concomitantly and patients should be monitored closely. There was no clinically relevant effect of nevirapine on fluconazole. The co-administration of fluconazole and nevirapine resulted in approximately 29% increase in nevirapine exposure compared to when nevirapine was administered alone	*i) Viramune Summary of Product Characteristics, Boehringer Ingelheim International GmbH, September 2008.* *ii)(Wakeham et al., 2009).Co-administration of fluconazole increases nevirapine concentrations in HIV-infected Ugandans, CROI, Montreal, 2009 Abstr no.700.*	**LHPG** **BHIVA**
NVP + prednisolone					**LHPG**
ZDV + dapsone			Concomitant treatment, especially acute therapy, with potentially nephrotoxic or myelosuppressive drugs (e.g. systemic pentamidine, dapsone, pyrimethamine, co-trimoxazole, amphotericin, flucytosine, ganciclovir, interferon, vincristine, vinblastine and doxorubicin) may also increase the risk of adverse reactions to	*i)Retrovir Summary of Product Characteristics, GlaxoSmithKline UK, July 2005. Ii)Zidovudine, trimethoprim and dapsone*	**LHPG**

			zidovudine. If concomitant therapy with any of these drugs is necessary then extra care should be taken in monitoring renal function and haematological parameters and, if required, the dosage of one or more agents should be reduced.	*pharmacokinetic interactions in patients with human immunodeficiency virus infection. Lee BL, et al. Antimicrob Agents Chemother, 1996, 40: 1231-1236.*	
ZDV + fluconazole			Fluconazole increases zidovudine AUC and Cmax by 74% and 84%, respectively. Routine dose modification of zidovudine is not warranted with coadministration, but monitor for potential zidovudine toxicity	*Sahai J, et al. J Infect Dis, 1994, 169: 1103-1107.*	**LHPG**
ZDV + fluconazole			Slight increase in zidovudine half-life. No change in fluconazole pharmacokinetics.	*Brockmeyer NH, Tillmann I, Mertins L, et al. Eur J Med Res. 1997; 2:377-383.*	**CCO**
ZDV + RIHAZ-E			i)Rifampicin significantly decreased zidovudine AUC (47%) and Cmax (43%). ---The concomitant use of rifampicin, ribavirin or stavudine with zidovudine should be avoided. Limited data suggests that co-administration of zidovudine with rifampicin decreases the AUC of zidovudine by 48% ± 34%. This may result in a partial loss or total loss of efficacy of zidovudine. ii)The effect of rifampicin (600 mg once daily) on the pharmacokinetics of zidovudine (200 mg three times daily) was studied in 8 HIV-infected subjects. Rifampicin induced zidovudine glucuronidation and amination pathways resulting in decreased plasma and urine exposures to zidovudine. Coadministration of rifampicin for 14 days significantly increased zidovudine oral clearance (89%) with corresponding decreases in Cmax (43%) and AUC (47%). After stopping rifampicin for 14 days, values returned to within 26% of baseline.	*i)Retrovir Summary of Product Characteristics, GlaxoSmithKline UK, July 2005* *ii)Pharmacokinetic interaction between rifampin and zidovudine. Burger DM, et al. Antimicrob Agents Chemother, 1993, 37: 1426-1431.*	**LHPG**

Table 5a Moderate interactions

Table 6a Minor interactions

Interaction	Interacting drug		Mechanism of interaction and potential clinical consequence	Reference	Source
	ARV	Other			
ABC + RIHAZ-E	ABC		rifampicin, via action on UDP-glucuronyltransferases slightly decrease the plasma concentrations of abacavir.	*Ziagen Summary of Product Characteristics, Glaxo Group Ltd, November 2005*	**LHPG**
Aluvia + SP		PYM	Inhibition/induction of CYP3A by RTV. Monitor blood counts for efficacy of PYM.	*Malaty LI et al.Drug Saf.1999 Feb;20(2):147-69.*	**LHPG de Maat**
Aluvia + prednisolone		PRED	PRED AUC ↑ 37% and 28% after 4 and 14 days of RTV, respectively. Careful monitoring of therapeutic and A/E is recommended when prednisolone is concomitantly administered with ritonavir.	*Norvir Summary of Product Characteristics, Abbott Laboratories Ltd, February 2007;Penzak SR, Formentini E, Alfaro RM, et al. J Acquir Immune Defic Syndr, 2005, 40(5):573-580.*	**LHPG**
DDI + SP		PYM			**LHPG**
EFV + prednisolone					**LHPG**
NFV + prednisolone			The effect of CHOP chemotherapy (adriamycin, cyclophosphamide, vincristine and prednisolone) on the pharmacokinetics of protease inhibitors was investigated in HIV+ subjects receiving nelfinavir (750 mg three times daily, n=3). Nelfinavir AUC and Cmax values were higher when given with CHOP, though this was not statistically significant.	*-Cruciani M, Gatti G, Vaccher E, et al. J Antimicrob Chemother, 2005, 55(4):546-549.*	**LHPG**

Chapter 3

Investigations of potential interactions between praziquantel and saquinavir in CEM and CEMVBL cells

Chapter 3

Investigations of potential interactions between praziquantel and saquinavir in CEM and CEM$_{VBL}$ cells

3.1 Introduction

3.2 Methods

3.2.1 Materials

3.2.2 Development of an HPLC method for simultaneous quantification of SQV and PZQ

3.2.2.1 Instrumentation and chromatographic conditions

3.2.3 Extraction Procedure and quantification

3.2.3.1 Preparation of stock QCs

3.2.3.2 Preparation of standards for HPLC assay and validation

3.2.3.3 Calibration curves

3.2.4 Method validation

3.2.4.1 Linearity, limit of quantification and limit of detection

3.2.4.2 Accuracy and precision

3.2.4.3 Recovery

3.2.4.4 Specificity and selectivity

3.2.4.5 Stability

3.2.5 Drug accumulation experiments

3.2.5.1 Substrate studies

3.2.5.2 Inhibition studies

3.2.5.3 Statistical analysis

3.3 Results

3.3.1 Chromatography and detection

3.1 Introduction

As previously discussed (1.7), anti-HIV treatment is complex and requires a cocktail of 3 to 4 antiretroviral (ARV) drugs for routine treatment in addition to other drugs used in the management of opportunistic infections (Ledergerber *et al.*, 1999; Temesgen *et al.*, 1997; Volberding *et al.*, 1998). The combination therapy increases the risk for drug-drug interactions which could impact on the pharmacokinetics of both the antiretroviral and the co-administered drugs (de Maat *et al.*, 2003; Piscitelli *et al.*, 2001; Storch *et al.*, 2007).

These interactions may result in an increase or decrease in the plasma concentrations of the drugs thereby increasing the risk of toxicity or development of resistance amongst other adverse effects (Young, 2005). It is therefore important to understand the potential interactions between antiretroviral and other drugs following their concomitant administration as this may require dosage adjustment in some instances (Perloff *et al.*, 2005; Pontali, 2007). Anthelminthics are one group of such drugs that may be co-administered with ARVs owing to the prevalence of AIDS in the tropics that are also afflicted by helminthic infections as discussed earlier in section 1.2.

Praziquantel (PZQ) is a common systemic anthelminthic drug used for the management of schistosomiasis, a chronic disease endemic in equatorial regions (Cioli *et al.*, 2003; WHO, 2007b). Protease inhibitors (PI) are a group of drugs widely used in the treatment of AIDS in Africa, and saquinavir (SQV) was the first PI to be approved for treatment of HIV (Baker, 1995; Collier *et al.*, 1996). As discussed earlier (section 1.2), it is not uncommon to find patients who are on both PIs and praziquantel owing to the geographic overlap of HIV/AIDS and

schistosomiasis (Kjetland *et al.*, 2006; WHO, 2004). The knowledge of any potential interactions between these drugs is therefore of importance in the optimisation of therapy of HIV. A possible mechanism for interactions between these drugs is on the modulation of the efflux drug transporter, P-gp. Several drugs are substrates of the efflux transporters and metabolising enzymes (especially CYP3A4) raising the possibility of interactions during absorption or metabolism as they are expressed in the GIT tract, enterocytes and liver (Alsenz *et al.*, 1998; Okamura *et al.*, 1993; Patel *et al.*, 2001). Most ARVs and anthelminthics are orally administered and therefore it may be possible for interactions between these two groups of drugs are likely to be at the levels of drug transporters and metabolism.

SQV and other PIs are known to be substrates of efflux transporters P-gp, ABCC1 and ABCC2 (Alsenz *et al.*, 1998; Gimenez *et al.*, 2004; Profit *et al.*, 1999) but PZQ has not been conclusively characterised to date. In previous studies the authors concluded that PZQ is an inhibitor of P-gp without being a substrate (Hayeshi *et al.*, 2006) whereas a different study indicated that PZQ did not show potential for interacting with cellular efflux pumps despite being a highly permeable substance (Gonzalez-Esquivel *et al.*, 2005).

P-gp functions to transport drugs from the intracellular to the extracellular domain, often against concentration gradients. The inhibition or potentiation of the transporter function will have an impact on the cellular accumulations of the drugs, and efficacy (Jones *et al.*, 2001b; Kim *et al.*, 1998b). The induction of P-gp has been reported to cause a reduction in plasma concentrations of PIs after multiple exposure (Huang *et al.*, 2001), and the interaction between saquinavir and cyclosporine has also been

attributed to P-gp (Brinkman *et al.*, 1998a). P-gp mediated interactions between drugs can be ascertained by investigating their intracellular accumulation in cell lines that express P-gp (Jones *et al.*, 2001b; Khoo *et al.*, 2002). Previous studies in our laboratory have investigated the intracellular accumulation of SQV in T-lymphoblastoid cells, CEM parental and CEMVBL cells which overexpress P-gp (Janneh *et al.*, 2005). Potential interactions between SQV and PZQ can therefore be studied by comparing their accumulation in these cell lines, using SQV as a positive control.

A number of assays for simultaneous quantification of various groups of anti-HIV drugs (especially protease inhibitors) have been reported in the literature (Bouley *et al.*, 2001; Holland *et al.*, 2006; Villani *et al.*, 2001; Yamada *et al.*, 2001). However, to date there is no reported assay for simultaneous quantification of ARVs and anthelminthics. The work described in this chapter had a double objective; to develop a suitable assay method for simultaneous quantification of both PZQ and SQV and to characterise PZQ with regards to substrate specificity of the transporter P-gp. This involved the validation of a high performance liquid chromatography (HPLC) based on assay for simultaneous determination of SQV and PZQ concentrations in cell culture media and cell pellets. The assay was then used to assess potential interactions between these drugs in CEM (parental) and CEMVBL cells [CEM cells were used as the control line whereas CEMVBL cells were derived from CEM cells treated with vinblastine](Beck *et al.*, 1979). In order to determine whether PZQ is a substrate of P-gp, the accumulation of PZQ in CEM and CEMVBL cells was compared to that of SQV. To ascertain whether it is an inhibitor, the

accumulation SQV in CEMVBL cells was compared to SQV in the presence of PZQ, and a known inhibitor, tariquidar (XR).

3.2 Methods

3.2.1 Materials

Saquinavir (SQV) [Formula weight, 670.86] was donated by Roche Pharmaceuticals (Welwyn Garden City, UK). Praziquantel (PZQ) [cat. no. P4668, Formula Weight, 312.41], clozapine (CLZ) [cat. no. C6305, Formula Weight, 326.82], Dulbecco's Modified Eagle's Medium, DMEM (cat. no. D6249, containing 4500 mg/L glucose, 4mM L-glutamine and 110 mg/L sodium pyruvate Hanks Balanced Salt Solution), HBSS (cat. no. H8264 [Modified, with sodium bicarbonate, without phenol red., liquid, sterile-filtered, cell culture tested]), Roswell Park Memorial Institute medium, RPMI (cat. no. R8758)], Foetal Bovine Medium, FBS (cat. F7524) and Trypsin-EDTA solution were purchased from Sigma Chemical Co. (Poole, UK). Acetonitrile (ACN) and methanol (MeOH) were purchased from VWR international (Leicestershire, UK) whereas diethyl ether was purchased from Fisher Scientific, (Leicestershire, UK). Tariquidar was kindly donated by Xenova Group plc (Berkshire, UK). All the other chemicals used were of analytical or HPLC grade. Deionised water used to prepare the solutions or mobile phase was purified in an Elga DV 25 pure lab option system (Elga, High Wycombe, Bucks, and UK). T-lymphoblastoid cell lines, CEM and CEM_{VBL} cells were gifts from Dr. R. Davey (University of Queensland, Australia), and the cells were counted using a Nucleo Counter (ChemoMetec, Denmark) cell counter.

3.2.2 Development of an HPLC method for simultaneous quantification of SQV and PZQ

3.2.2.1 Instrumentation and chromatographic conditions

The HPLC consisted of a Dionex (Dionex Softron GmbH, Germany) HPLC system with a P 680 pump, an ASI-100 automated sample injector and a UVD 1704 detector. A 250µl injector with a 20µl loop was used. Reversed-phase-liquid chromatography was carried out using a Hypurity^{TM} C_{18} analytical column, 5µm x 4.6mm (Thermo Electron Corporation, Runcorn, UK 22105-154630). A column guard (Thermo electron 60140-412) was used to protect the analytical column. The ultraviolet detector was set to monitor the 215 nm wavelength. The mobile phase for the analysis was composed of ammonium formate 20mM (pH = 4.2), ACN and MeOH (57:38:5 v/v) and was prepared fresh for each assay. The separation was facilitated via isocratic elution at 1.5 ml/min flow rate and the run time was eight minutes for each separation. 20µl of the samples was injected for each run by means of an automated injector. The peak area ratios for the calibration curves and quantification were obtained and analysed using Chromelon software (version 6.5).

3.2.3 Extraction Procedure and quantification

Various concentrations of the stock solutions of PZQ, SQV and CLZ were prepared by dissolving in methanol.

3.2.3.1 Preparation of the Stock quality controls (QCs)

Quality control samples (250 µl for each assay) were prepared by diluting separately the stock SQV/PZQ solutions (25.6µM in methanol) in DMEM media (in duplicate)

to give concentrations of 6.4 μM (low quality control [LQC]), 12.8 μM (medium quality control [MQC]) and 19.2μM (high quality control [HQC]).

3.2.3.2 Preparation of standards for HPLC assay and validation

Working internal standard clozapine (CLZ), 5μg/ml

Sufficient volume of the working internal standard was prepared fresh for each assay in a serial dilution of the stock CLZ solution in methanol (1mg/ml in methanol), first 1 in 10 to give 10μg/ml and then 1 in 2.

3.2.3.3 Calibration curves

Working stock standard solutions of PZQ and SQV were prepared by appropriate dilution in DMEM media. For the construction of the calibration curve, 100 μl of the calibration standards were prepared by serial dilution of the stock SQV/PZQ solutions in DMEM (in duplicate), after thawing the samples resulting in seven concentrations of 0, 1.6, 3.2, 6.4, 12.8, 19.2 and 25.6μM in 10ml labelled glass tubes (Table 3.1). Quality control samples (100 μl) were prepared by thawing the stock QC samples and pipetting into separate 10ml labelled glass tubes (in duplicate) to give concentrations of 6.4 low QC (LQC), 12.8 medium QC (MQC) and 19.2μM high QC (HQC).

Tube No.	Levels	SQV/PZQ Conc.(μM)	Stock(μl)	DMEM(μl)
1,2	1	0	0	100
3,4	2	1.6	6.25	93.75
5,6	3	3.2	12.5	87.5
7,8	4	6.4	25	75
9,10	5	12.8	50	50
11,12	6	19.2	75	25
13,14	7	25.6	100	0
	Total		268.75(μl)	431.25(μl)
	QCs			
15,16	LQC	6.4	100	0
17,18	MQC	12.8	100	0
19,20	HQC	19.2	100	0

Table 3.1 Concentrations of the standards and QCs

The next step involved the addition of 20 μl of 5μg/ml of internal standard CLZ and 2mls of the extraction solvent diethyl ether to each individual 100μl samples of the standards and QCs using a Finn repeater pipette. The tubes were then capped and tumbled for 30min using a rotary mixer and then centrifuged for 5 min at 4000 × *g*.

The aqueous phase was then frozen in a cryogenic bath and the solvent phases transferred to labelled 5 ml clean tubes, followed by the evaporation of the solvent to dryness using a centrifugal rotary evaporator (Jouan RC.10.10). The residues were reconstituted by addition of 100 μl mobile phase and vortexing of all the tubes. 100 μl of the samples were then aliquoted into autosampler vials, securely capped and

centrifuged for 4 minutes at 4000 × *g*. They were then placed in corresponding numbered wells of the autosampler tray, transferred to the HPLC column and analysed as described in 3.2.2.

3.2.4 Method validation

3.2.4.1 Linearity, limit of quantification and limit of detection

To evaluate the linearity of the assay, ten six-point calibration curves were analysed on separate days. The standard samples were extracted as described in section 3.2.3.2 and the standard curves plotted as the peak area ratio (PAR) of the respective compound to the internal standard versus the concentration. The curves were obtained using DMEM spiked with 1.6, 3.2, 6.4, 12.8, 19.2 and 25.6 μM of both SQV and PZQ on the same run, and each point on the calibration curve was run in duplicate (two separate extractions). The curves were constructed by calculating the PARs of each compound to the internal standard and plotting against the respective concentration of the sample. To assess linearity, the line of best fit was determined by least squares regression.

The lowest limit of quantification (LLQ) for each drug was the minimal concentration that was within the range of the nominal concentration, the acceptance criteria for each calculated standard concentration limited to not more than 20% deviation from the nominal value. Calibration curves were established with standard solutions for the concentration points 1.6, 3.2, 6.4, 12.8, 19.2 and 25.6 μM, all analysed in quadruplicate. To determine the limit of detection (LLD), quadruplicate standard solutions for the concentration points of 1.6, 0.8, 0.4, 0.2, 0.1 and 0.05 μM were analysed and the peak areas of the respective drug concentration compared to

that in DMEM (blank). The lowest concentration that produced a signal three or more times above the noise level of a blank preparation was designated as the limit of detection (LOD).

3.2.4.2 Accuracy and precision

The accuracy and precision of the analytical method was based on the fact that the relative standard deviation of each concentration should be within ± 20% of the nominal concentration. Inter-day accuracy and precision ware evaluated by comparing ten replicate low, medium and high QC levels analysed on different days. The intra-day accuracy and precision was determined by the analysis of the three concentrations of the QC samples in six replicates in the same day evaluated in duplicate. The results obtained were expressed as relative standard deviations to the mean and the CV expressed as a percentage;

Coefficient of variation, CV = (SD/Mean) × 100.

3.2.4.3 Recovery

The recovery or extraction efficiency of the analyte after the liquid-liquid extraction was determined by comparing the peak areas of six replicate samples of the QCs of each compound in extracted DMEM to those of non-processed standard solutions (standard solutions spiked in mobile phase). Recovery was expressed as a percentage of the peak area of the extracted drug to that injected in mobile phase.

3.2.4.4 Specificity and selectivity

The separation from endogenous compounds was investigated by analysing six different samples of DMEM.

3.2.4.5 Stability

Short and long term stability was investigated by comparing the peak areas of six freshly prepared QC assay samples to those of the freeze-thawed samples, and it was expressed as a percentage.

3.2.5 Drug accumulation experiments

3.2.5.1 Substrate studies

The parental cell line was CEM (T-lymphoblastoid cell line) was used as the parental cell line. CEMVBL cells that have increased expression of P-gp were selected under vinblastine (Janneh *et al.*, 2005). The selection of drug resistant cells was carried out by using increasing concentrations of vinblastine up to 100ng/mL. CEM and CEMVBL cells were maintained in 175cm^2 flasks containing RPMI media supplemented with 10% FBS at 37°C in a humidified 5% CO_2 -gassed incubator, conditions which led to doubling after approximately every 24 hours. The cells were passaged 1:5 upon reaching confluence every three to four days. This involved washing the cells in RPMI (+10% FBS), discarding the supernatant followed by resuspension in RPMI (+10% FBS). CEMVBL cells were incubated with vinblastine (100ng/mL) after every 8 weeks to mainatain drug pressure. The cells were passaged for at least three times before achieving confluence suitable for experiments.

Aliquots (100µl) of CEM and CEMVBL Cells were then counted using Nucleo Counter (Chemometec, Denmark) and the appropriate volume containing 10 million cells was transferred into eight 20 ml sterilin tubes appropriately labelled for the following samples; CEM PZQ, VBL PZQ, CEM SQV and VBL SQV. The cell samples were then centrifuged (2000 × *g* for 5 min at 4°C) and the supernatant fraction discarded. A total of 10ml of fresh RPMI media was added to the resulting pellets to give a concentration of 1 million cells/ml, and 100 µl of 1mg/ml of both PZQ and SQV to each respective tube resulting in a concentration of 10µg/ml. The cells were then incubated at 37°C for 30 min in a shaking water bath. The resulting cell suspensions were then centrifuged (2000 × *g* for 5 min at 4°C) and 100µl

samples of the supernatant fraction were removed and the aliquots used to determine the extracellular (EXT) concentration. The excess supernatant fraction was then discarded and the resulting cell pellet was washed three times in 10ml of ice cold HBSS and centrifuged (2000 × *g* for 5 min). The resulting pellets were solubilized by reconstituting in 100µl of distilled water and used to determine intracellular (INT) concentrations, as described in a method previously validated in our laboratory (Janneh *et al.*, 2007). The experiment was carried out in quadruplicate.

The samples were then assayed by HPLC and the data expressed as cellular accumulation ratio (CAR), the ratio of the intracellular to the extracellular accumulation calculated by the formula, CAR = INT/EXT [intracellular concentrations were calculated using the volume of a single CEM and CEMVBL cell to be 1 picolitre (pl)] (Ford *et al.*, 2004; Jones *et al.*, 2001b).

3.2.5.2 Inhibition studies

In the inhibition study, the cells (CEM and CEMVBL) spiked with a concentration of 10µg/ml of SQV were incubated in the absence or presence of 10µg/ml PZQ and 1µM tariquidar (XR) respectively.

3.2.5.3 Statistical analysis

All results were presented as mean ± SD with 95% confidence intervals for the difference between the means, where appropriate. The assessment of normality was done using Shapiro – Wilk test and the statistical analysis performed using the unpaired *t*-test. A two-tailed *p* value of <0.05 was accepted as being significant.

3.3 Results

3.3.1 Chromatography and detection

The analysis of SQV and PZQ on the same mobile phase was highly dependent on the pH value of the mobile phase. Thus the reverse-phase analysis was initially performed with various mixtures of ammonium formate, ACN and MeOH. The final mobile phase was 57% ammonium formate, 38% ACN and 5% MeOH. These conditions yielded satisfactory and reproducibility of the retention times of both PZQ and SQV. CLZ was found to be the suitable internal standard. The retention time for SQV was 5.1 minutes. PZQ 6.2 minutes and CLZ 2.2 minutes. The detection at 215 nm provided adequate sensitivity. A sample chromatogram of SQV, PZQ and CLZ is represented in Fig.3.1.

3.3.2 Linearity, limit of quantification and limit of detection

The lower limit of quantification for both SQV and PZQ on the same run was 1.6μM whereas the upper limit of quantification was 25.6μM. The concentration-response relationship for both SQV and PZQ standards was found to be linear in the concentration range of 1.6 – 25.6μM (r = 0.99773 PZQ and r = 0.9962 SQV) [Fig 3.3]. This linear relationship was demonstrated by a coefficient of variation obtained from the daily standard curves used for the analysis of unknown samples. The lowest limit of quantification (LLQ) detection was 1.6 μM, while the LLD was 0.1μM for both drugs.

3.3.3 Accuracy and precision.

The mean inter-day precision was within the range for both drugs with average CVs of between 3.28 and 5.89 % for PZQ, and 3.67 and 9.32 for SQV (Table 3.2).

Similarly, the intra-day assay values were between 0.97 to 2.13 for PZQ and 0.86 to 2.15 for SQV (Table 3.3).

3.3.4 Recovery

The mean recovery for both drugs in DMEM was always greater than 92% for both drugs within the analysed concentration range of 6.4 μM (MQC) to 19.2 μM (HQC) [Table 3.4].

3.3.5 Specificity and selectivity

The selectivity of the chromatographic separation was demonstrated by the absence of interfering endogenous peaks in DMEM (Fig 3.2).

3.3.6 Stability

Freeze thawing DMEM samples containing SQV and PZQ did not appear to significantly affect the concentrations when compared to fresh samples (Table 3.5).

3.3.7 Accumulation experiments

The chromatograms illustrating the extracellular and intracellular accumulation of both SQV and PZQ in CEM and CEMVBL cells are depicted in Fig 3.4. The accumulation of SQV was significantly lower in CEMVBL than CEM cells (0.1 ± 0.031 versus 0.52 ± 0.046, $p = 0.03$ [$p < 0.05$]), whereas similar accumulation of PZQ occurred in both cell lines (0.05 ± 0.005 versus 0.04 ± 0.009, $p = 0.4$) [Table 3.6; Fig 3.5]. PZQ did not significantly affect the accumulation of SQV in either CEM (0.52 ± 0.068 versus 0.54 ± 0.061, $p = 0.77$), or in CEMVBL cells (0.09 ± 0.015 versus 0.1 ± 0.031, $p = 0.89$) cells as compared to tariquidar; 0.52 ± 0.068 versus 0.61 ± 0.102,

$p = 0.34$ in CEM cells and 0.09 ± 0.015 versus 0.56 ± 0.089, $p = 0.029$ [$p<0.05$] in CEM_{VBL} cells (Table 3.7; Fig 3.6).

Date	Validation	LQC		MQC		HQC	
		PZQ	SQV	PZQ	SQV	PZQ	SQV
20/08/07	Validation1	6.31	6.05	12.80	12.70	19.85	18.88
		5.71	6.07	12.78	13.51	20.17	18.88
21/08/07	Validation2	6.96	7.01	12.18	12.33	19.42	19.16
		6.17	5.63	13.26	12.69	20.12	20.14
22/08/07	Validation3	5.63	5.17	11.86	10.16	17.77	17.15
		6.33	5.87	12.38	13.73	19.34	18.96
23/08/07	Validation4	6.27	5.78	12.70	12.55	19.28	19.07
		5.86	5.71	12.91	12.93	19.00	19.02
24/08/07	Validation5	6.14	5.79	12.48	12.68	19.48	19.65
		6.43	6.22	12.77	12.90	19.48	19.65
28/09/07	Validation6	6.23	5.73	12.70	12.69	19.42	18.28
		5.76	6.27	13.16	12.57	19.19	18.45
03/09/07	Validation7	6.67	5.78	13.13	11.60	19.17	18.37
		6.53	7.13	12.65	13.33	18.99	18.78
04/09/07	Validation8	6.18	6.47	13.48	13.72	18.90	19.09
		6.96	6.97	11.91	11.84	18.79	18.24
12/09/07	Validation9	6.31	6.87	12.40	13.26	18.71	18.38
		6.65	6.92	12.91	12.52	20.01	19.18
13/09/07	Validation10	6.49	7.04	13.21	13.87	20.66	20.10
		6.14	6.04	14.02	14.43	19.85	19.36
	Mean	6.29	6.23	12.78	12.80	19.38	18.94
	STDEV	0.37	0.58	0.52	0.93	0.64	0.69
	CV (%)	5.89	9.32	4.06	7.24	3.28	3.67

Table 3.2 Inter-day precision of the analytical method

Sample	LQC		MQC		HQC	
	PZQ	SQV	PZQ	SQV	PZQ	SQV
1	2.77	3.28	6.42	8.29	9.67	11.25
2	2.77	3.24	6.72	8.64	9.74	11.30
3	2.68	3.28	6.73	8.66	9.82	11.35
4	2.75	3.29	6.75	8.68	9.81	11.37
5	2.75	3.29	6.71	8.70	10.01	11.63
6	2.73	3.33	6.77	8.75	10.03	11.60
7	2.71	3.31	6.74	8.71	10.05	11.63
8	2.72	3.30	6.78	8.70	10.09	11.67
9	2.72	3.34	6.39	8.22	10.08	11.67
10	2.73	3.28	6.71	8.61	9.92	11.50
Mean	2.73	3.29	6.67	8.60	9.92	11.50
STDEV	0.03	0.03	0.14	0.18	0.15	0.16
CV (%)	0.97	0.86	2.13	2.15	1.54	1.42

Table 3.3 Intra-day precision of the analytical method

Sample	MP		DMEM		PZQ		SQV	
	PZQ	**SQV**	**PZQ**	**SQV**	**Recovery**	**Mean recovery**	**Recovery**	**Mean recovery**
LQC 1	2.31	3.04	2.68	2.92	116.18	114.84	96.11	95.41
LQC 2	2.42	3.17	2.74	3.00	113.50		94.70	
LQC 3	2.37	3.12	2.68	3.02	113.01	99.01	96.79	92.97
LQC 4	2.79	3.31	2.37	2.95	85.02		89.14	
LQC 5	2.68	3.22	2.42	2.82	90.40	105.06	87.48	97.25
LQC 6	2.33	3.04	2.79	3.26	119.72		107.03	
MQC 1	5.14	6.54	6.02	7.82	117.10	118.09	119.51	119.33
MQC 2	5.21	6.37	6.20	7.59	119.08		119.15	
MQC 3	5.24	6.51	6.25	7.47	119.32	117.00	114.71	110.35
MQC 4	5.10	6.73	5.84	7.13	114.68		105.98	
MQC 5	5.69	7.52	5.84	6.88	102.71	110.97	91.52	102.49
MQC 6	5.14	6.78	6.13	7.69	119.24		113.46	
HQC 1	7.72	9.70	8.92	9.69	115.42	117.23	99.86	101.63
HQC 2	7.90	9.91	9.40	10.25	119.05		103.40	
HQC 3	7.92	9.97	9.03	9.72	113.96	115.03	97.43	99.80
HQC 4	8.29	10.45	9.63	10.68	116.10		102.17	
HQC 5	8.87	11.25	9.71	10.84	109.44	113.96	96.29	102.49
HQC 6	8.03	10.16	9.52	11.04	118.49		108.70	

Table 3.4 Recovery of PZQ and SQV

Sample	MP		DMEM		PZQ		SQV	
	PZQ	**SQV**	**PZQ**	**SQV**	**Stability**	**Mean stability (%)**	**Stability (%)**	**Mean stability (%)**
LQC 1	2.31	3.04	2.69	2.77	116.78	106.55	91.14	92.04
LQC 2	2.42	3.17	2.33	2.95	96.31		92.94	
LQC 3	2.37	3.12	2.68	2.70	113.13	101.94	86.53	85.45
LQC 4	2.79	3.31	2.53	2.79	90.75		84.37	
LQC 5	2.68	3.22	2.67	2.81	99.70	108.27	87.23	95.67
LQC 6	2.33	3.04	2.72	3.17	116.84		104.11	
MQC 1	5.14	6.54	5.41	6.59	105.23	107.08	100.70	100.90
MQC 2	5.21	6.37	5.67	6.44	108.93		101.10	
MQC 3	5.24	6.51	6.20	6.69	118.37	118.41	102.80	105.19
MQC 4	5.10	6.73	6.04	7.24	118.45		107.58	
MQC 5	5.69	7.52	6.35	6.87	111.64	114.35	91.33	94.46
MQC 6	5.14	6.78	6.02	6.62	117.06		97.58	
HQC 1	7.72	9.70	8.48	8.45	109.77	110.69	87.07	85.65
HQC 2	7.90	9.91	8.81	8.35	111.60		84.23	
HQC 3	7.92	9.97	8.99	8.91	113.43	112.78	89.34	88.62
HQC 4	8.29	10.45	9.30	9.18	112.13		87.89	
HQC 5	8.87	11.25	8.97	9.40	101.08	106.31	83.52	86.90
HQC 6	8.03	10.16	8.96	9.17	111.54		90.27	

Table 3.5 Stability data for PZQ and SQV

Sample	CEMSQV	VBL SQV	CEM PZQ	VBL PZQ
1	0.54	0.12	0.03	0.06
2	0.52	0.14	0.05	0.05
3	0.45	0.07	0.05	0.05
4	0.55	0.08	0.04	0.05
Mean	0.52	0.10	0.04	0.05
STDEV	0.046	0.031	0.009	0.005
***p* value**	$p = 0.03$		$p = 0.4$	

Table 3.6 Cellular accumulation ratio (CAR) values for the substrate studies

Sample	CEM SQV	CEMSQV PZQ	CEMSQV XR	VBL SQV	VBL SQV PZQ	VBL SQV XR
1	0.57	0.45	0.55	0.07	0.08	0.53
2	0.50	0.57	0.51	0.10	0.07	0.50
3	0.59	0.59	0.73	0.10	0.13	0.69
4	0.44	0.53	0.66	0.11	0.13	0.52
Mean	0.52	0.54	0.61	0.09	0.10	0.56
STDEV	0.068	0.061	0.102	0.015	0.031	0.089
p value		$p = 0.77$	$p = 0.34$		$p = 0.89$	$p = 0.03$

Table 3.7 Cellular accumulation ratio values for inhibition studies

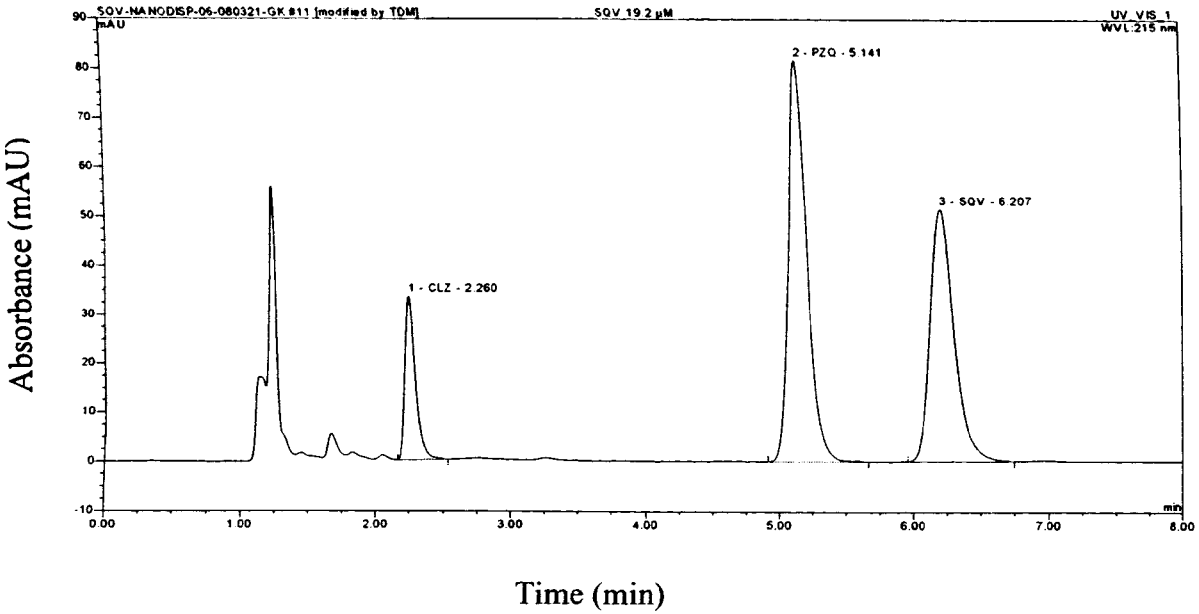


Fig 3.1 Chromatogram depicting the retention times of CLZ (internal standard), PZQ and SQV at a concentration of 19.2 µM for both drugs.

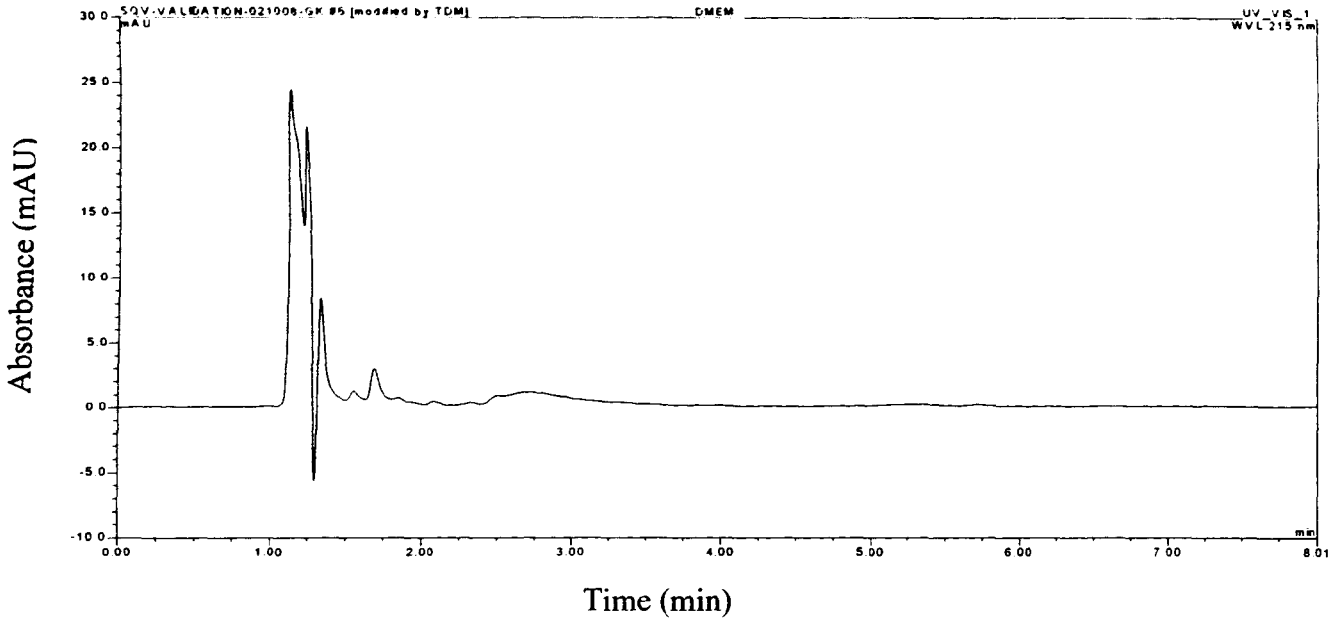
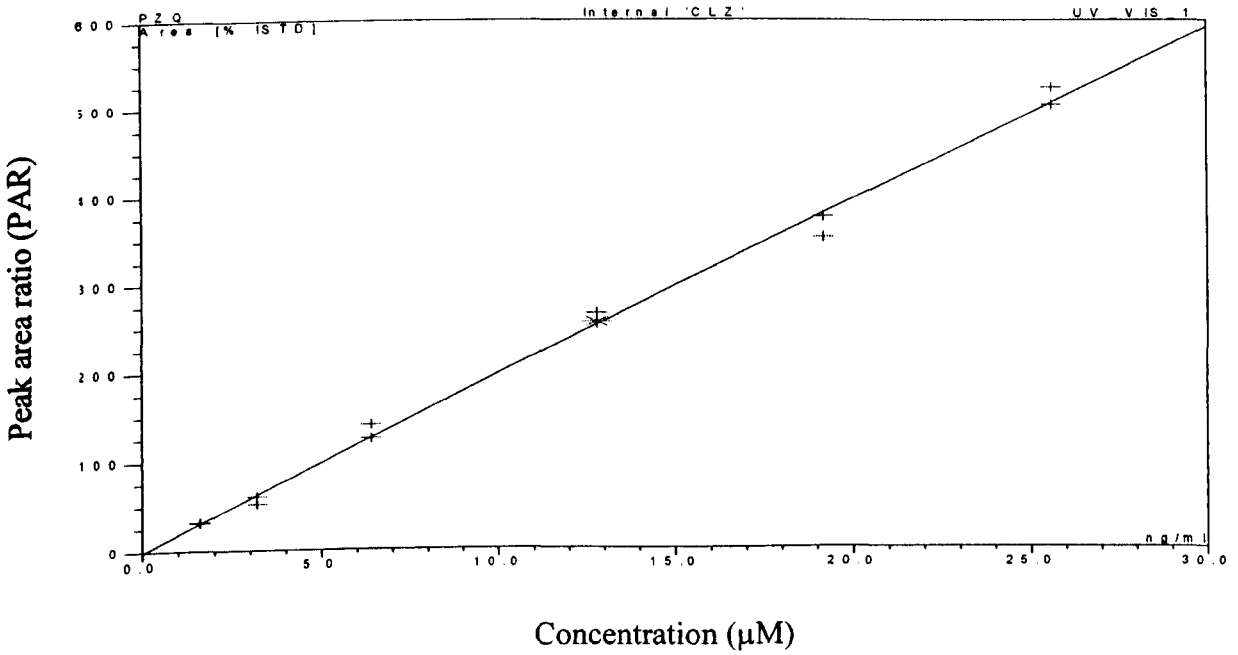


Fig 3.2 Chromatogram of a blank extract (DMEM), showing the injection peak and absence of any other interfering peaks.

A. PZQ standard curve



B. SQV standard curve

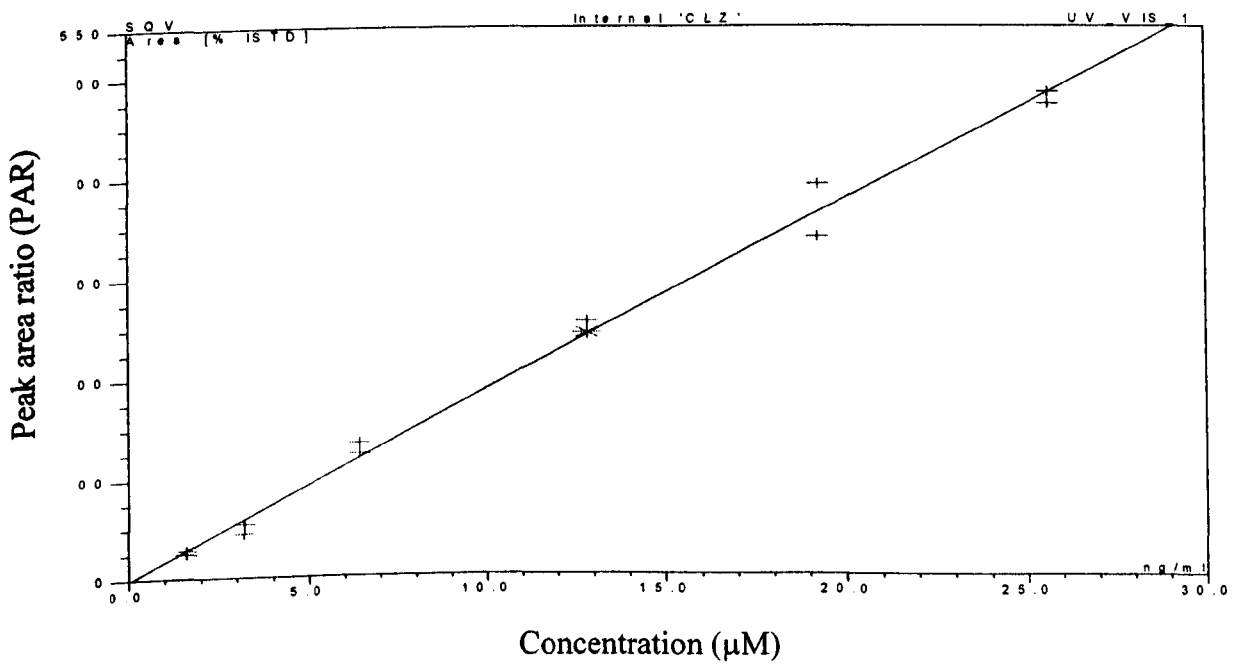
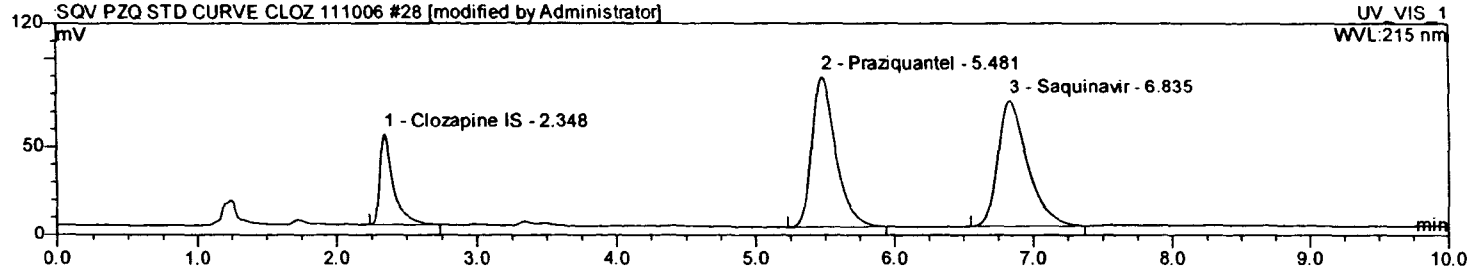
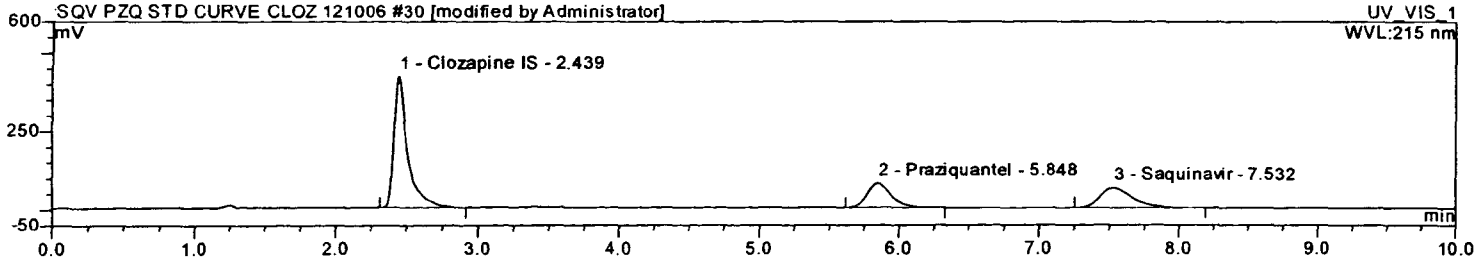


Fig 3.3 Plots of the calibration curves showing the concentration response relationships of A (PZQ) and B (SQV) on the same run.

CEMSQV/PZQ extracellular



VBLSQV/PZQ extracellular



VBL SQV/PZQ intracellular

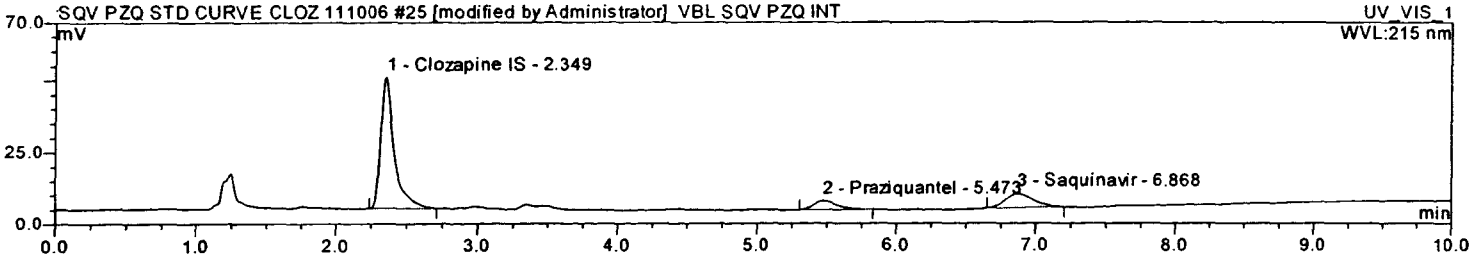


Fig 3.4 Chromatograms showing the extracellular and intracellular accumulation of SQV/PZQ in CEM and CEMVBL cells

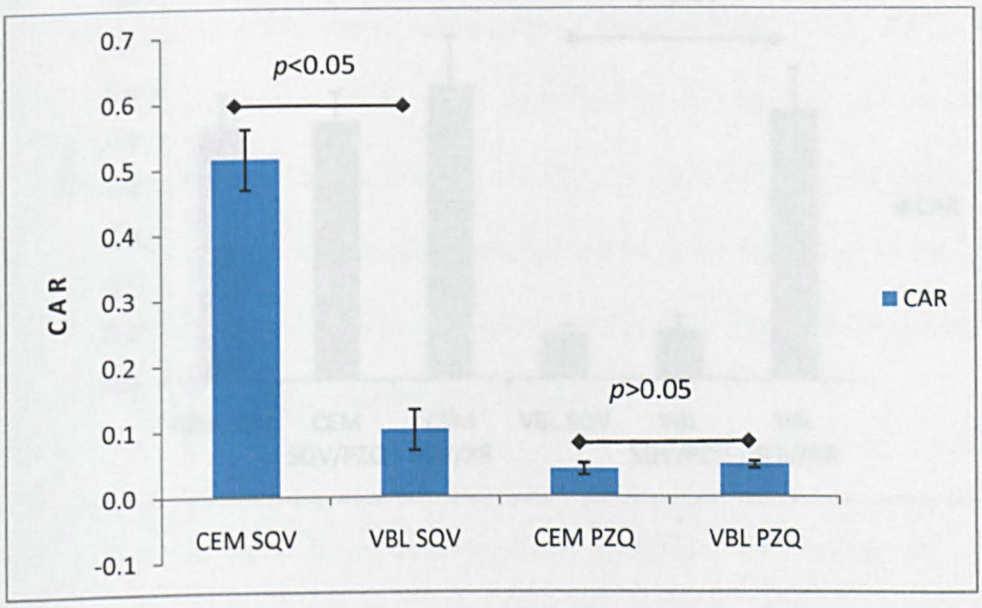


Fig 3.5 Intracellular accumulation of SQV and PZQ in CEM and CEM$_{VBL}$ cells, Mean ± SD (n=4).

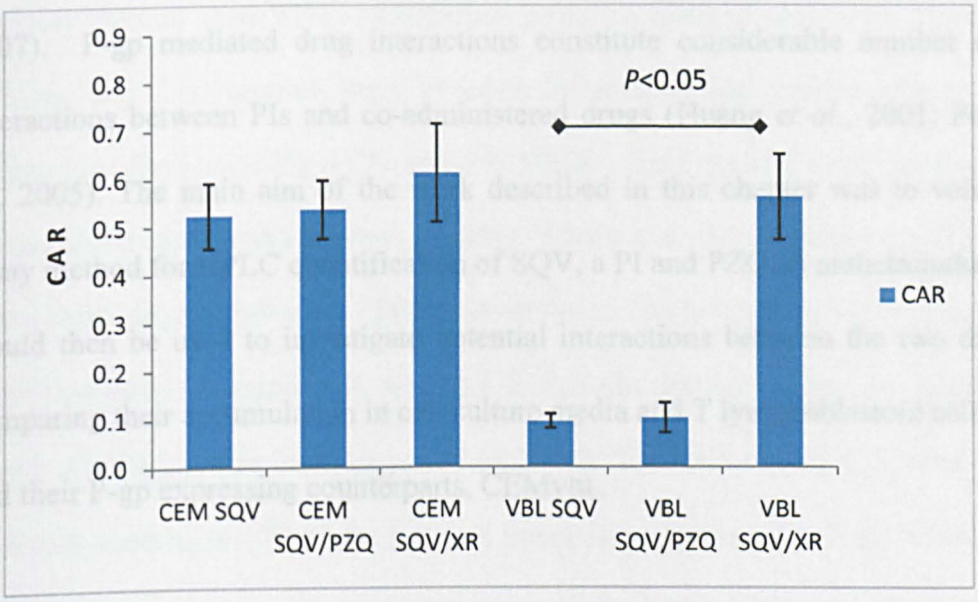


Fig 3.6 Effect of PZQ and tariquidar on the intracellular accumulation of SQV in CEM and CEM$_{VBL}$ cells, Mean ± SD (n=4).

3.3 Discussion

Managing drug interactions is now a major concern in the optimization of therapy of HIV/AIDS. This is driven by the large number of drugs that patients are required to take simultaneously, in order to prevent treatment failure (Kashuba, 2005; Pontali, 2007). P-gp mediated drug interactions constitute considerable number of drug interactions between PIs and co-administered drugs (Huang *et al.*, 2001; Perloff *et al.*, 2005). The main aim of the work described in this chapter was to validate an assay method for HPLC quantification of SQV, a PI and PZQ an anthelminthic. This would then be used to investigate potential interactions between the two drugs by comparing their accumulation in cell culture media and T lymphoblastoid cells, CEM and their P-gp expressing counterparts, CEMVBL.

Several methods have been developed to quantify ARV drugs and other co-administered drugs (Bonato *et al.*, 2007; Gonzalez-Esquivel *et al.*, 2005; Hanpitakpong *et al.*, 2004). To date none have been reported in the literature that is suitable for simultaneous quantification of ARVs and PZQ or indeed any anthelminthics. We have developed a simple reliable and reproducible method that can potentially be used to investigate the interactions between SQV and PZQ. This assay provides a sensitive method for simultaneous and specific quantification of PZQ and SQV using HPLC, by employing the same extraction procedures (a simple liquid-liquid extraction) and the same chromatographic conditions (mobile phase, column and UV wavelength). In addition our method is also unique as it does not involve LC/MS. The isocratic elution as well as the short run time (8 minutes) and the small sample size required provide additional advantages to the method.

The assay was developed from a method used in our laboratories for TDM of PIs, and CLZ was chosen as the internal standard due to cost and ease of availability (Clarke *et al.*, 2001; Dickinson *et al.*, 2005). The method was validated with mean average CVs less than 10% for the two drugs, intra-assay precision less than 5%, and recovery greater than 92%. Interfering peaks in DMEM that elute at about 1.8 minutes and 2.5 minutes were avoided by maintaining mobile phase at 57% ammonium formate, 38% ACN and 5% MeOH. This method could potentially be extended for the quantification of other ARVs and anthelminthics and also for therapeutic drug monitoring (TDM) in resource limited places such as in Africa. From our earlier discussion in section 1.2, it is not uncommon to have patients that are on ARV treatment including SQV and PZQ. The method has the potential to be routinely used in the TDM for patients undergoing therapy for both conditions. It would be particularly useful in monitoring patients with schistosomiasis and HIV co-infection who develop TB or fungal infections. The two diseases are commonly associated with HIV/AIDS in Africa and rifampicin is widely used in Africa to treat TB. Ketoconazole is used to manage fungal infections and the two drugs have been reported to dramatically alter the plasma concentrations of PZQ upon co-administration. In a study, rifampicin reduced the concentration of PZQ by 80% in healthy subjects (Ridtitid *et al.*, 2002), while ketoconazole increased PZQ concentration by 90% in a related study (Ridtitid *et al.*, 2007). Both actions were attributed to the induction and inhibition of the CYP3A4 enzyme that metabolizes PZQ.

As discussed earlier in section 3.1, SQV is a substrate of efflux transporters P-gp, ABCC1 and ABCC2 (Janneh *et al.*, 2005; Profit *et al.*, 1999). PZQ has not been

conclusively characterised to date despite its widespread use in the management of schistosomiasis. In a previous study, the authors concluded that PZQ is an inhibitor of P-gp without being a substrate (Hayeshi *et al.*, 2006). A related study indicated that PZQ did not show potential for interacting with cellular efflux pumps (Gonzalez-Esquivel *et al.*, 2005). From our results PZQ does appear to be either a substrate or an inhibitor of P-gp. In addition, PZQ did not appear to impact on the accumulation of SQV suggesting that it may not interact with SQV by influencing the P-gp mediated transport of SQV.

The intracellular accumulation of drugs is controlled by several factors including ion trapping, lipophilicity and plasma protein binding as well as influx and efflux transporters. With regards to PIs, drug transporters P-gp, ABCC1, ABCC2 and BCRP play an important role in their accumulation (Hoggard *et al.*, 2003; Janneh *et al.*, 2005). Being an efflux transporter P-gp transports PIs from the intracellular to extracellular compartments, and the differences in their accumulation may be used to study their pharmacokinetics (Jones *et al.*, 2001b). CEM cells treated with vinblastine (CEMVBL) overexpress P-gp (Beck *et al.*, 1979) and the comparison of the *in vitro* accumulation of PIs in CEM parental and CEMVBL cells has been used to investigate the effects of active transport (Jones *et al.*, 2001b; Khoo *et al.*, 2002).

Similarly, the interactions with other drugs may be assessed by investigating their ability to inhibit or potentiate their accumulation (Brinkman *et al.*, 1998a; Hoggard *et al.*, 2003; Perloff *et al.*, 2005). In our experiment SQV was used as a positive control to investigate whether PZQ is a substrate of P-gp, and tariquidar a known inhibitor of P-gp was used as a positive control to determine whether PZQ is an inhibitor of P-gp.

The results obtained from our experiments indicate that there was similar accumulation of PZQ in both CEMVBL and CEM cells as compared to SQV whose accumulation was significantly lower in CEMVBL than CEM cells in line with previous studies (Jones *et al.*, 2001a), suggesting that PZQ is not a substrate for P-gp. PZQ did not significantly affect the accumulation of SQV in either CEM or CEMVBL cells as compared to tariquidar, suggesting that the PZQ is not an inhibitor of P-gp. We can therefore conclude that PZQ is neither a substrate nor an inhibitor of P-gp. From these findings, it would appear that the earlier interpretation that PZQ as an inhibitor of P-gp without being a substrate based on transport along the Caco-2 cell monolayers may be incorrect (Hayeshi *et al.*, 2006). This could be explained in part by the fact that Caco-2 cells express many other transporters.

In summary, it appears that PZQ does not interact with SQV at the level of the drug transporter P-gp. However it should be noted that CEMVBL cells are assumed to only overexpress P-gp and as discussed earlier, there are many other transporters influx and efflux that may modulate the transport and possible interactions between the drugs. Caco-2 cells express a wider range of transporters and metabolic enzymes and these cells may be more suitable to study interactions involving other transporters (Hidalgo *et al.*, 1989; Hilgers *et al.*, 1990).

Chapter 4

Investigation of the interactions between antiretroviral and anthelminthic drugs in Caco-2 cell monolayers

Chapter 4

Investigation of the interactions between antiretroviral and anthelminthic drugs in Caco-2 cell monolayers

4.1 Introduction

The majority of the drugs in use are orally administered and their absorption from the gastrointestinal tract (GIT) is pivotal for their success in therapy (Lennernas, 1998). The ability of a drug to cross the intestinal wall in order to reach portal circulation is to a large extent dependent on the permeability coefficient of the drug. The Caco-2 cell model provides a simple, reliable method to assay in vitro permeability of drugs (Adson *et al.*, 1995; Artursson, 1990). The permeability of drugs through the Caco-2 cell monolayers (CCM) correlates well with *in vivo* absorption in humans thus making the CCM an invaluable analytical tool in the screening of orally administered drugs (Artursson *et al.*, 1991; Artursson *et al.*, 2001; Gres *et al.*, 1998).

CCM are derived from human colonic adenocarcinoma and have morphological as well as functional similarities to intestinal (absorptive) enterocytes (Hidalgo *et al.*, 1989; Hilgers *et al.*, 1990). They have adherent properties and so form a monolayer with tight junctions which prevent paracellular diffusion so that drugs or other solutes can only pass through the cell, as illustrated in the cartoon (Fig 4.1). This also results in the development of cell polarity and the efflux transporter P-glycoprotein (P-gp) has been shown to be localised on the apical brush border, approximately 20 microns above the base of the cells (Hunter *et al.*, 1993), while certain MRPs also efflux transporters are expressed on the basolateral side of the monolayer (Hirohashi *et al.*, 2000)[Fig 4.1].

Apart from P-gp and MRPs, CCM express a wide array of transporters (efflux and influx) as well as metabolic enzymes, thus making them suitable for the study of drug-drug interactions based on the permeability of drugs through the monolayers

(Alsenz *et al.*, 1998; Hidalgo, 2001; Hidalgo *et al.*, 1989; Hilgers *et al.*, 1990; Peters *et al.*, 1989). The permeation of drugs through the monolayers allows the study of the major absorptive mechanisms for drugs, such as passive transcellular transport and carrier-mediated influx as well as efflux mechanisms (Alsenz *et al.*, 2003). In this system, the passage of drugs from apical (AP) to basolateral (BL) compartment is attributable to passive diffusion and occurs at a lower rate, whereas the BL to AP passage occurs by active transport, presumed to be mediated by transporters (Alsenz *et al.*, 1998; Hidalgo *et al.*, 1990). The carrier-mediated transport is a saturable process, which raises a possibility that when two drugs are co-administered they may compete for a transporter (influx or efflux) which would lead to drug interactions leading to lower or higher exposure than when dosed alone (Alsenz *et al.*, 1998; Okamura *et al.*, 1993).

The cells are grown on a porous membrane and form differentiated monolayers after about 20 days. The membranes are polarised and the evaluation of the monolayers can be performed by measuring the transepithelial resistance (TEER) using a volt-ohm meter equipped with electrodes placed in the upper and lower chambers of the insert. TEER increases with culture reaching a maximum in about 10-15 days (Anderle *et al.*, 1998; Bravo *et al.*, 2004; D'Souza *et al.*, 2003), and depends on the number of cells seeded, and the surface area of the filter. TEER values range from 150 to 1600 ohm.cm^2 as compared to human ileum which is about 50 ohm (Legen *et al.*, 2005).

The PIs, saquinavir (SQV) and lopinavir (LPV) are substrates and inhibitors of drug transporters (Alsenz *et al.*, 1998; Kim *et al.*, 1998a), while NNRTIs have not been

conclusively characterised (Storch *et al.*, 2007; Stormer *et al.*, 2002). From our accumulation studies (Chapter 3), we demonstrated that PZQ is neither a substrate nor an inhibitor of P-gp, but as earlier discussed the CCM express several other transporters. Indeed, earlier work on transport of antiparasitic drugs along the CCM suggested that PZQ among others were inhibitors of P-gp without being its substrates. Although it was not clear how the investigators concluded that this was mediated specifically by P-gp using the CCM model, which expresses many other transporters (Hayeshi *et al.*, 2006). In two related studies, there was dramatic reduction in plasma concentrations of PZQ by rifampicin and increase by ketoconazole upon co-administration and the authors concluded that the effects were as a result of the induction and inhibition of cytochrome P 450 (CYP 450) enzymes, mainly CYP 3A4 (Ridtitid *et al.*, 2007; Ridtitid *et al.*, 2002). IVM has been described as a substrate and inhibitor of drug transporters (Lespine *et al.*, 2006; Pouliot *et al.*, 1997). IVM interacts with P-gp modulators (Alvinerie *et al.*, 2008; Ballent *et al.*, 2006) and recently the inhibition of P-gp has been described as a strategy to counter the emerging resistance to ivermectin (Lespine *et al.*, 2008). However, there is no information on interactions between either PZQ or IVM with antiretrovials to date, despite the likelihood of co-administration of these drugs in tropical regions.

In this chapter, the CCM was used to evaluate potential interactions between antiretrovirals (ARVs) and anthelminthics. PZQ and IVM were used as prototypes for the anthelminthics, and PIs, SQV and LPV as well as NNRTIs, efavirenz (EFV) and nevirapine (NVP) as prototypes of ARVs. The impact of SQV, EFV and NVP on the transport of PZQ and conversely, the impact of PZQ on the transport of SQV was assessed. Likewise the influence of SQV, NVP, LPV and EFV on the transport of

IVM, and IVM on the transport of SQV, NVP, LPV and EFV were investigated. The impact of the anthelminthics on the ARVs transport was determined by assessing the AP→BL and BL→AP directions alone, and in the presence of an anthelminthic, and the reverse was conducted for the influence of ARVs on anthelminthics. Quantification was performed using an HPLC method described earlier (section 3.2) or radiolabeled assay using a liquid scintillating counter.

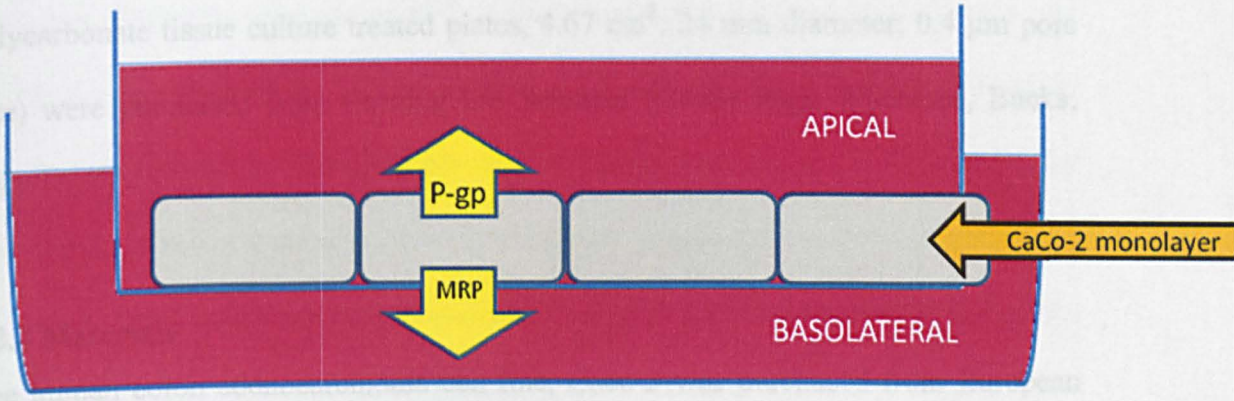


Figure 4.1 The diagram represents a transwell for growing Caco-2 cells including the compartments and the positions of the transporters

4.2 Methods

4.2.1 Equipment

The HPLC consisted of a Dionex (Dionex Softron GmbH, Germany) HPLC system with a P 680 pump, an ASI-100 automated sample injector and a UVD 1704 detector. A 250µl injector with a 20µl loop was used. Reversed-phase-liquid chromatography was carried out using a Hypurity^{TM} C_{18} analytical column, 5µm x 4.6mm (Thermo Electron Corporation, Runcorn, UK 22105-154630). A column guard (Thermo electron 60140-412) was used to protect the analytical column. The ultraviolet detector was set to monitor the 215 nm wavelength. A Packard Tri-Carb Liquid Scintillation Analyzer model 1900 TR (Packard instrument Co.) was used for radioactivity counting. A Millicell Electrical Resistance System (Fisher Scientific, Leicestershire, UK) was used for measuring TEER. Transwells (six-well transwell polycarbonate tissue culture treated plates, 4.67 cm^2, 24 mm diameter; 0.4 µm pore size) were purchased from Corning life Sciences (Costar High Wycombe, Bucks; UK).

4.2.2 Materials

The human colon adenocarcinoma cell line, Caco-2 was purchased from European collection of cell cultures (ECACC No. 286010202), and the cells were counted using a Nucleo Counter (ChemoMetec, Denmark) cell counter. SQV was provided by Roche Discovery (Welwyn Garden City, UK), LPV by Abbott Laboratories (Chicago, USA), EFV by Dupont Bristol Myers Squibb (New Brunswick, NJ, USA), and NVP by Boehringer Ingelheim (Berkshire, UK). Radiolabelled SQV (^{3H} SQV), LPV (^{3}H LPV), EFV (^{14}C EFV) and NVP (^{3}H NVP) were purchased from Moravek Biochemicals (Brea California, USA). ^{3}H IVM was kindly donated by Dr Iain

Gardner of Pfizer (Sandwich, Kent, UK). PZQ, CLZ, DMEM, HBSS, FBS, DMSO and Trypsin-EDTA solution were purchased from Sigma Aldrich (Poole, UK). ACN and MeOH were purchased from VWR Laboratory Supplies (Poole, UK) whereas diethyl ether was purchased from Fisher Scientific, (Loughborough, UK). Ultima Gold liquid scintillation cocktail was obtained from Packard (Groningen, Netherlands). All the other chemicals used were of analytical or HPLC grade. Deionised water used to prepare the solutions or mobile phase was purified in an Elga DV 25 pure lab option system (Elga, High Wycombe, Bucks, and UK).

4.2.3 Caco-2 cell lines

4.2.3.1 Cell culture

Caco-2 cells were cultured in DMEM supplemented with foetal bovine serum (15%v/v). The cells were grown and routinely seeded in tissue cultured treated 162 cm^2 flasks in a humidified chamber (37°C, 10% CO_2 incubator) and harvested by regular trypsinization. The medium was changed every 2 to 3 days until the confluence of the cell monolayer was achieved. Trypsinization involved decanting the media, followed by washing twice with 6 ml of HBSS and the detachment of the monolayer by addition of 4 ml of trypsin EDTA. The cells were then incubated for 10 minutes. The resulting suspension was then centrifuged (2000 $g \times 5$ min, 4°C), the supernatant removed and the resulting pellet re-suspended in 20ml of fresh DMEM (+15% FBS), and 10ml transferred two new flasks and each made to 20ml.

4.2.3.2 Storage of Caco-2 cells

The cells were trypsinised as described earlier after attaining confluence (section 4.2.3.1). The pellets were then re-suspended in DMEM (15%v/v), counted using a nucleocounter and centrifuged (2000 *g* × 5 min, 4°C). The cells were then re-suspended in warm FBS (FBS + 10% DMSO), mixed thoroughly and made up to a concentration of 5×10^6 cells per ml. 1 ml of the cell suspension was then transferred to pre-labelled 1.5ml cryovials and frozen at -80°C for use as and when required. The viable semi-frozen cells are thawed by placing the cryovials rapidly in a waterbath (37°C) or by simply holding in the vials in the hands for a few minutes and re-suspending the pellets in 9 ml DMEM (+15%FBS) followed by culturing as described in section 4.2.3.1.

4.2.4 Determination of drug transport in Caco-2 cells

4.2.4.1 Cell seeding

The monolayers used were between passage 20 and 65 after 15 days of growth. Each experiment was performed in duplicate using two six well-transwell culture plates. The cells were trypsinized as described earlier (4.2.3.1), and after centrifugation the pellet was re-suspended in fresh DMEM (+15%), and the cells counted using a cell counter. A volume of DMEM was added to give a cell count of 2×10^6 cells per ml, and cells were seeded on the transwell culture plates at a density of 2×10^4 cells/cm^2 (~100,000 cells per well, since insert membrane growth area = 4.67 cm^2). The plates were then incubated at 37°C and 10% CO_2 in a humidified chamber and the media was changed every 2-3 days, by aspirating using a suction pump and replacing with an equal volume of DMEM. Transport experiments were conducted 15 to 20 days after seeding. The TEER across the cell monolayers was monitored using a Millicell-

ERS to assess cell monolayer integrity and the monolayers considered appropriate for the experiment when the TEER values were typically above 500 cm^2.

4.2.4.2 Transport experiments

Prior to transport studies, each monolayer was washed and equilibrated with the transport medium (DMEM without FBS). The medium was removed from all AP and BL compartments of the transwells and replaced with 2 ml of the transport medium (DMEM alone) to both compartments and equilibrated for 1 h (37°C, 10% CO_2 incubator), after which the TEER was re-assessed and labelled. The medium was then removed from both compartments and replaced with an equal volume of pre-warmed medium containing the compound of interest at the appropriate concentration. For the AP →BL transport, 2ml of medium containing the desired drug was placed in the AP chamber and 2 ml of the medium alone in the BL chamber, whereas 2ml of medium containing the drug was placed in the BL and 2ml of medium on the AP chamber for the BL→AP transport. The effect of the second drug was then assessed by adding the medium containing the original drug and the drug under study to the AP side for AP →BL transport with the medium containing the original drug alone in the BL chamber, and vice-versa for the BL→AP transport. Transport in each direction was done in triplicate. The transwell plates were then incubated (37°C, 10% CO_2 incubator), and 100µl of the samples from the AP and BL compartments were taken at 60, 120, 180 and 240 min and quantified either by HPLC method described earlier (section 3.2) or by using a liquid scintillating counter depending on the drugs under study. HPLC was used in the assay of interactions between SQV/PZQ, IVM/SQV, EFV/PZQ and NVP/PZQ. Radiolabeled assay was utilised in the investigations of the interactions between

PZQ/SQV, SQV/IVM, NVP/IVM, IVM/NVP, LPV/IVM, IVM/LPV, EFV/IVM and IVM/EFV. The integrity of the CCM during the experiment was monitored by measuring the TEER at the beginning (0 min) and the end of the experiment (240min).

4.2.4.3 Apparent permeability

The results were expressed as apparent permeability coefficient (*P*app, unit: cms^{-1}), the amount of compound transported per second. *P*app values were calculated for both AP to BL ($Papp_{AtoB}$), and BL to AP ($Papp_{BtoA}$) movement of the compound. *P*app was calculated using the following equation:

$$Papp\ (cm/s) = \frac{dQ/dt}{(1 / (AC_O)}$$

dQ/dt = Steady-state flux (dpm s^{-1} or µmol s^{-1})

A = Surface area of the filter (cm^2)

C_O = Initial concentration in the donor chamber (dpm litre $^{-1}$ or µM)

The quotient of secretory permeability and absorptive permeability ($Papp_{BL-AP}/Papp_{AP-BL}$) constitutes the efflux ratio, while the reverse ($Papp_{AP-BL}/Papp_{BL-AP}$) is the uptake ratio (Hubatsch *et al.*, 2007). This calculation requires that the receiver concentration should not exceed 10% of the donor concentration, and therefore it was applied for the samples taken at 60 minutes. The permeability is a saturable process and depends on several physiological conditions such as accumulation, pH, and lipophilicity (sink conditions), which have an effect on *P*app values with incubations over longer periods of time (Hubatsch *et al.*, 2007; Youdim *et al.*, 2003).

4.2.4.4 Statistical analysis

The results were presented as mean ± standard deviation (SD) of five experiments with 95% confidence intervals for differences between the means where appropriate. The assessment of normality was done using Shapiro – Wilk test and the analysis performed using the unpaired t-test. A two-tailed p value of <0.05 was accepted as being significant.

4.3 Results

The results of the transport experiments are summarised in Table 1. IVM inhibited the efflux transport of SQV and significantly facilitated the uptake of LPV. LPV increased the uptake transport of IVM, while NVP inhibited the uptake transport of IVM.

4.3.1 Impact of SQV on the transport of PZQ

The transport of 20μg/ml (64μM) PZQ across the CCM (n=3) was determined in both AP→BL and BL→AP directions alone, and in presence of 20μg/ml (29.8 μM) SQV. The cells used were at passage 20 and the TEER values were 798 ± 16 for the PZQ and 785 ± 22 for PZQ + SQV (29.8 μM), and the TEER values measured at the beginning and the end of the experiment remained constant, consistent with intact monolayers (Artursson, 1990; Sulzbacher *et al.*, 1998). Quantification was performed by HPLC.

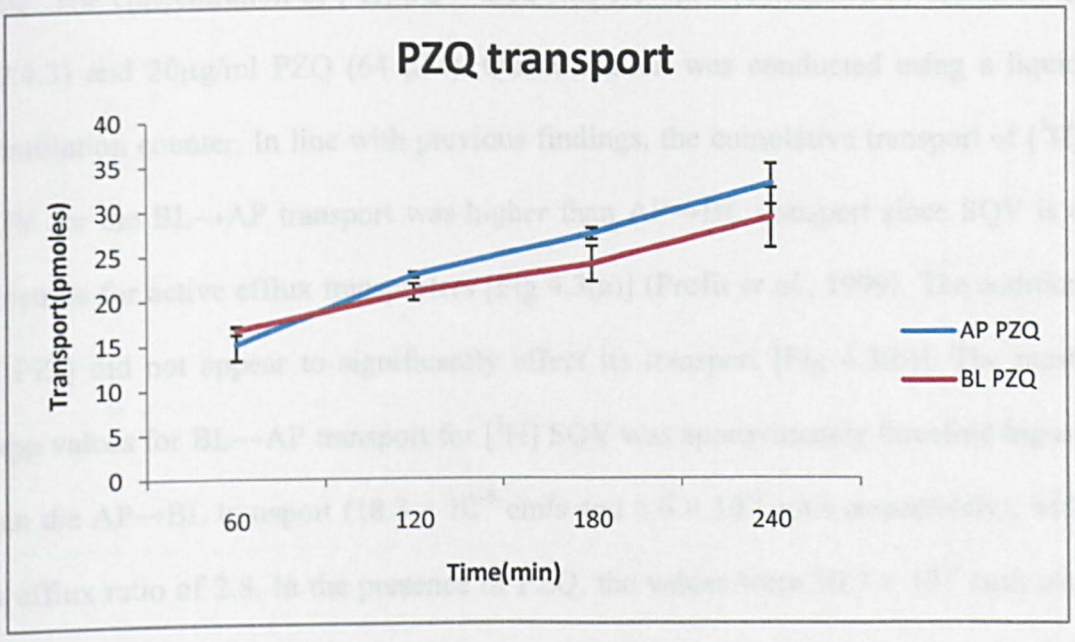
The cumulative BL→AP transport of PZQ was not markedly different from the AP→BL transport indicating that active efflux pump did not occur. The inclusion of SQV did not significantly alter its transport in either direction (Fig 4.2). The mean *P*app values for AP→BL and BL→AP transport for PZQ alone was calculated as 38.2×10^{-6} cm/s and 42.9×10^{-6} cm/s respectively resulting in an efflux ratio of 1.1. The introduction of SQV resulted in *P*app values of 27.1×10^{-6} cm/s for the AP→BL transport and 32.9×10^{-6} cm/s for BL→AP and an efflux ratio of 1.2, thereby insignificantly affecting the ratio.

Drug A	Drug B	Observed effect	Implication
PZQ	SQV	Similar efflux ratio	No significant influence on PZQ transport
SQV	PZQ	Similar efflux ratio	No significant influence on SQV transport
IVM	SQV	Similar efflux ratio	No significant influence on IVM transport
SQV	IVM	BL→AP transport of SQV inhibited, efflux ratio reduced (3.1 to 1.2)	**Potential for interaction; efflux transport of SQV inhibited**
PZQ	EFV	Similar efflux ratio	No significant influence on PZQ transport
PZQ	NVP	Similar efflux ratio	No significant influence on PZQ transport
IVM	NVP	AP→BL transport of IVM inhibited, efflux ratio increased (0.8 to 1.8)	**Potential for interaction; uptake transport of IVM inhibited**
NVP	IVM	Similar efflux ratio	No significant influence on NVP transport
IVM	LPV	AP→BL transport of IVM facilitated, efflux ratio decreased	**Potential for interaction; uptake transport of IVM increased**
LPV	IVM	AP→BL transport of LPV facilitated, efflux ratio decreased (6.7 to 0.8)	**Potential for interaction; uptake transport of LPV increased**
IVM	EFV	Similar efflux ratio	No significant influence on IVM transport
EFV	IVM	Similar efflux ratio	No significant influence on EFV transport

Drug A: Drug whose transport is under investigation
Drug B: Interacting drug

Table 4.1 Summary of potential interactions

(a)



(b)

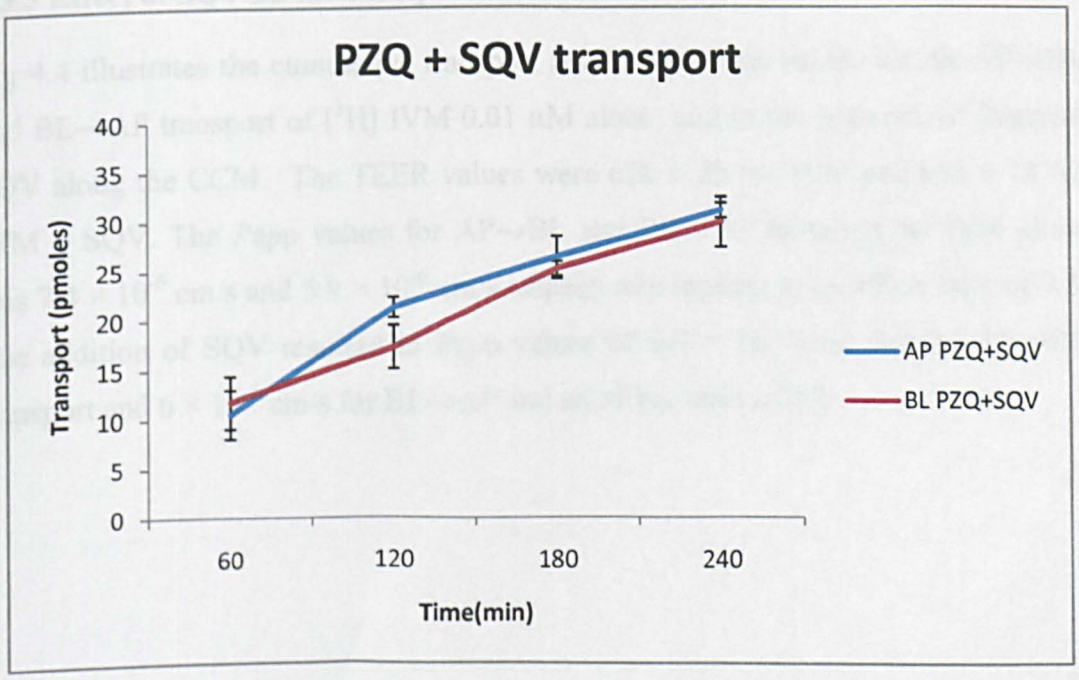


Figure 4.2 Cumulative transport of PZQ, a) alone and b) in presence of SQV. The results are expressed as mean ± s.d (n=3).

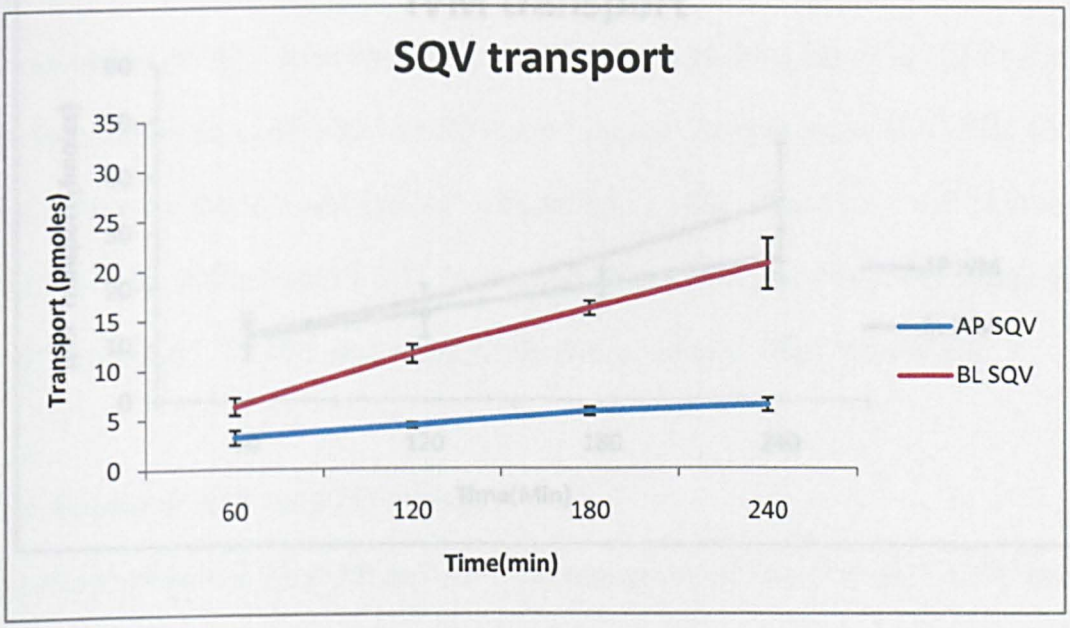
4.3.2 Impact of PZQ on the transport of SQV

The TEER values were 767 ± 34 for the [^{3}H] SQV and 801 ± 29 for [^{3}H] SQV + PZQ. The concentration of [^{3}H] SQV used was 1.07 μM (calculated as described in 4.2.4.3) and 20μg/ml PZQ (64 μM). Quantification was conducted using a liquid scintillation counter. In line with previous findings, the cumulative transport of [^{3}H] SQV for the BL→AP transport was higher than AP→BL transport since SQV is a substrate for active efflux transporters [Fig 4.3(a)] (Profit *et al.*, 1999). The addition of PZQ did not appear to significantly affect its transport [Fig 4.3(b)]. The mean *P*app values for BL→AP transport for [^{3}H] SQV was approximately threefold higher than the AP→BL transport (18.3 × 10^{-6} cm/s and 6.6 × 10^{-6} cm/s respectively), with an efflux ratio of 2.8. In the presence of PZQ, the values were 10.7 × 10^{-6} cm/s and 27 × 10^{-6} cm/s with a comparable efflux ratio of 2.5.

4.3.3 Effect of SQV on the transport of IVM

Fig 4.4 illustrates the cumulative transport results and *P*app values for the AP→BL and BL→AP transport of [^{3}H] IVM 0.01 μM alone, and in the presence of 20μg/ml SQV along the CCM. The TEER values were 658 ± 25 for IVM and 648 ± 18 for IVM + SQV. The *P*app values for AP→BL and BL→AP transport for IVM alone was 7.4 × 10^{-6} cm/s and 5.9 × 10^{-6} cm/s respectively leading to an efflux ratio of 0.8. The addition of SQV resulted in *P*app values of 6.4 × 10^{-6} cm/s for the AP→BL transport and 6 × 10^{-6} cm/s for BL→AP and an efflux ratio of 0.9.

(a)



(b)

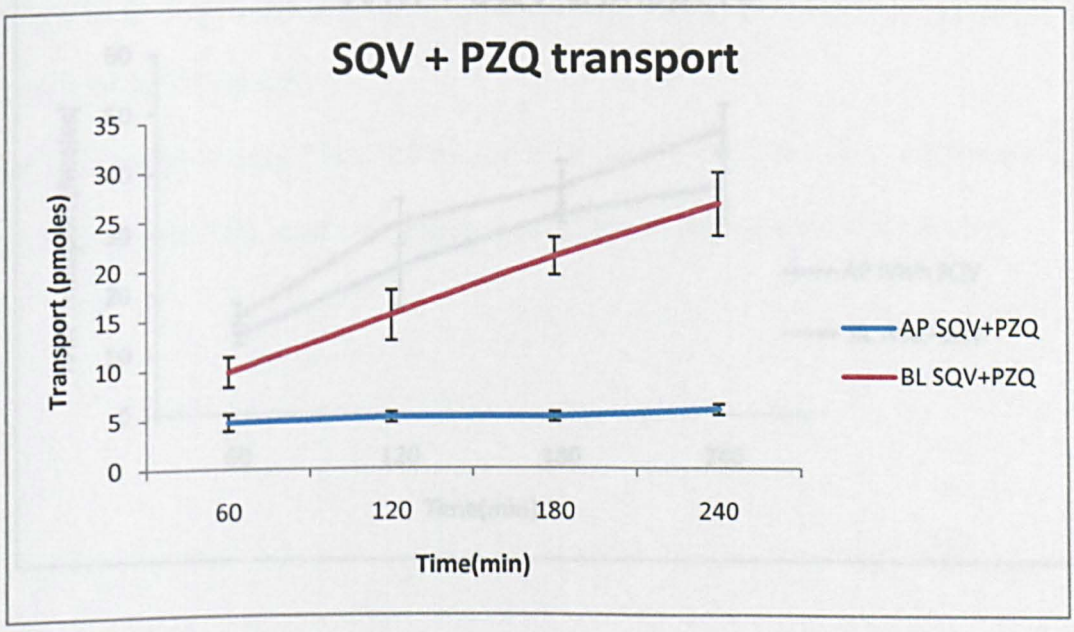
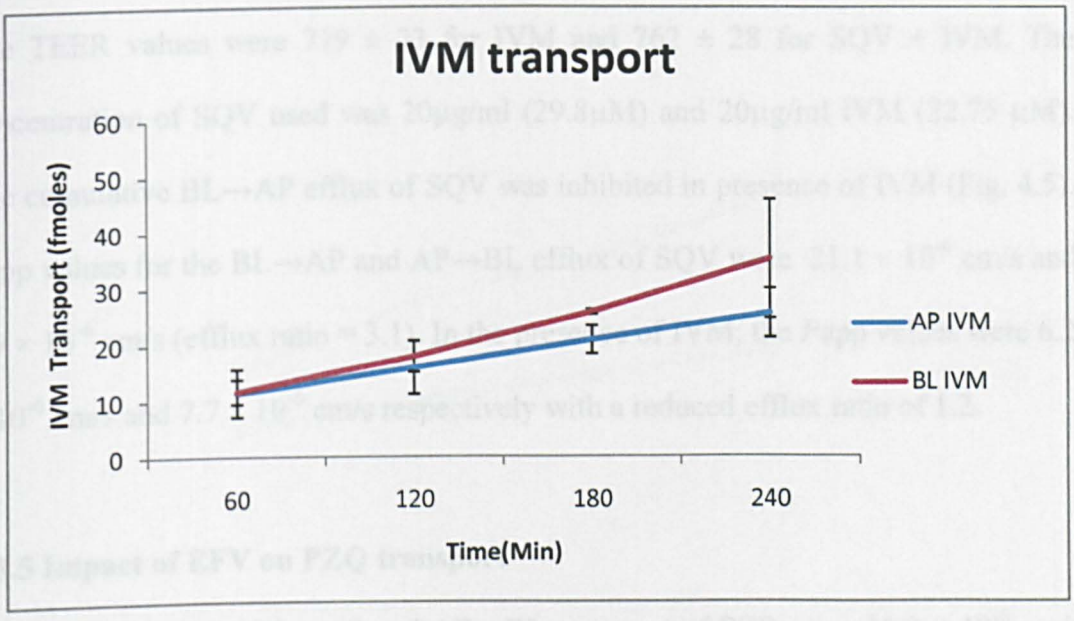


Figure 4.3 Cumulative transport of SQV, a) alone and b) in presence of PZQ. The results are expressed as mean ± s.d (n=3).

(a)



(b)

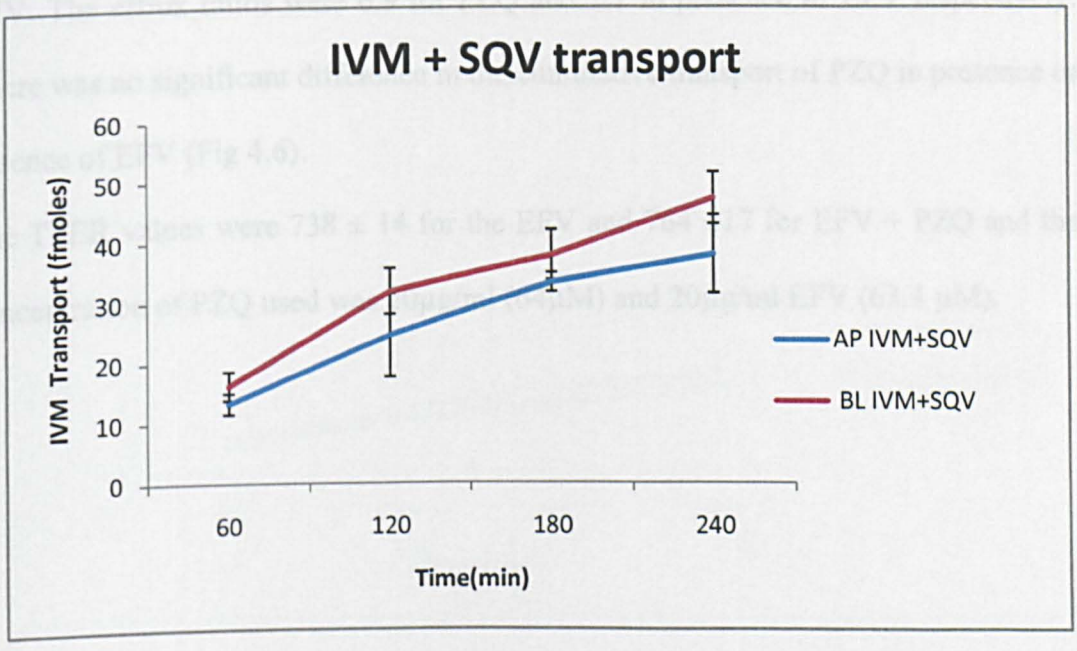


Figure 4.4 Cumulative transport of [^{3}H] IVM, a) alone and b) in presence of SQV. The results are expressed as mean ± s.d (n=3).

4.3.4 Effect of IVM on the transport of SQV

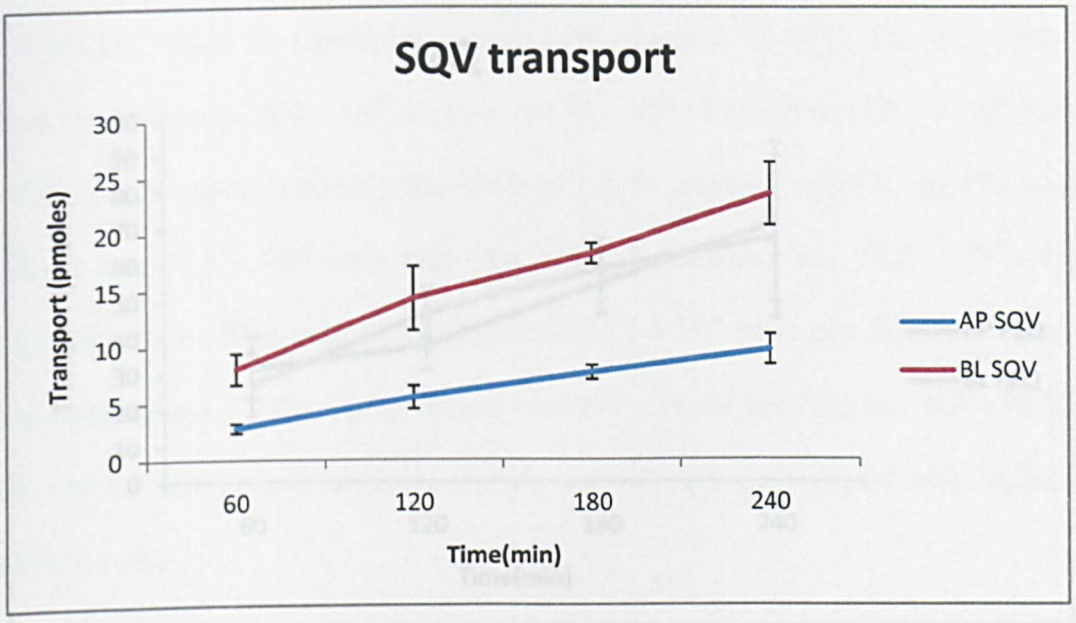
The TEER values were 719 ± 23 for IVM and 762 ± 28 for SQV + IVM. The concentration of SQV used was 20μg/ml (29.8μM) and 20μg/ml IVM (22.75 μM). The cumulative BL→AP efflux of SQV was inhibited in presence of IVM (Fig. 4.5). *P*app values for the BL→AP and AP→BL efflux of SQV were 21.1×10^{-6} cm/s and 6.9×10^{-6} cm/s (efflux ratio = 3.1). In the presence of IVM, the *P*app values were 6.2 $\times 10^{-6}$ cm/s and 7.7×10^{-6} cm/s respectively with a reduced efflux ratio of 1.2.

4.3.5 Impact of EFV on PZQ transport

The *P*app values for BL→AP and AP→BL transport of PZQ were 30.3×10^{-6} cm/s and 35×10^{-6} cm/s alone, and 39.2×10^{-6} cm/s and 35.1×10^{-6} cm/s in presence of EFV. The efflux ratios were 0.9 for PZQ and 1.1 in presence of EFV respectively. There was no significant difference in the cumulative transport of PZQ in presence or absence of EFV (Fig 4.6).

The TEER values were 738 ± 14 for the EFV and 764 ±17 for EFV + PZQ and the concentration of PZQ used was 20μg/ml (64μM) and 20μg/ml EFV (63.4 μM).

(a)



(b)

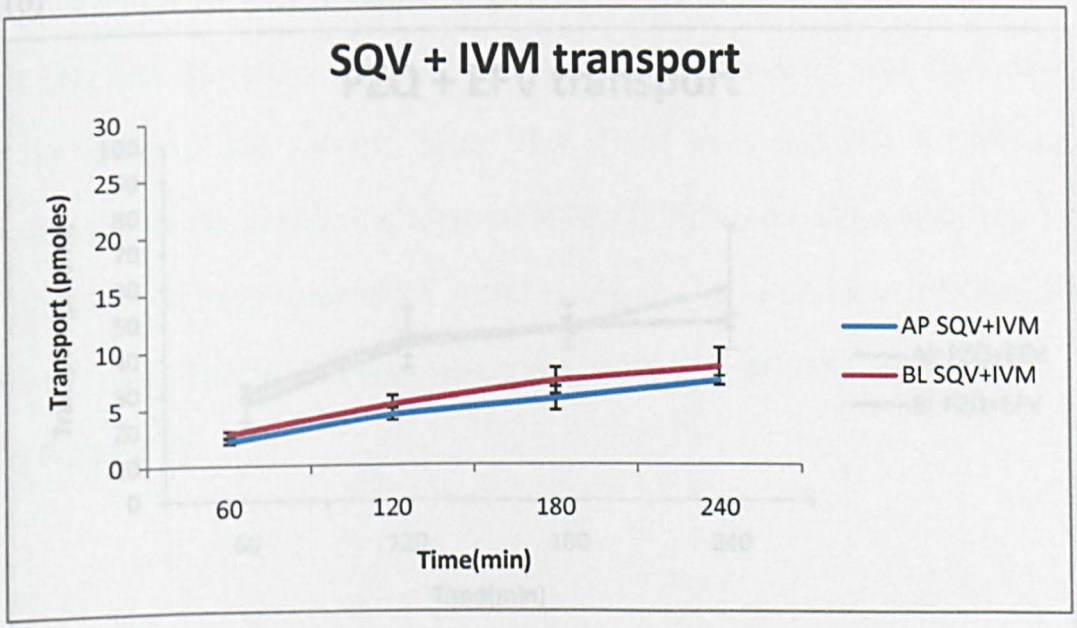
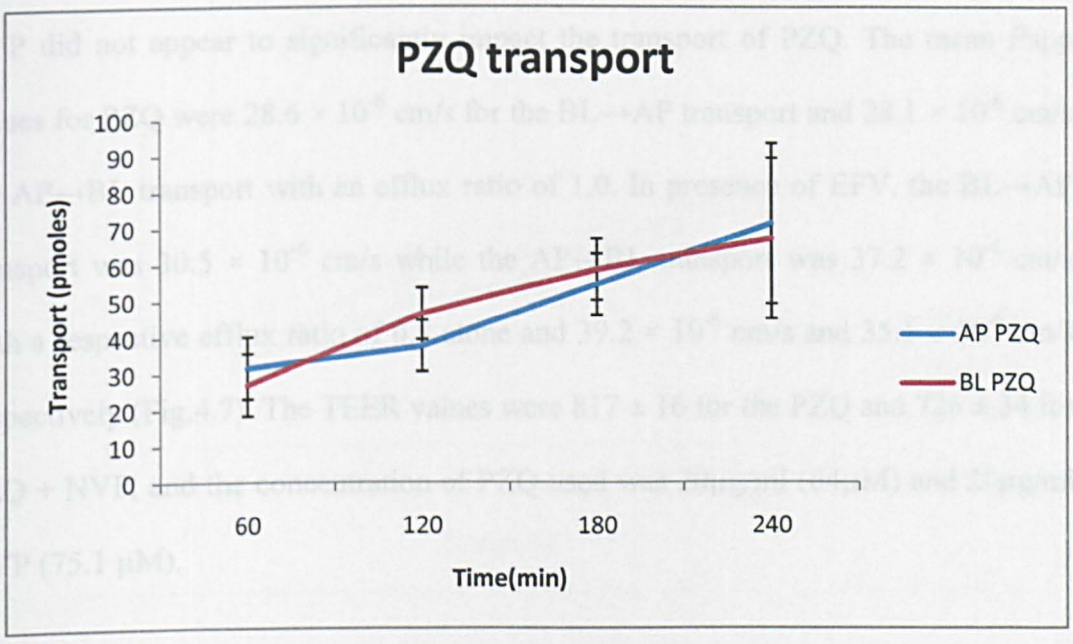


Figure 4.5 Cumulative transport of SQV, a) alone and b) in presence of IVM. The results are expressed as mean ± s.d (n=3).

(a)



(b)

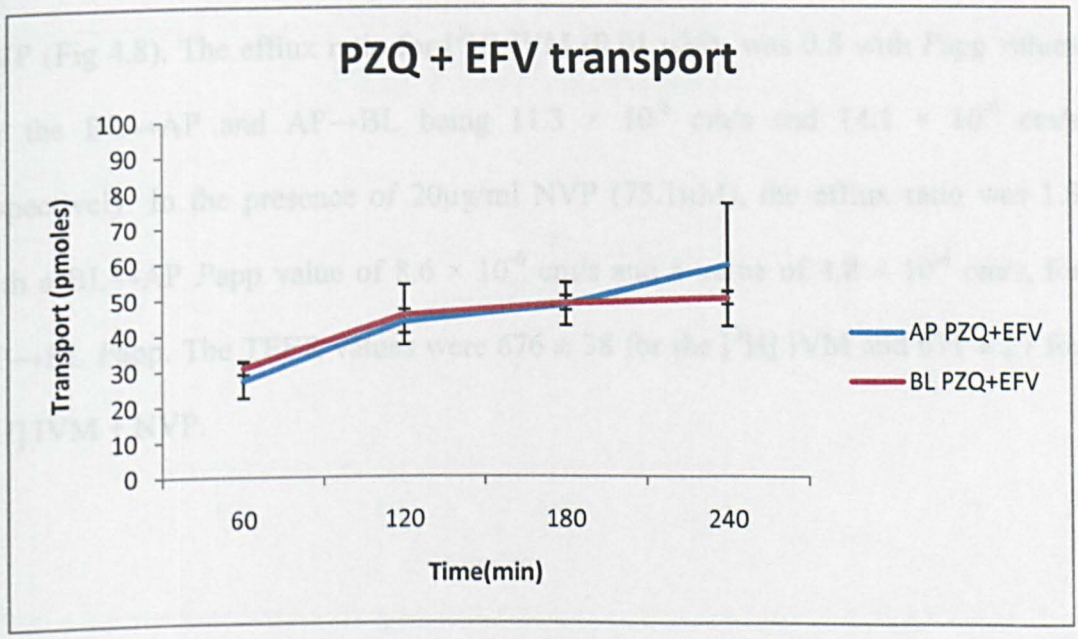


Figure 4.6 Cumulative transport of PZQ, a) alone and b) in presence of EFV. The results are expressed as mean ± s.d (n=3).

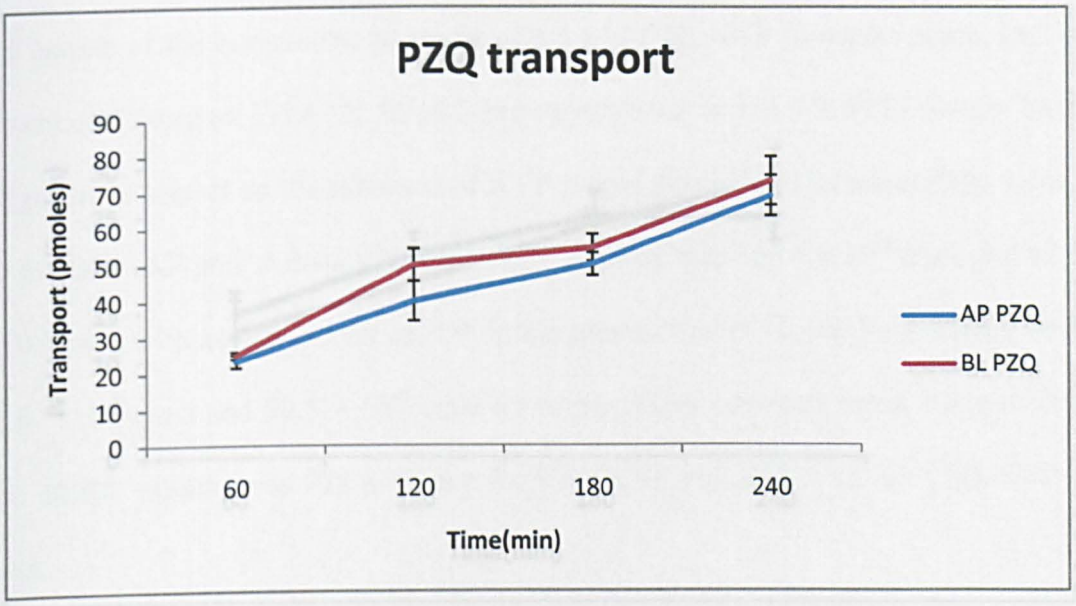
4.3.6 Impact of NVP on PZQ transport

NVP did not appear to significantly impact the transport of PZQ. The mean *P*app values for PZQ were 28.6 × 10^{-6} cm/s for the BL→AP transport and 28.1 × 10^{-6} cm/s for AP→BL transport with an efflux ratio of 1.0. In presence of EFV, the BL→AP transport was 30.5 × 10^{-6} cm/s while the AP→BL transport was 37.2 × 10^{-6} cm/s with a respective efflux ratio of 0.8 alone and 39.2 × 10^{-6} cm/s and 35.1 × 10^{-6} cm/s respectively (Fig.4.7). The TEER values were 817 ± 16 for the PZQ and 726 ± 34 for PZQ + NVP, and the concentration of PZQ used was 20μg/ml (64μM) and 20μg/ml NVP (75.1 μM).

4.3.7 Effect of NVP on IVM transport

The cumulative AP→BL transport of [^{3}H] IVM was inhibited in the presence of NVP (Fig 4.8). The efflux ratio for [^{3}H] IVM (0.01 μM) was 0.8 with *P*app values for the BL→AP and AP→BL being 11.3 × 10^{-6} cm/s and 14.1 × 10^{-6} cm/s respectively. In the presence of 20μg/ml NVP (75.1μM), the efflux ratio was 1.8 with a BL→AP *P*app value of 8.6 × 10^{-6} cm/s and a value of 4.8 × 10^{-6} cm/s, for AP→BL *P*app. The TEER values were 676 ± 38 for the [^{3}H] IVM and 671 ± 27 for [^{3}H] IVM + NVP.

(a)



(b)

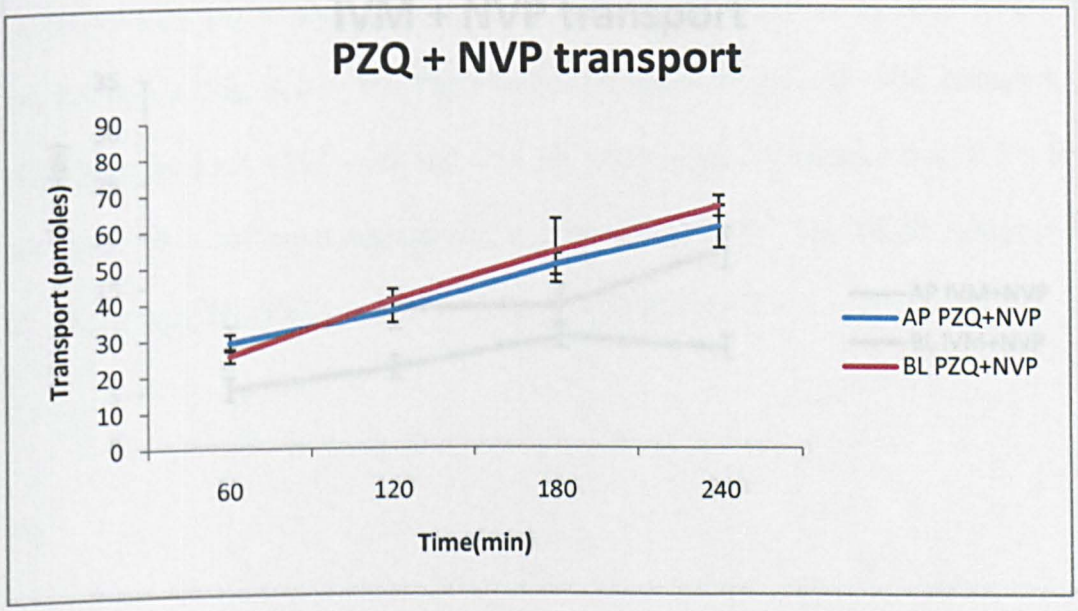
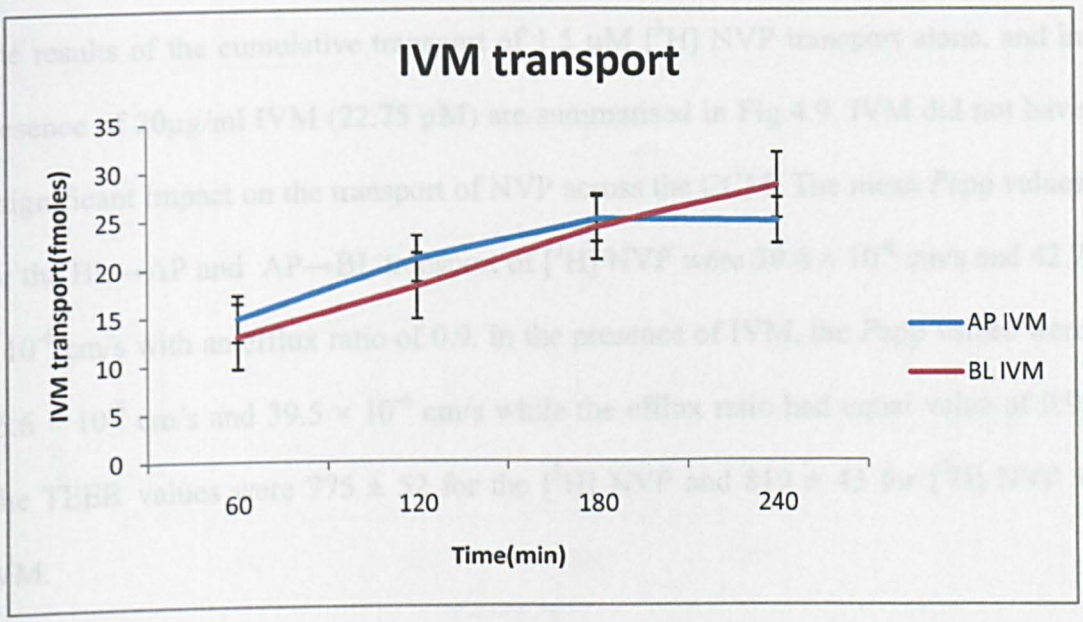


Figure 4.7 Cumulative transport of PZQ, a) alone and b) in presence of NVP. The results are expressed as mean ± s.d (n=3).

(a)



(b)

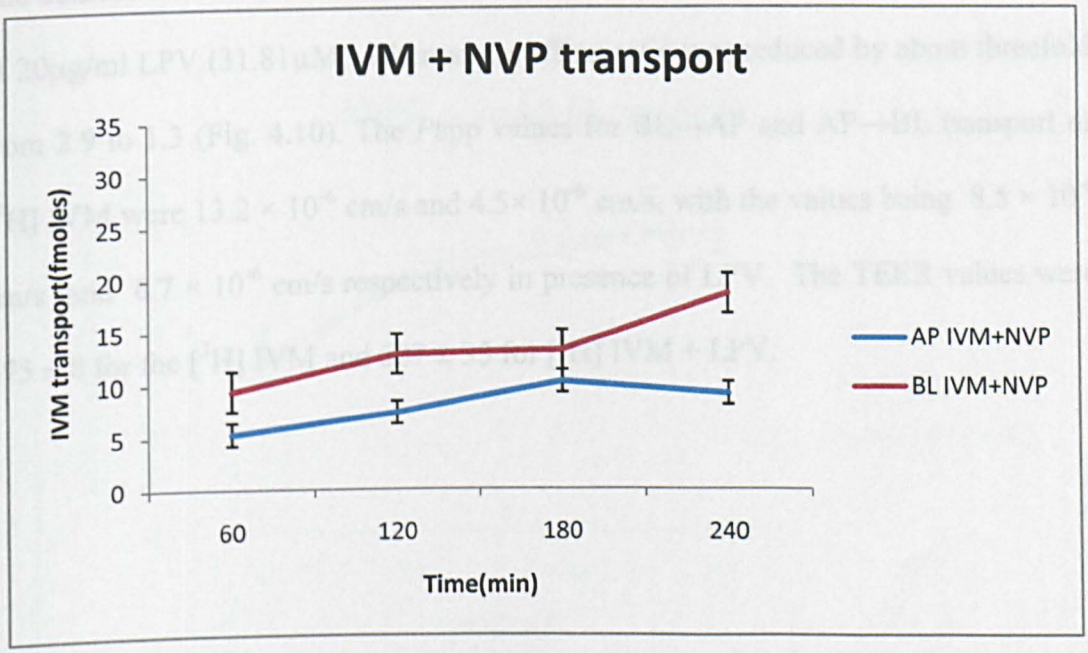


Figure 4.8 Cumulative transport of [^{3}H] IVM, a) alone and in b) presence of NVP. The results are expressed as mean ± s.d (n=3).

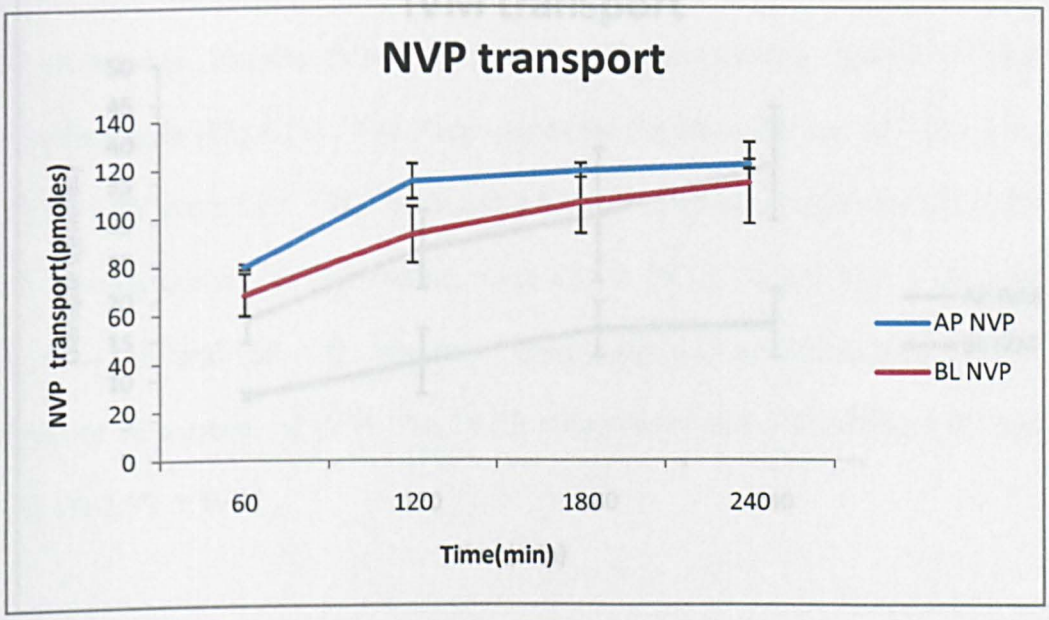
4.3.8 Effect of IVM on NVP transport

The results of the cumulative transport of 1.5 μM [^{3}H] NVP transport alone, and in presence of 20μg/ml IVM (22.75 μM) are summarised in Fig 4.9. IVM did not have a significant impact on the transport of NVP across the CCM. The mean *P*app values for the BL→AP and AP→BL transport of [^{3}H] NVP were 39.4 × 10^{-6} cm/s and 42.7 × 10^{-6} cm/s with an efflux ratio of 0.9. In the presence of IVM, the *P*app values were 36.6 × 10^{-6} cm/s and 39.5 × 10^{-6} cm/s while the efflux ratio had equal value of 0.9. The TEER values were 775 ± 52 for the [^{3}H] NVP and 819 ± 43 for [^{3}H] NVP + IVM.

4.3.9 Impact of LPV on IVM transport

The cumulative AP→BL transport of [^{3}H] IVM (0.02 μM) increased in the presence of 20μg/ml LPV (31.81μM), whereas the efflux ratio was reduced by about threefold from 2.9 to 1.3 (Fig. 4.10). The *P*app values for BL→AP and AP→BL transport of [^{3}H] IVM were 13.2 × 10^{-6} cm/s and 4.5× 10^{-6} cm/s, with the values being 8.5 × 10^{-6} cm/s and 6.7 × 10^{-6} cm/s respectively in presence of LPV. The TEER values were 675 ± 8 for the [^{3}H] IVM and 607 ± 35 for [^{3}H] IVM + LPV.

(a)



(b)

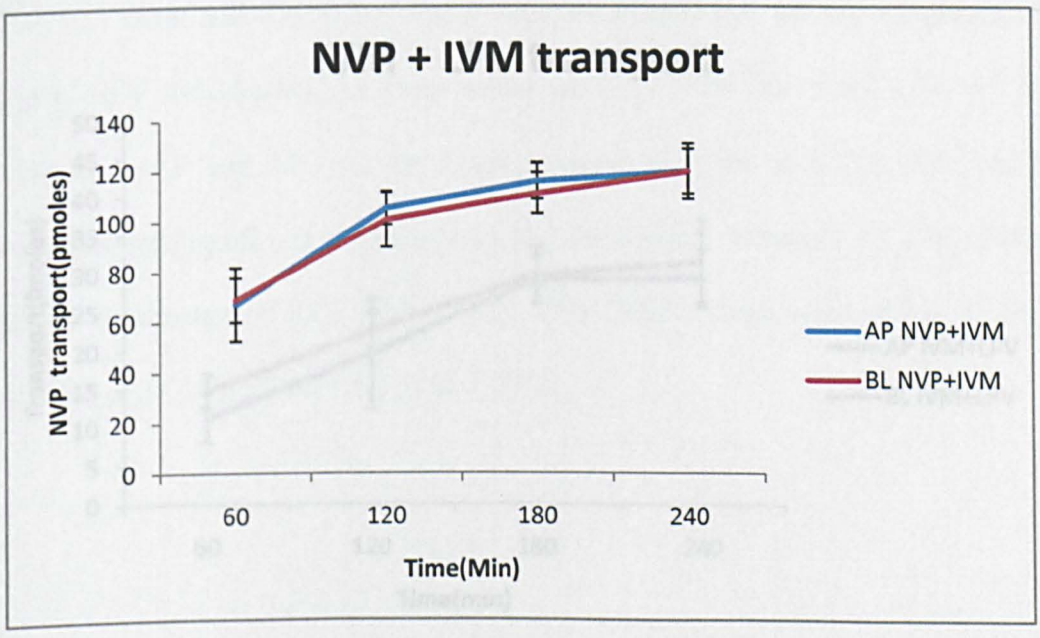
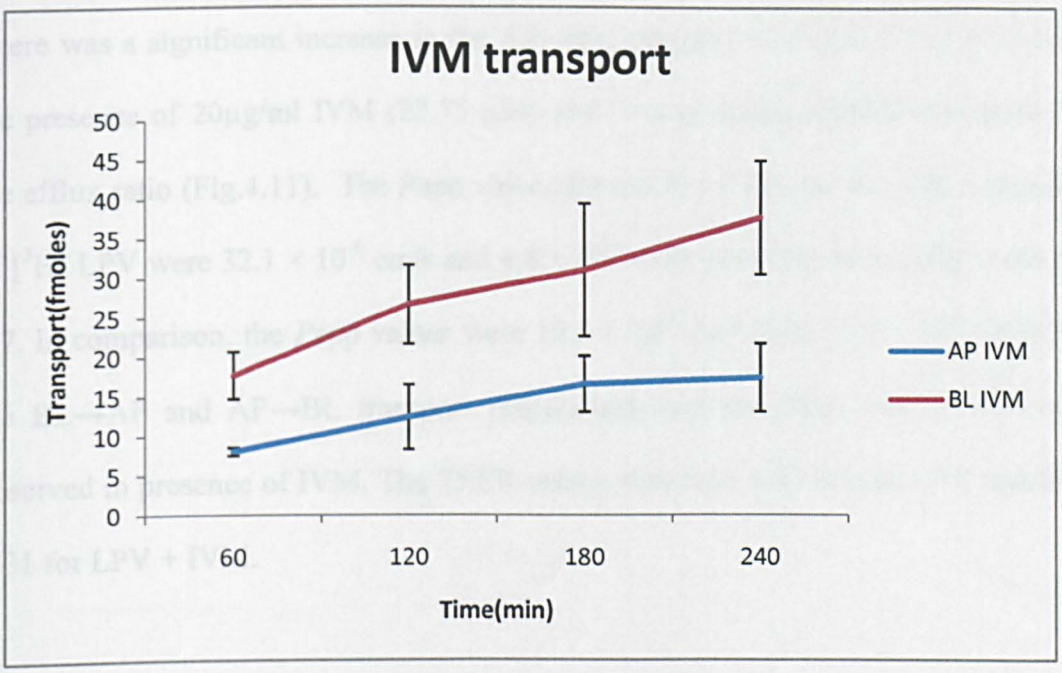


Figure 4.9 Cumulative transport of [^{3}H] NVP, a) alone and b) in presence of IVM. The results are expressed as mean ± s.d (n=3).

(a)



(b)

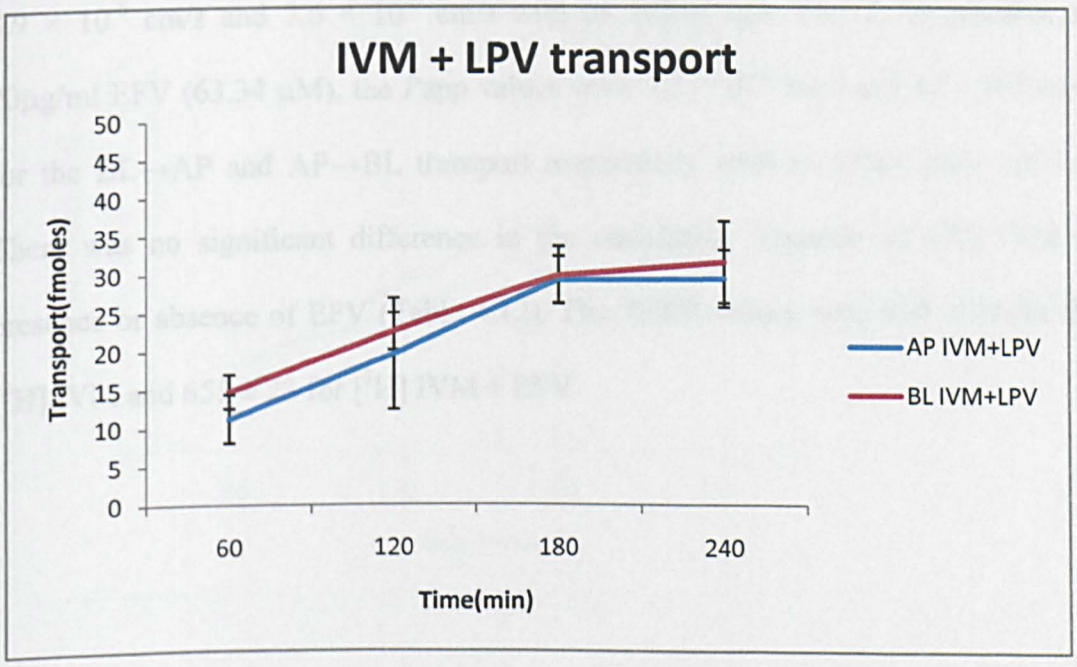


Figure 4.10 Cumulative transport of [^{3}H] IVM, a) alone and b) in presence of LPV. The results are expressed as mean ± s.d (n=3).

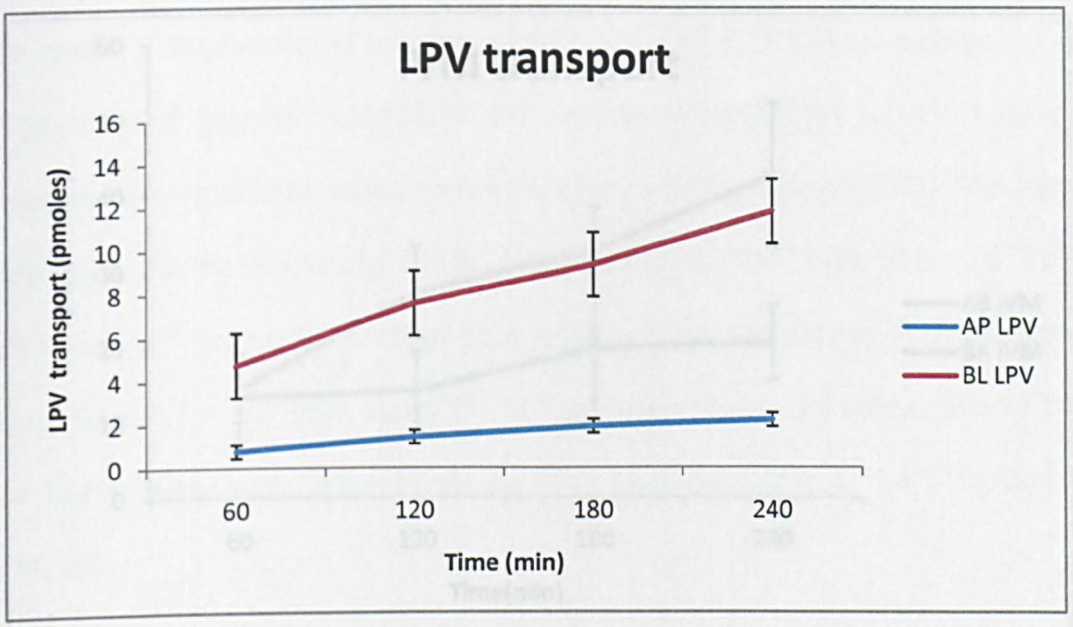
4.3.10 Impact of IVM on LPV transport

There was a significant increase in the AP→BL transport of [^{3}H] LPV (1.5 μM) in the presence of 20μg/ml IVM (22.75 μM) and corresponding eightfold decrease in the efflux ratio (Fig.4.11). The *P*app values for the BL→AP and AP→BL transport of [^{3}H] LPV were 32.1 × 10^{-6} cm/s and 4.8 × 10^{-6} cm/s resulting in an efflux ratio of 6.7. In comparison, the *P*app values were 12.3 × 10^{-6} cm/s and 15.9 × 10^{-6} cm/s for the BL→AP and AP→BL transport respectively and an efflux ratio of 0.8 was observed in presence of IVM. The TEER values were 654 ± 33 for the LPV and 635 ± 31 for LPV + IVM.

4.3.11 Impact of EFV on IVM transport

The *P*app values for the BL-AP and AP-BL transport of [^{3}H] IVM (0.01 μM) were 8.9 × 10^{-6} cm/s and 7.6 × 10^{-6} cm/s with an efflux ratio of 1.2. In presence of 20μg/ml EFV (63.34 μM), the *P*app values were 7.3 × 10^{-6} cm/s and 4.7× 10^{-6} cm/s for the BL→AP and AP→BL transport respectively with an efflux ratio of 1.6. There was no significant difference in the cumulative transport of [^{3}H] IVM in presence or absence of EFV (Table 4.12). The TEER values were 650 ± 20 for the [^{3}H] IVM and 655 ± 23 for [^{3}H] IVM + EFV.

(a)



(b)

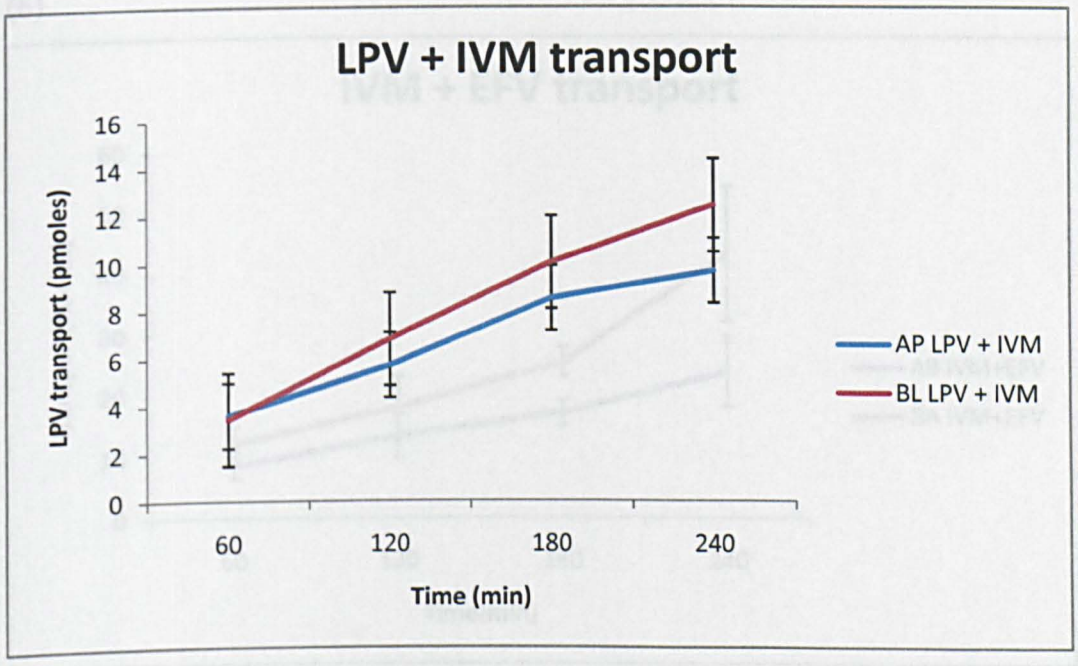
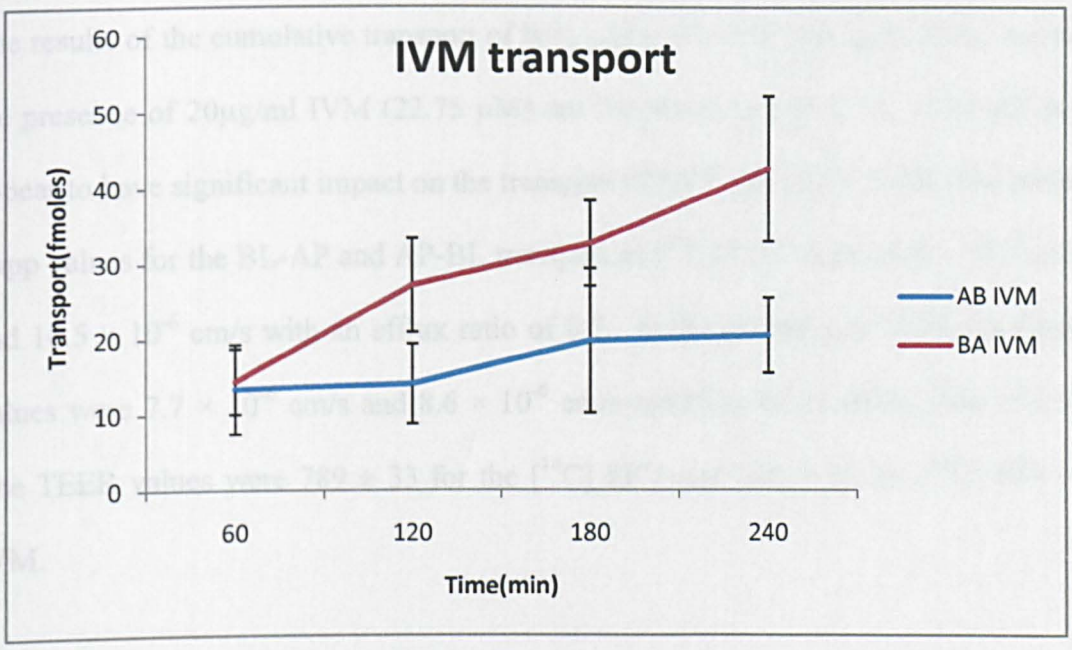


Figure 4.11 Cumulative transports of [^{3}H] LPV, a) alone and b) in presence of IVM. The results are expressed as mean ± s.d (n=3).

(a)



(b)

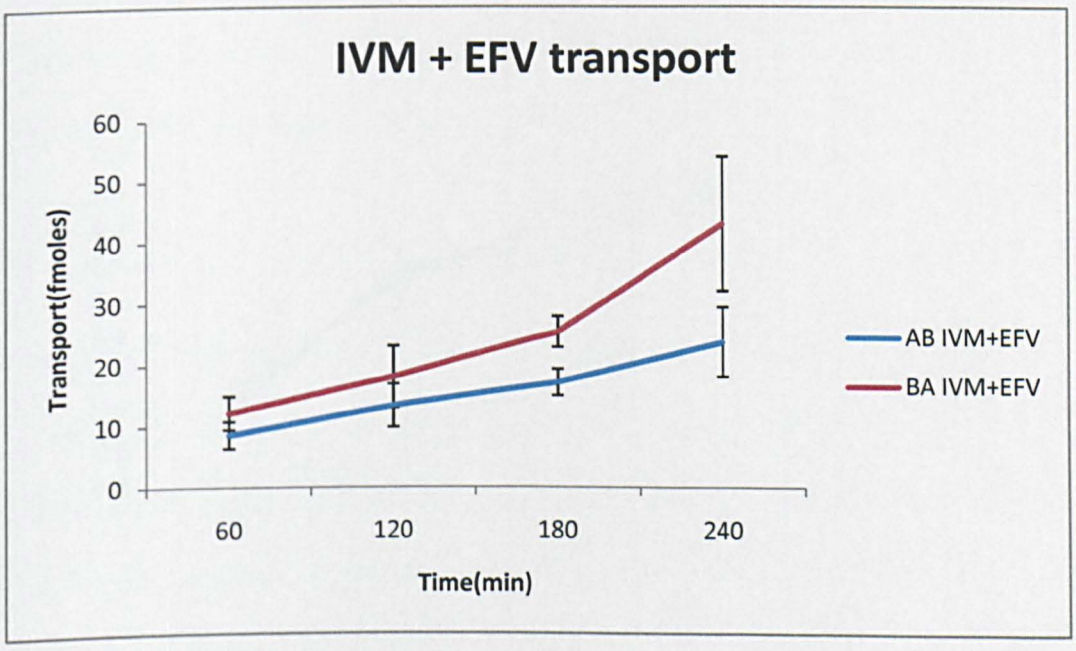
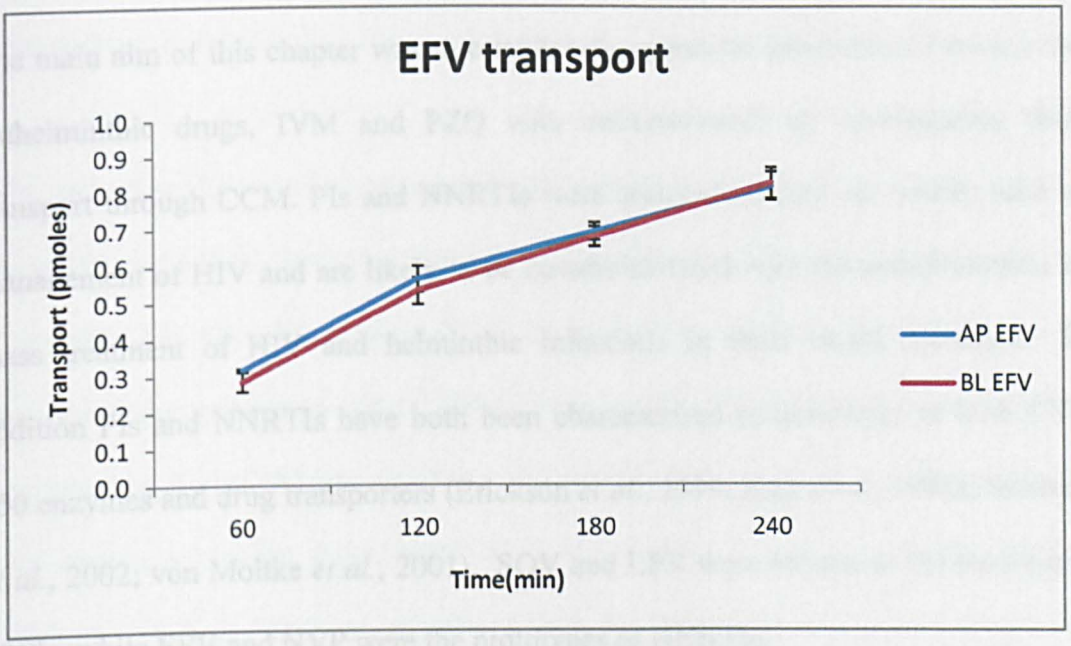


Figure 4.12 Cumulative transport of [^{3}H] IVM, a) alone and b) in presence of EFV. The results are expressed as mean ± s.d (n=3).

4.3.12 Impact of IVM on EFV transport

The results of the cumulative transport of 0.01 μM [^{14}C] EFV transport alone, and in the presence of 20μg/ml IVM (22.75 μM) are illustrated in Fig 4.13. IVM did not appear to have significant impact on the transport of EVF across the CCM. The mean *P*app values for the BL-AP and AP-BL transport of [^{14}C] EFV were 10.8 × 10^{-6} cm/s and 16.5 × 10^{-6} cm/s with an efflux ratio of 0.7. In the presence of IVM, the *P*app values were 7.7 × 10^{-6} cm/s and 8.6 × 10^{-6} cm/s resulting in an efflux ratio of 0.9. The TEER values were 789 ± 33 for the [^{14}C] EFV and 738 ± 42 for [^{14}C] EFV + IVM.

(a)



(b)

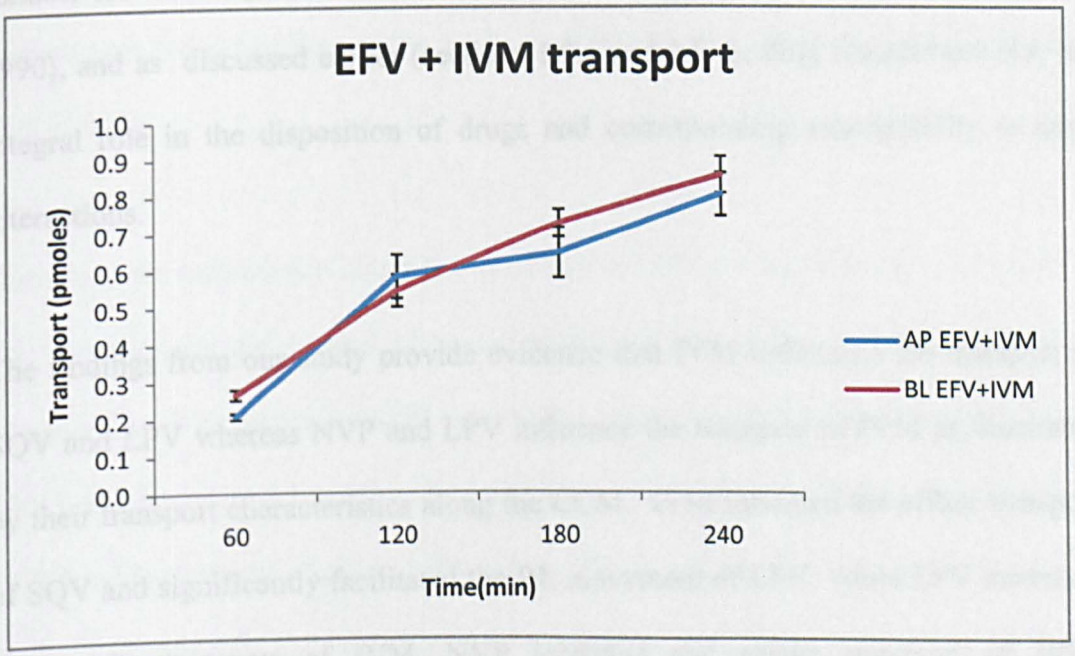


Figure 4.13 Cumulative transport of [^{14}C] EFV, a) alone and b) in presence IVM. The results are expressed as mean ± s.d (n=3).

4.4 Discussion

The main aim of this chapter was to establish the potential interactions between the anthelminthic drugs, IVM and PZQ with antiretrovirals by investigating their transport through CCM. PIs and NNRTIs were selected as they are widely used in management of HIV and are likely to be co-administered with the anthelminthics in mass treatment of HIV and helminthic infections in third world countries. In addition PIs and NNRTIs have both been characterised as substrates of both CYP 450 enzymes and drug transporters (Erickson *et al.*, 1999; Kim *et al.*, 1998a; Stormer *et al.*, 2002; von Moltke *et al.*, 2001). SQV and LPV were chosen as the prototypes of PIs, while EFV and NVP were the prototypes of NNRTIs.

The CCM as outlined earlier express a wide range of transporters making them suitable for the study of drug-drug interactions (Hidalgo *et al.*, 1989; Hilgers *et al.*, 1990), and as discussed earlier (sections 1.9.5 and 1.9.6), drug transporters play an integral role in the disposition of drugs and corresponding susceptibility to drug interactions.

The findings from our study provide evidence that IVM influences the transport of SQV and LPV whereas NVP and LPV influence the transport of IVM as illustrated by their transport characteristics along the CCM. IVM inhibited the efflux transport of SQV and significantly facilitated the BL movement of LPV, while LPV increased the uptake transport of IVM. NVP inhibited the uptake transport of IVM. Conversely, SQV did not appear to influence the transport of IVM. PZQ did not appear to significantly influence the transport of ARVs and the ARVs did not affect the transport of PZQ. IVM inhibited the BL→AP (efflux) transport of SQV by a

threefold decrease of the efflux ratio, raising the possibility of an interaction between the two drugs involving drug transporters.

SQV and other PIs have been demonstrated to be substrates of efflux transporters P-gp, MRP1 and MRP2 that are expressed by Caco-2 cells (Kim *et al.*, 1998a; Profit *et al.*, 1999). We also did confirm SQV as a substrate of P-gp, from our cellular accumulation experiments using CEM parental and CEM_{VBL} cells which overexpress P-gp (section 3.3). IVM has also been characterized as a substrate of P-gp and has been shown to inhibit P-gp, MRP 1, 2 and 3 (Lespine *et al.*, 2006). Altered expression of P-gp has been associated with the neurotoxicity of IVM (Edwards, 2003). In experiments on collie dogs, the dogs that have a deletion of ABCB-1 gene display neurotoxicity when dosed with IVM whereas normal dogs do not. The authors concluded that P-gp plays a role in effluxing the IVM from the CNS(Mealey *et al.*, 2001).

There is scant information about the interactions between IVM and other drugs from the literature. In the control of onchoerciasis, doxycycline has been reported to enhance the ivermectin-induced suppression of microfiladermia (Hoerauf *et al.*, 2001), and levamisole has been shown to increase the plasma bioavailability of IVM though without increasing its antiparasitory effects (Awadzi *et al.*, 2004). In a recent study, ketoconazole substantially increased IVM plasma concentrations in sheep upon co-administration and this was attributed to the reversal of P-gp effects by the authors (Alvinerie *et al.*, 2008). P-gp modulators itraconazole and valspodar have also been shown to increase the concentration of IVM in plasma and gastrointestinal tissues of rats (Ballent *et al.*, 2006).

There was a significant increase in the AP→BL (influx) transport of LPV in the presence of IVM, suggesting that IVM facilitated the uptake of LPV accompanied by a corresponding eightfold decrease in the efflux ratio. The facilitated absorption could possibly have resulted from inhibition of P-gp or other LPV transporters. The transport of LPV and other PIs has been shown to be modulated by efflux transporters P-gp and MRP (Agarwal *et al.*, 2007; Gimenez *et al.*, 2004). Whereas studies have demonstrated that these efflux transporters limit the uptake of ARVs, the influence of influx transporters such as OATP on their modulation and indeed most drugs has not been fully elucidated.

A recent study concluded that interplay of influx transporter (OATP), efflux transporters (P-gp and MRP) and lipophilicity had implications on the cellular uptake and retention of SQV and LPV in some T-cell lines, CEM, CEM_{VBL} and CEM_{1000} as well as peripheral blood mononuclear cells [PBMCs] (Janneh *et al.*, 2008). In this study, the pre-treatment of the cells with P-gp and MRP inhibitors, tariquidar (XR9576) for P-gp and MK571 with frusemide for MRP respectively; followed by subsequent co-incubation with a human OATP substrate, estrone-3-sulphate (E-3-S) resulted in a reduction of the cellular accumulation of SQV and LPV. This may indicate the involvement of OATP in the effect IVM on the disposition of LPV. It is worth noting that LPV is more lipophilic than SQV, which may contribute to the difference in response to IVM between the two PIs.

A related study concluded that, IVM may influence the absorption of fexofenadine by interfering with influx and efflux pumps OATP and P-gp (Olsen *et al.*, 2006). Our study also revealed that LPV facilitated the influx transport of IVM, though to a

lesser degree. There was an increase in the AP→BL transport of IVM in presence of LPV accompanied by a threefold decrease in the efflux ratio. It is therefore evident from these findings that there is likelihood of interactions between LPV and IVM, and that these interactions are most likely influenced by drug transporters (influx and efflux). Further work should be carried out to determine the specific transporters responsible for the interactions, and the range of the dosage that would exhibit these interactions.

NVP inhibited the influx transport of IVM, with the cumulative AP→BL transport of IVM reduced in the presence of NVP, and the calculated efflux ratio was doubled. In contrast, NVP did not appear to influence the transport of IVM. In a study to investigate the influence of NNRTIs on P-gp activity, NVP significantly reduced the uptake of rhodamine 123, a P-gp substrate into LS180V cells signifying decreased efflux as a result of inhibition of transport (Stormer *et al.*, 2002). In a related study, Weiss concluded that NNRTIs induced P-gp in LS 180 cells (Weiss *et al.*, 2007b; Weiss *et al.*, 2008). The observed interactions between NVP and IVM may therefore be attributed to the activities of influx and efflux drug transporters. With regards to the interactions between IVM and EFV, there were no significant interactions observed. The cumulative transport of IVM was unaffected in presence of EFV. Likewise the transport of EFV remained fairly constant in presence of IVM. To date there is no conclusive data with respect to substrate specificity of EFV for drug transporters. However, EFV has been reported to decrease plasma concentrations of co-administered drugs that are metabolized by CYP 450 enzymes without modifying intestinal absorption of co-administered substrates of P-gp (Berruet *et al.*, 2005).

PZQ did not influence the transport of ARVs and equally ARVs did not significantly affect the transport of PZQ. The presence of PZQ did not alter the transport of SQV, whereas SQV, EFV and NVP did not affect the transport of PZQ. This is consistent with our earlier findings whereby we established that PZQ is neither a substrate nor an inhibitor of P-gp in our accumulation experiments involving CEM parental and CEMVBL cells (section 3.3). PZQ has not been fully characterised in relationship to drug transporters and metabolic enzymes. In fact, its mechanism of action is also not very clear. A recent publication hypothesizes that it is an adenosine antagonist, and that it inhibits nucleoside uptake in schistosomes leading to calcium influx and subsequent muscular contraction without having any effect on mammalian cells (Angelucci *et al.*, 2007). However, there is also evidence that the molecular target of PZQ are Ca^{2+} ions and that the efficacy is due to spastic paralysis and vacuolisation probably due to increased influx of the ions (Doenhoff *et al.*, 2008; Greenberg, 2005b; Mehlhorn *et al.*, 1981).

In a study involving the transport of PZQ and other antiparasitic drugs along Caco-2 cell monolayers, PZQ appeared to be an inhibitor of P-gp without being a substrate, based on inhibition of Pgp mediated [^{3}H]-taxol transport in Caco-2 cells (Hayeshi *et al.*, 2006). Ketoconazole, a CYP-450 inhibitor has been reported to double the plasma concentration of PZQ in humans, while rifampicin an inducer has been reported to dramatically reduce its concentration, and the authors have recommended dose adjustment upon co-administration (Ridtitid *et al.*, 2007; Ridtitid *et al.*, 2002).

In summary, these studies provide evidence that there may be interactions between ARVs and anthelminthics, and that IVM interacts with LPV, SQV and NVP. Further

experiments should be conducted to investigate the possibility of in vivo interactions. From a clinical perspective, the co-administration of IVM with these drugs may require dose adjustments to reduce the incidences of drug-drug interactions. However, this requires confirmation in clinical studies.

Chapter 5

Induction of ABCB1, CYP2B6 and CYP3A4 genes in Caco-2 cell culture by the anthelminthics, praziquantel (PZQ) and ivermectin (IVM)

Chapter 5

Induction of ABCB1, CYP2B6 and CYP3A4 genes in Caco-2 cell culture by the anthelminthics, praziquantel (PZQ) and ivermectin (IVM)

5.1 Introduction

P-glycoprotein (ABCB1), an efflux protein limits the bioavailability of drugs by lowering intracellular drug levels (Sparreboom *et al.*, 1997). P-gp is expressed on the apical surface of normal epithelial and endothelial cells of the intestine, liver, kidney, brain and the placenta whereby it has role in modulating the pharmacokinetics of substrate drugs (Thiebaut *et al.*, 1987). It belongs to the ATP-binding cassette (ABC) family (Gottesman *et al.*, 1993) and in humans it is encoded by the ABCB1 gene (Schinkel *et al.*, 1994). The induction or inhibition of P-gp therefore alters the absorption and distribution of the drugs and has a role in drug interactions with co-administered drugs (Collett *et al.*, 2004b; de Maat *et al.*, 2003; Perloff *et al.*, 2005).

Cytochrome P450 (CYP 450) enzymes are expressed in the same anatomical areas as drug transporters predominantly in the liver, intestines and kidney and are responsible for the metabolism of several drugs (Guengerich, 1999). They are also involved in drug-drug interactions (Thummel *et al.*, 1998). Many drugs are substrates of both enzymes and drug transporters, and because of the co-localization there exists an overlap of substrate specificity and functional interplay (Benet *et al.*, 2004; Zhang *et al.*, 1998). They may also act synergistically in reducing the bioavailability of orally administered drugs by promoting the efflux and metabolism (Chiou *et al.*, 2000; Perloff *et al.*, 2005). Many drugs are capable of inducing CYP enzymes especially CYP 3A4 (Guengerich, 1999) and P-gp (Matheny *et al.*, 2001). The induction of the enzymes or drug transporters will therefore have an impact on the pharmacokinetics of drugs that are substrates of either or both (Matheny *et al.*, 2001; Perloff *et al.*, 2005).

From our earlier experiments described in section 3.3 on the intracellular accumulation in CEM and CEMVBL, we demonstrated that PZQ is neither a substrate nor an inhibitor of P-gp. The results from the interactions between anthelminthics and ARVs based on the transport along the Caco-2 cell monolayers (CCM) described in the previous chapter have shown that PZQ does not interact with SQV, NVP or EFV transport. IVM interacted with SQV, NVP and LPV. PZQ has not been fully characterized with regards to drug transporters though some authors concluded that it is an inhibitor of P-gp without being a substrate of P-gp based on the transport along the CCM (Hayeshi *et al.*, 2006). In two related studies, there was a dramatic reduction in plasma concentrations of PZQ when co-administered with rifampicin and marked increase when co-administered with ketoconazole. The authors concluded that it was due to the induction of CYP3A4 by rifampicin and inhibition by ketoconazole (Ridtitid *et al.*, 2007; Ridtitid *et al.*, 2002), since PZQ is metabolised by CYP3A4. IVM has been characterized as a substrate and inhibitor of P-gp (Brayden *et al.*, 2008; Lespine *et al.*, 2006; Pouliot *et al.*, 1997). However, to date there is limited information relating to the induction of P-gp and metabolic enzymes by both drugs despite their widespread use.

The aim of the experimental work described in this chapter was to investigate whether PZQ and IVM are capable of inducing P-gp, CYP3A4 and CYP2B6 genes in Caco-2 cells. Rifampicin (RIF) was used as positive control for CYP3A4 and ABCB1, while Phenobarbital (PB) was used for CYP2B6. Caco-2 cells are suitable for prediction of the *in vivo* bioavailability of orally administered drugs (Collett *et al.*, 2004a; Cummins *et al.*, 2004). In addition, they may be used to evaluate the induction of the transporters and the enzymes (Collett *et al.*, 2004b; Huang *et al.*,

2001; Vermeir *et al.*, 2005). The analysis of the PCR results data was done by comparative C_T method (Schmittgen *et al.*, 2008).

5.2 Materials and methods

5.2.1 Materials

Caco-2 cells were purchased from the European collection of cell cultures (ECACC No. 286010202), and the cells were counted using a Nucleo Counter (ChemoMetec, Denmark) cell counter. Reverse transcription and real time components, PCR buffer (100mM Tris-Hcl, pH [8.3], 500mM KCl, 15mM $MgCl_2$, 0.01% [w/v] gelatine), 25mM $MgCl_2$, 10mM dNTP Mix, 50µM Random Hexamers, 40/µL RNAse Inhibitor, 50u/µL RT scribe, 18µM each of GAPDH, CYP2B6, CYP3A4, ABCB1 primers and 5µM probes were purchased from Applied Biosystems (Warrington, Cheshire, UK). ABsolute® QPCR Mix was supplied by Thermo Scientific (Epsom, Surrey). TRIzol® reagent was obtained from Gibco Life Technologies Ltd (Paisley, UK). PZQ, IVM, DMEM, RPMI, HBSS, FBS, DMSO, Trypsin-EDTA solution Nuclease-free water and Thiazolyl Blue Tetrazolium and Chloroform were purchased from Sigma Aldrich (Poole, UK). Isopropyl alcohol was purchased from Fisher Scientific, (Loughborough, UK).

5.2.2 Cell culture

The cells were cultured in DMEM supplemented with foetal bovine serum (15%) as described in section 4.2.3.1. They were incubated at 37°C and 10% CO_2 in a humidified chamber with media change after every 2-3 days until confluence was achieved.

5.2.3 Toxicity

An assay for the toxicity of PZQ and IVM in Caco-2 cells was conducted by the methyl thiazolyl diphenyl-tetrazolium bromide (MTT) assay to determine cell death (Mosmann, 1983) at concentration range of 0.01 μM to 100 μM. 1 ml solutions of IVM and PZQ (20mM) were prepared by dissolving in DMSO (vehicle). Serial dilution in DMSO (1:10) was then performed to obtain the concentration points of 2mM, 0.2mM, 0.02mM, 0.002mM and DMSO alone, the last point consisting of cells alone. The test solutions were then prepared by diluting in DMEM keeping the DMSO concentration at 0.5%. 1ml of the top point, 100μM was prepared by adding 5μl of the 20mM to 995μl of DMEM. The rest of the concentration points 10, 1, 0.1 and 0.01μM were prepared by the addition of 5μl of the next drug concentrations (2 - 0.002mM) to 995μl of DMEM. The concentration of vehicle was prepared by the addition of 5μl of DMSO to 995 μl of DMEM.

Two sets of samples of each drug were prepared in quadruplicate, one for a 24 hr and the second for a five-day assay. After trypsinization, the pellets from the Caco-2 cells (passage 38) were re-suspended in DMEM and seeded at a density of 1×10^6 cells per ml in DMEM. 50,000 cells (50 μl) were then transferred into each well in a 96-well flat-bottomed Nunclon® plate (Rosklide, Denmark) and the cells were incubated for 24 hrs at 37°C and 10% CO_2 in a humidified chamber, one plate for each drug. After 24 hours, the media was aspirated out of each well and replaced with 50 μl aliquots of DMEM containing the various drug concentrations. This was followed by the addition of 20 μl of MTT solution into each well and the plates incubated for a further 24 hrs (MTT assay solution was prepared by the adding 5mg of MTT to 1 ml of HBSS). 100 μl of lysis buffer (50% dimethylformaldehyde v/v

and 20% SDS) was then added into each well followed by incubation for a further two hours. Quantification of the surviving cells was then done by reading at 560 nm on a Tecan® multiwell scanning spectrophotometer. The toxicity of the second set of samples was assessed after incubation for a period of five days (Mosmann, 1983).

5.2.4 Treatment of Caco-2 cells for induction experiments

Caco-2 cells at passage number 39 were seeded at 1×10^6 cells per ml in DMEM supplemented with 15% FBS, into 50 ml sterile tubes. The cells were then aspirated into appropriately labelled wells in a six-well Nunclon® plate (3ml per well) and the plates were then incubated for 24 hours. After 24 hours, 100 µl of pre-prepared various drug concentrations (0-10µM) of the two drugs and 10µM of a positive controls were added to the wells. The positive controls used were rifampicin (RIF) and phenobarbitol (PB). 100 µM concentrations for both drugs were not used as it was toxic for both from the MTT results. This was followed by a further incubation of 24 hours after which the cells were then washed twice with 1 ml HBSS per well. 1 ml of TRIzol® was then added to each well and the cells were aspirated to 2ml labelled eppendorf tubes and stored at -80°C.

5.2.5 Isolation of total RNA

Isolation of total RNA and synthesis of cDNA was conducted as described previously (Owen *et al.*, 2004a; Owen *et al.*, 2004b). The samples were left to thaw at room temperature and the cells were lysed by repetitive pipetting. 200 µl of chloroform per 1ml of TRIzol® was then added to each tube followed by vigorous shaking for 15 seconds and centrifuging at 12,000 *g* for 15 mins at 2-8°C. The colourless upper aqueous phase was then carefully transferred to a new 2 ml

eppendorf and 500 µl of isopropyl alcohol added to each tube in order to precipitate the total RNA. The tubes were then incubated at 15 to 30°C for 10 mins and centrifuged at 12,000 *g* for 10 mins at 4°C. The supernatant was discarded and the resulting RNA pellet was washed in 1ml of ethanol [(75 % (v/v)], vortexed and centrifuged at 7500 g for 5 mins at 2-8°C. The supernatant was removed (as much as possible) and the pellet was air dried for 5 min (taking precaution that it was not over dried). It was then dissolved in RNA free water (50 µl Sigma H_2O) and incubated at 55-57°C for 10 mins. The concentration and purity of total RNA (n=3) was then determined using a spectrophotometer (Sci-tek instruments, Buckinghamshire, UK) and the total RNA stored at -80°C.

5.2.6 Synthesis of complementary DNA (cDNA)

Reverse transcription of total RNA samples to synthesize cDNA was carried out using Taqman® reverse transcription system on a Gene Amp® PCR System 9700 (Roche Molecular Systems, Branchburg, NJ,USA). The master mix for each reaction consisted of 5µl of 10 × PCR buffer, 11µl of 25mM $MgCl_2$, 1.75 µl of 50u/µL RT scribe, 10µl of 10mM dNTPmix, 1 µl of 40u/µL RNAse Inhibitor and 2.5 µl of 50µM Random Hexamers primer and 2µg of total RNA. Nuclease free water (Sigma H_2O) was added to make a total volume of 50 µl. Samples were prepared for each of the five concentrations points of both IVM and PZQ, and the two controls. The mixture was incubated for 10 min at 25°C and subsequently for 30 min at 48°C and the reverse transcription was terminated by heating for 5 min at 95°C and cooling at 4°C for the same period. The cDNA was then labelled and stored at 4°C.

5.2.7 Amplification of ABCB1, CYP3A4 and CYP2B6 by RT-PCR

RT-PCR assays were developed for quantification of mRNA expression relative to Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the housekeeping gene (ΔΔCT) as described previously and validated in our laboratory (Owen *et al.*, 2004a; Schmittgen *et al.*, 2008). Primers for ABCB1, CYP3A4 and CYP2B6 were used. The sequences for the primers and probes of ABCB1 and GAPDH genes are as outlined in Table 5.1. Genes for CYP3A4 and CYP2B6 were as per assay on demand and their assay identity numbers of the batches that were used are listed in the Table. Each primer had a final concentration of 0.9 μM while each probe had a final concentration of 0.25μM. The reaction mixture consisted of 40 ng (2 μl) of cDNA of each concentration point of the desired drug and a positive control in a master mix preparation. RIF was used as positive control for ABCB1 and CYP3A4 genes while PB was used for CYP2B6 gene. The master mix contained QPCR mixture (12.5 μl), primers and probes and topped up by nuclease free water to a final volume of 25 μl. The master mix of each reaction of the house housekeeping gene contained 12.5 μl QPCR mixtures, 1.25 μl each of GAPDH primer and probe, 8 μl of nuclease free water. This was substituted with the same volume of ABCB1 primers (0.9 μM) and probes (0.25μM) for ABCB1 gene. In the case of CYP3A4 and CYP2B6 genes, 1.25 μl of a mixture of 0.9μM ABCB1 primer and 0.25μmM probes was added to 12.5 μl of QPCR mixture and 9.25 μl of nuclease free water. A negative control contained water as a substitute for cDNA. The amplification was carried out on Opticron ® Fluorescence detector (MJ Research, UK) with initial denaturation at 95°C for 15 minutes. This was followed by 50 cycles of denaturation at 95°C for 15 sec and primer annealing with combined extension at 60°C for 1 minute.

Table 5.1 Sequences for the primers and probe of ABCB1, GAPDH and references of CYP2B6 and CYP3A4 genes.

Primer and probes sequences	
ABCB1-F	5'-GGAAGCCAATGCCTATGACTTTAT-3'
ABCB1-R	5'-TCAACTGGGCCCCTCTCTCT-3'
ABCB1 probe	5'-TGAAACTGCCTCATAAATTTGACACCCTGG-3'
GAPDH-F	5′-GAAGGTGAAGGTCGGAGTC-3′
GAPDH- R	5′-GAAGATGGTGATGGGATTTC-3′
GAPDH probe	5′-CAAGCTTCCCGTTCTCAGCC-3′
Gene Symbol	**Assay ID**
CYP2B6	Hs03044634_m1
CYP3A4	Hs00430021_m1

5.2.8 Analysis of the real time PCR data

The analysis of the PCR results data was carried out by comparative C_T method (Liptrott *et al.*, 2009; Schmittgen *et al.*, 2008). Relative quantification was based on the expression of mRNA in Caco-2 cells alone and in presence of the desired drug concentration. The expression data were normalised to GAPDH expression using the comparative ΔΔCt method consisting of 2 raised to the power of the difference in the Ct between the reference gene (GAPDH) and the test gene.

5.2.9 Statistical analysis

The results were presented as mean ± standard deviation (SD) of at least four experiments conducted in duplicate with 95% confidence intervals for differences between the means where appropriate. The assessment of normality was done using Shapiro – Wilk test and the differences in mRNA expression and toxicity determined using the t-test. A two-tailed p value of <0.05 was accepted as being statistically significant.

5.3 Results

5.3.1 Toxicity

PZQ was not toxic at all concentrations (0 to 100 μM) after 24 hour incubation in comparison to controls, as demonstrated by the cell viability results following the MTT assay (Fig 5.1). It was however toxic at 100 μM after incubation for 5 days (p = 0.006) [Fig 5.1]. In contrast, significant toxicity was observed for IVM at 100 μM after a 24 hour, and at 10 μM after a 5-day incubation respectively ($p < 0.0001$ and $p = 0.003$). The data generated at these concentrations were therefore interpreted with caution.

5.3.2 Positive controls

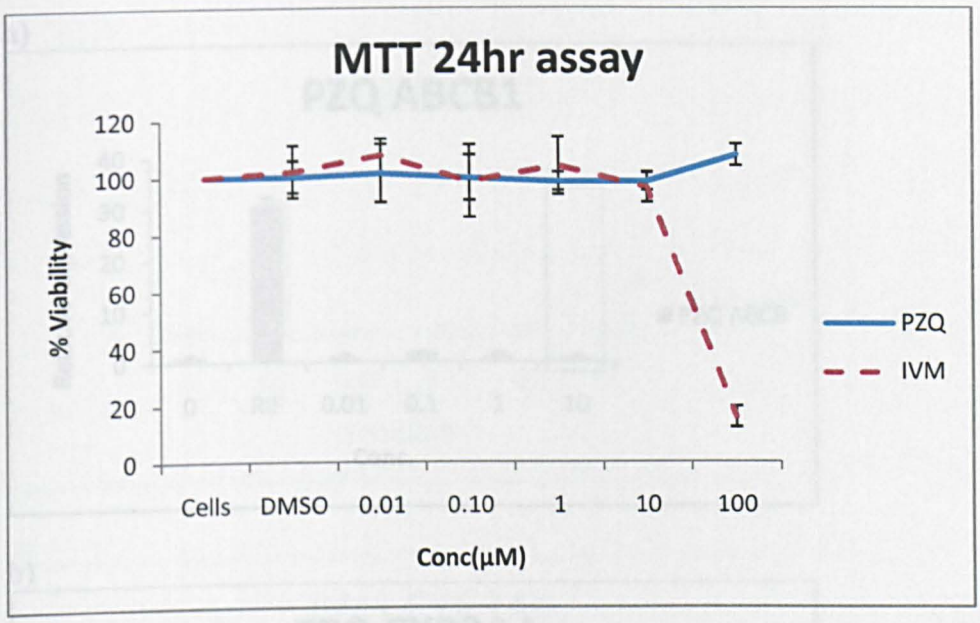
The mean fold change data for the induction of ABCB1 gene by RIF was 30.7 ± 2.58 ($p > 0.0001$) and 13.34 ± 1.56 ($p > 0.0001$) for CYP3A4. For PB the mean fold change was 119.47 ± 15.31 ($p > 0.0001$) for the induction of CYP2B6 gene.

5.3.3 Impact of PZQ on ABCB1, CYP3A4 and CYP2B6

In comparison with RIF, PZQ did not appear to cause and induction of ABCB1 or CYP3A4 genes in the concentrations in the study. Likewise, there was no induction of CYP2B6 gene (Fig 5.2).

5.3.4 Impact of IVM on ABCB1, CYP3A4 and CYP2B6

Concentration dependent induction of ABCB1 and CYP3A4 genes by IVM was not observed in the concentrations of 0.01 to 10 μM, in comparison with the positive control RIF. In comparison to PB, it did not induce CYP2B6 gene (Fig 5.3).



b)

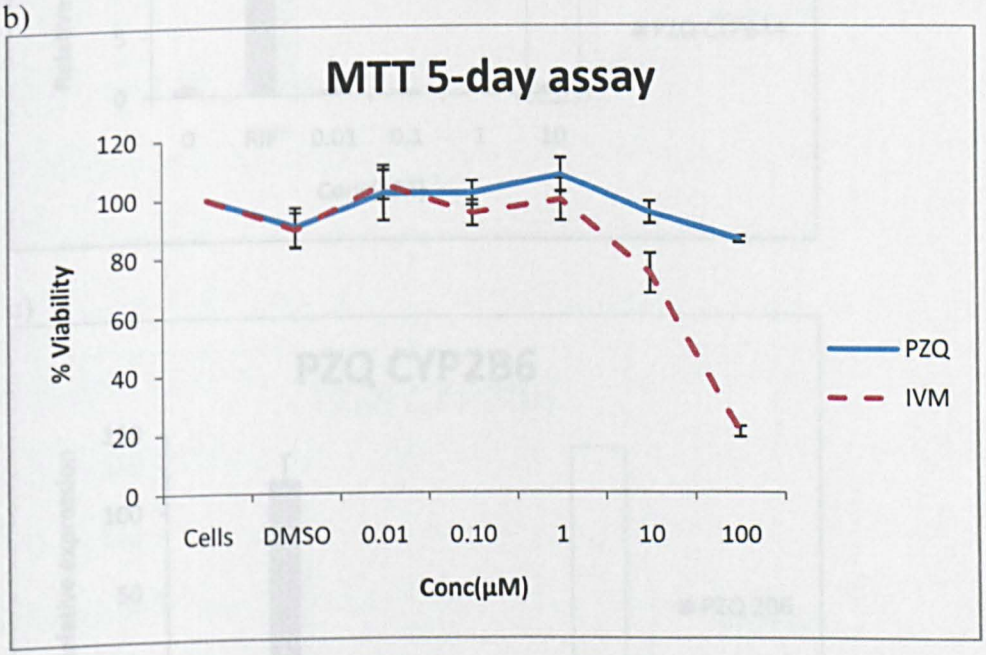
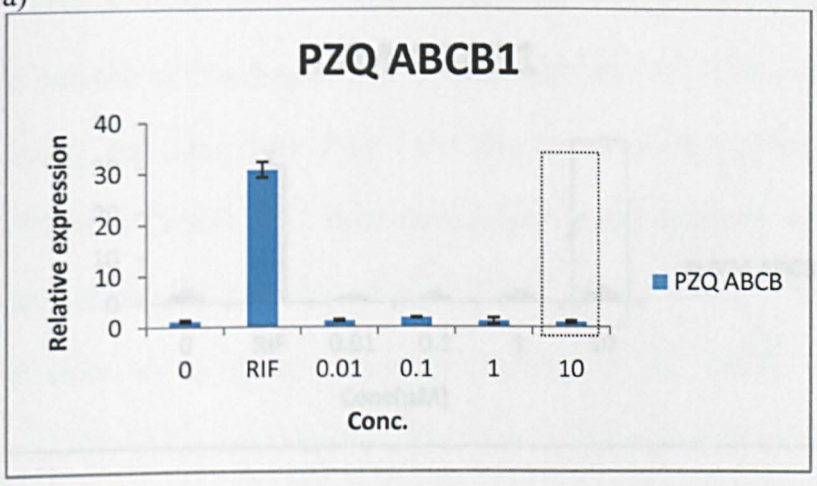
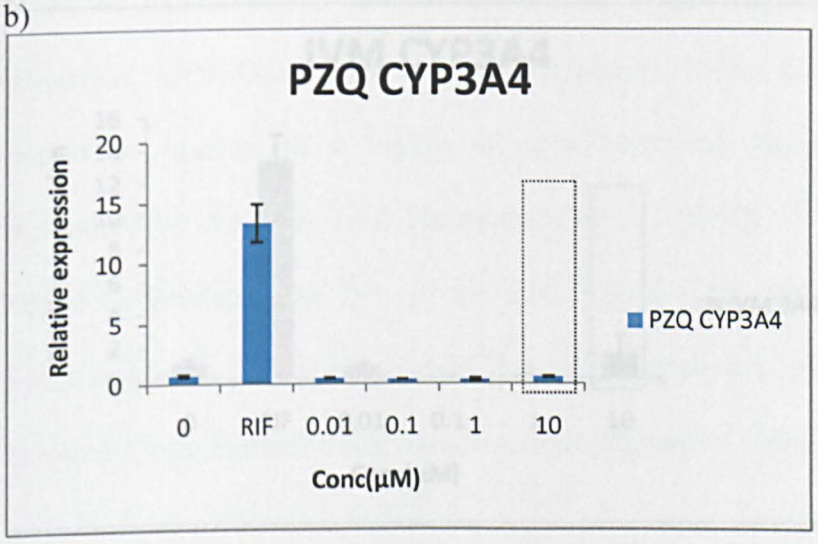


Fig 5.1 Effect of PZQ and IVM (0 - 100 µM) on the viability of Caco-2 cells expressed percentage viability after a) 24hrs and b) 5-days (Mean ± SD, n=4).

a)



b)



c)

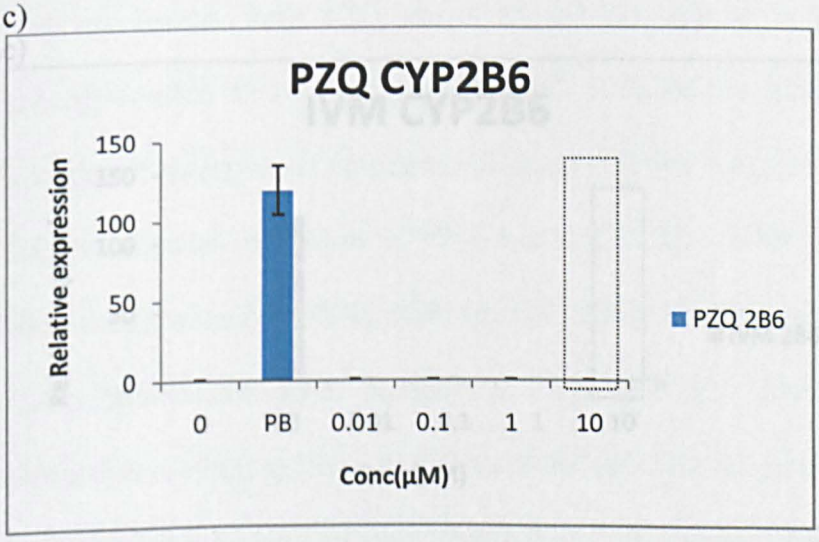
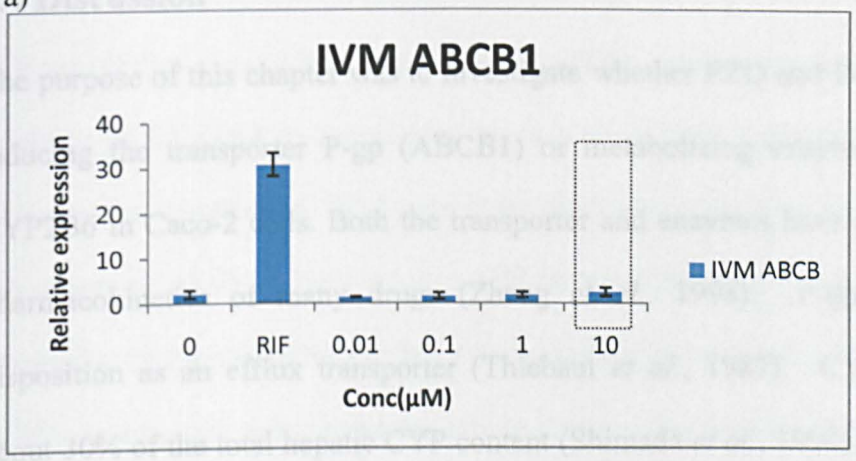
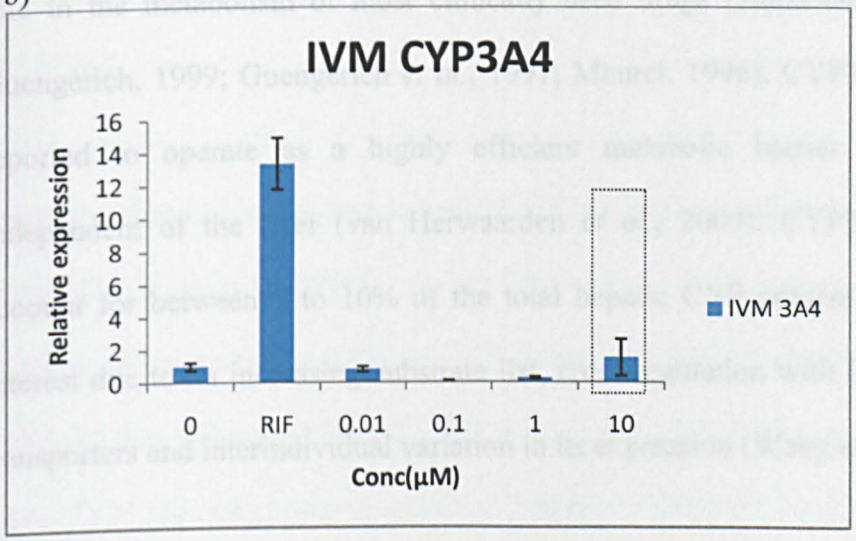


Fig 5.2 Impact of PZQ (0-10µM) on mRNA expression of a) ABCB1, b) 2B6 and c) 3A4 (Mean ± SD, n=4).

a)



b)



c)

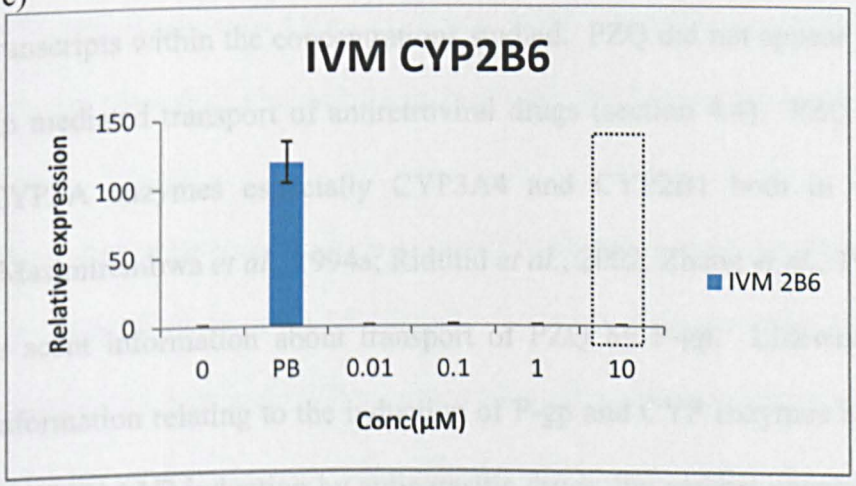


Fig 5.3 Impact of IVM (0-10µM) on mRNA expression of a) ABCB1, b) 2B6 and c) 3A4 (Mean ± SD, n=4).

5.4 Discussion

The purpose of this chapter was to investigate whether PZQ and IVM are capable of inducing the transporter P-gp (ABCB1) or metabolizing enzymes, CYP3A4 and CYP2B6 in Caco-2 cells. Both the transporter and enzymes have major roles in the pharmacokinetics of many drugs (Zhang *et al.*, 1998). P-gp influences their disposition as an efflux transporter (Thiebaut *et al.*, 1987). CYP3A4 constitutes about 30% of the total hepatic CYP content (Shimada *et al.*, 1994) and plays a major role in the metabolism of most clinically used drugs (Anzenbacher *et al.*, 2001; Guengerich, 1999; Guengerich *et al.*, 1991; Maurel, 1996). CYP3A4 has also been reported to operate as a highly efficient metabolic barrier in the intestine, independent of the liver (van Herwaarden *et al.*, 2009). CYP2B6 is thought to account for between 2 to 10% of the total hepatic CYP content, and is attracting interest due to its increasing substrate list, cross-regulation with CYP3A4 and drug transporters and interindividual variation in its expression (Wang *et al.*, 2008).

From our results, both PZQ and IVM did not appear to induce any of these transcripts within the concentrations studied. PZQ did not appear to influence the P-gp mediated transport of antiretroviral drugs (section 4.4). PZQ is metabolised by CYP3A enzymes especially CYP3A4 and CYP2B1 both in *vivo* and in *vitro* (Masimirembwa *et al.*, 1994a; Ridtitid *et al.*, 2002; Zhang *et al.*, 1997). To date there is scant information about transport of PZQ by P-gp. Likewise, there is limited information relating to the induction of P-gp and CYP enzymes by PZQ. In a study on CYP1A1/2 induction by antiparasitic drugs, the authors concluded that PZQ does not induce CYP1A1 enzyme (Bapiro *et al.*, 2002).

IVM is a known substrate (Brayden *et al.*, 2008; Pouliot *et al.*, 1997), and inhibitor of P-gp (Didier *et al.*, 1996; Lespine *et al.*, 2006). It has been reported to be a weak inhibitor of CYP3A4 in comparison to its inhibition of P-gp (Perloff *et al.*, 2003). The co-administration of IVM with P-gp modulators has been reported to increase the secretion of IVM both in *vivo* and in *vitro* (Ballent et al., 2006). Altered expression in P-gp function has been postulated to be responsible for the elevation of IVM in brain, with subsequent toxicity in some collie dogs (Edwards, 2003; Mealey *et al.*, 2001). It is metabolised by CYP enzymes, predominantly CYP3A4 (Zeng *et al.*, 1998).

Presently, there is conflicting information regarding the induction of both P-gp and metabolic enzymes by IVM. It has been reported to induce CYP enzymes in mouflon sheep including CYP1A, CYP2B and CYP3A sub-families (Skalova *et al.*, 2001), or that it does not induce CYP1A1 or CYP 1A2 (Bapiro *et al.*, 2002). In our study, IVM did not appear to induce either P-gp or any of the CYP enzymes. From our previous experiments on the interactions between ARVs and IVM (section 4.4), IVM interacted with LPV, SQV and NVP. Therefore, any potential interaction between these drugs is likely to be mediated by inhibition rather than induction.

There should be some caution however in the interpretation of these results as there are some limitations in this study, including the suitability of Caco-2 cells in induction of P-gp and CYP enzymes. A study comparing the expression of CYP3A4 and ABC transporter genes in different colonic carcinoma cell lines concluded that LS180 cells had significantly higher mRNA levels than Caco-2 cells (Pfrunder *et al.*, 2003). In addition, temporal induction and subsequent downregulation of ABCB1

mRNA with increasing passage number during culture of Caco-2 cells has been reported (Goto *et al.*, 2003). The expression of Caco-2 cells has also been reported to be influenced by culture conditions (Sambuy *et al.*, 2005). Further to that, although the P-gp expression in Caco-2 cells closely resembles that of the colon, some variability in predicting oral variability of drugs has been reported (Calcagno *et al.*, 2006). However, Caco-2 cells can be transformed to express large amounts of CYP3A4 by culturing in the presence of vitamin D3 (Engman *et al.*, 2001), indicating that induction by at least same chemical stimuli is possible in these cells. It has also been reported that P-gp expression in Caco-2 cells may be enhanced by culturing in a medium containing vinblastine (Shirasaka *et al.*, 2006). From our toxicity results IVM appeared to be more toxic of the two drugs, showing toxicity at 10 μM after incubation for 24 hours. Rifampicin was used as positive control of CYP3A4 as it induces the genes in primary cell cultures (Huang *et al.*, 2006; Kostrubsky *et al.*, 1998; Nishimura *et al.*, 2002; Schuetz *et al.*, 1993). Phenonobarbital was used as positive control of CYP2B6 (Faucette *et al.*, 2004; Rencurel *et al.*, 2005). The concentration of DMSO was kept at below 0.5% as higher concentrations reduce mRNA levels (Nishimura *et al.*, 2003).

The clinical relevance of the study relates to the role of drug transporters in drug-drug interactions and development of resistance. This is important in relation to the two anthelminthic drugs owing to their mass use. PZQ is a cheap and effective drug used in individual and mass treatment of schistosomiasis (Ferrari *et al.*, 2003). This disease is endemic in 74 countries and 200 million people are infected with 600 million at risk (Fenwick *et al.*, 2006; WHO, 2007c). IVM is used alone and in combination with albendazole and/or diethylcabamazine in mass treatment and

control of lymphatic filariasis (Molyneux *et al.*, 2003). Between 2000 and 2007 more than 1.9 billion doses were administered to at least 570 million people in 48 countries mainly in Africa, Asia and Latin America (Ottesen *et al.*, 2008). Most of the patients with these conditions are also likely to be co-infected with other diseases such as malaria, TB and HIV/AIDS. As a result apart from the mass use, IVM and PZQ are likely to be co-administered with other drugs raising the issue of drug-drug interactions and resistance (Collett *et al.*, 2004b; Lespine *et al.*, 2008). This may arise via the modulation of P-gp and metabolic enzymes leading to the alteration of their pharmacokinetic profiles (Baron *et al.*, 2001; Benet *et al.*, 2004). In fact the emergence resistance to IVM in humans has already been reported (Osei-Atweneboana *et al.*, 2007). The modulation of P-gp has been postulated as a possible strategy to enhance the efficacy and reverse the resistance of helminths to IVM (Lespine *et al.*, 2008).

In summary, although both drugs did not appear to induce P-gp and CYP enzymes, further induction/inhibition experiments should be conducted in different cell lines that express these transporters and enzymes. This will help in understanding the pharmacokinetics of these and other drugs in order to prevent the development of drug-drug interactions, toxicity and resistance.

Chapter 6

Effect of nanodispersion on the transport of SQV along the Caco-2 cell monolayers (CCM)

Chapter 6

Effect of nanodispersion on the transport of SQV along the Caco-2 cell monolayers (CCM)

6.1 Introduction

Despite the success of HAART in the management of HIV/AIDS, there are still several challenges. These include compliance and the ability of drugs to cross physiological barriers in order to reach the cellular reservoirs of HIV in sufficient quantities to exert maximum virucidal effect. Together these contribute to development of resistance to antiretroviral drugs (Obaru *et al.*, 1998; Wainberg *et al.*, 1998). Though the current therapy is able to lower the viral load below detectable limits, the HIV virus continues to survive in anatomical areas with poor drug permeation (sanctuary sites) such as CD4+ macrophages and T-lymphocytes, the CNS, cerebrospinal fluid and lymphatic system. This may result in relapses and increased likelihood of the development of resistance (Ohkura *et al.*, 1997; Vyas *et al.*, 2006). Maximal suppression of this virus therefore requires the drugs to adequately penetrate these reservoir sites, and current research indicates nanotechnology as a potential method towards the achievement of this goal (Bakker-Woudenberg *et al.*, 1994; Vyas *et al.*, 2006).

Nanomedicine (nanotechnology application in healthcare) has generated considerable interest as a drug delivery system owing to its numerous advantages. These include increased solubility, improved delivery to the sites of action, bioavailability and tissue specificity coupled with reduced metabolism which reduces the side effects. This system may also allow simultaneous drug delivery of two or more drugs (Allen *et al.*, 2004a; Emerich *et al.*, 2006; Zhang *et al.*, 2008b). In addition, the intracellular nanoparticle delivery of drugs has been demonstrated to circumvent the transporter mediated drug efflux, allowing drugs to gain entry into cells through endocytosis/phagocytosis (Kidane *et al.*, 2005; Vauthier *et al.*, 2003). The potential

for nanoparticles to deliver drugs to the CNS with minimal adverse effects has also been reported (Tosi *et al.*, 2008). Biodegradable acrylate nanoparticles with bound doxorubicin have been observed to have a higher therapeutic index compared to free doxorubicin owing to improved delivery of the drugs to the sites of action, increased accumulation and retention, with a net result of a reduction of adverse effects and resistance (Kattan *et al.*, 1992; Wong *et al.*, 2006).

Currently, there are two nanotechnology methods that are utilized in the improvement of the physical properties of hydrophobic drugs with a view to enhancing their solubility. The first method involves the attachment or encapsulation of the drug to a vehicle in order to form ''nano-carriers'' which have better solubility (Alonso, 2004). In the second method, the drug is manipulated to form a particle dispersion (''nano-engineering'').

The nanocarrier approach entails the use of polymeric materials and devices of nanometric size range (1-100nm [nanoparticles]) in drug and gene deliveries. Examples of these include liposomes (El Maghraby *et al.*, 2008), dendrimers (Perumal *et al.*, 2008), micelles (Liu *et al.*, 2008), polymer conjugation (Duncan *et al.*, 2005), nanoemulsions (Sadurni *et al.*, 2005), magnetic nanoparticles and quantum dots. Research on this area has mainly focused on cancer, neuropathologies as well as cardiovascular disorders, and some products are currently undergoing evaluation for clinical use (Sanvicens *et al.*, 2008; van Vlerken *et al.*, 2006; Zhang *et al.*, 2008b).

Nanodispersion technology involves the dispersion and de-agglomeration of nanopowders and reduction of particle size to micron and submicron range. This overcomes the bonding forces after wettening the nanopowder or micropowder leading to increased surface area and solubility (Kesisoglou *et al.*, 2007). The nano-engineering process entails the manipulation of pure drug via several techniques such as milling, homogenization and precipitation to small particles that are suspended in a liquid which in most cases is water (Horn *et al.*, 2001). The milling process is the most effective and has been used in the development of several drug formulations from poorly soluble drugs that are capable of being administered through all the common routes of administration (Merisko-Liversidge *et al.*, 2003). However, one of the major constraints in this process is the likelihood of contamination resulting from the damage of the milling equipment (Rabinow, 2004).

Recently, researchers at the University of Liverpool have developed a new non-attrition method that involves the conversion of emulsions to stable soluble template porous structures. This method has been applied to more than 220 hydrophobic compounds with different physical properties that include waxes. This technology is protected by two university patent filings *[WO 2004011537, WO2005014704]* and has led to the foundation of IOTA NanoSolutions Limited®, a nanomaterials engineering company associated with the University of Liverpool that specializes in the formulation of poorly soluble ingredients. The process involves the formation of oil-in-water emulsions by dissolving hydrophobic drugs in a volatile water immiscible organic solvent and the water-soluble materials in water. Dry powder composites are then formed through freeze-drying the emulsions in order to remove the volatile/aqueous phases (Fig 6.1).

The resultant product composed of the drug dispersed throughout the water soluble material is stable, dry and highly porous unlike those from previous emulsion technologies. The drugs are blended with hydrophilic matrices and kinetically trapped but do not form macroscopic crystals during the drying process, and readily dissolves upon addition of water to form nanodispersions. It has been demonstrated that an aqueous nanodispersion of the drug Triclosan® developed in this manner has higher efficacy than the solutions of the parent drug (Zhang *et al.*, 2008a).

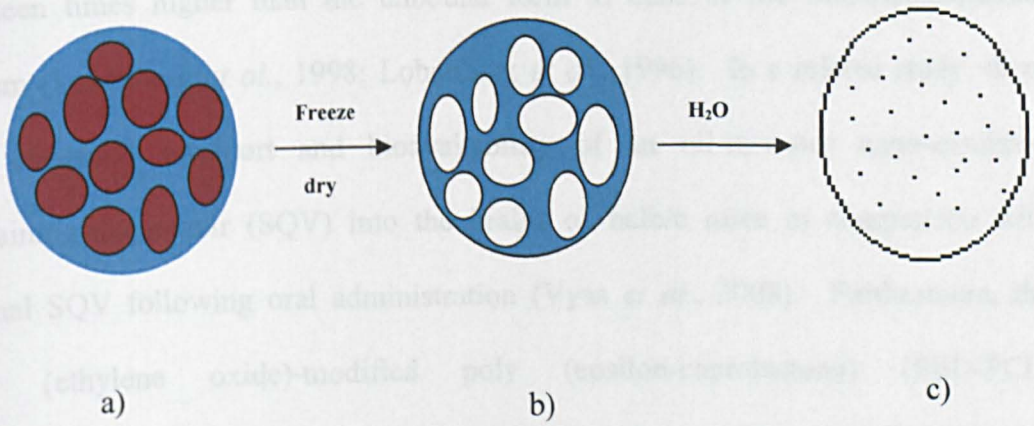


Fig 6.1: Illustration of the nanodispersion process showing: (a) the formation of an oil in water emulsion by dissolution, (b) dry powder composite and (c) dispersion of nanoparticles

Presently, the use of nanotechnology in drug delivery of antiretrovirals (ARV) is still limited despite the numerous potential benefits that would be achieved as a result of improved delivery of the drugs to sanctuary sites, especially since nanoparticles may gain entry to cells through endocytosis/phagocytosis (Kidane *et al.*, 2005; Vyas *et al.*, 2006). Most of the ARV drugs are highly hydrophobic and liposomes and

conjugates have been investigated as possible nanocarriers for the drugs (Bakker-Woudenberg *et al.*, 1994; Castor, 2005). Liposomes have an advantage of lipophilicity, are able to deliver more than one drug and provide protection for drugs against degradation (Allen *et al.*, 2004b). In addition it has been demonstrated that they are readily phagocytosed by macrophages thus making them suitable for drug delivery of ARVs to HIV-infected macrophages (Lanao *et al.*, 2007). However, use of liposomes has been limited by long term stability and safety (Gupta *et al.*, 2005; Voinea *et al.*, 2002).

The concentration of AZT (Azidothymidine) bound to nanoparticles was found to be eighteen times higher than the unbound form in cells of the reticuloendothelial system (Lobenberg *et al.*, 1998; Lobenberg *et al.*, 1996). In a related study, there was enhanced transport and bioavailability of an oil-in-water nano-emulsion containing saquinavir (SQV) into the brains of balb/c mice in comparison with normal SQV following oral administration (Vyas *et al.*, 2008). Furthermore, the poly (ethylene oxide)-modified poly (epsilon-caprolactone) (PEO-PCL) nanoparticulate system has been reported to be a suitable nanocarrier for SQV into human microcyte / monophage cell lines (Shah *et al.*, 2006).

The main objective of the work described in this chapter was to investigate the effects of nanodispersion on the transport of SQV along Caco-2 cell monolayers (CCM). As discussed earlier (section 4.1), the CCM have similar morphological and functional properties to intestinal enterocytes and are therefore well suited since the permeability of drugs through the CCM correlates well with in vivo absorption in humans.

The transport along the CCM of five nanodispersed samples of SQV obtained from IOTA in comparison to normal SQV was investigated. Cellular accumulation experiments using CEM parental (Foley *et al.*, 1965) and CEMVBL cells that overexpress P-gp (Beck *et al.*, 1979) was also assessed to ascertain whether the nanodispersion affects the accumulation of SQV, a substrate of P-gp (Janneh *et al.*, 2005).

6.2 Materials and methods

6.2.1 Materials

Caco-2 cells were purchased from the European collection of cell cultures (ECACC No. 286010202), and the cells were counted using a Nucleo Counter (ChemoMetec, Denmark) cell counter. SQV and the various formulations of nanodispersed SQV were provided by IOTA NanoSolutions Ltd (MerseyBIO, Liverpool, UK). DMEM, RPMI, HBSS, FBS, DMSO and Trypsin-EDTA solution were purchased from Sigma Aldrich (Poole, UK). ACN and MeOH were purchased from VWR Laboratory Supplies (Poole, UK) whereas diethyl ether was purchased from Fisher Scientific, (Loughborough, UK). All the other chemicals used were of analytical or HPLC grade. Deionised water used to prepare the solutions or mobile phase was purified in an Elga DV 25 pure lab option system (Elga, High Wycombe, Bucks, and UK).

6.2.2 Cell culture

The cells were cultured in DMEM and plated onto the transwells as described in section 4.2.4 at a density of 2×10^4 cells/cm^2 followed by incubation at 37°C and 10% CO_2 in a humidified chamber with media change after every 2-3 days. Transport experiments were conducted 15 to 20 days after seeding and the trans-epithelial resistance (TEER) across the cell monolayers was monitored until they were considered appropriate for the experiment.

6.2.3 Cumulative trans-epithelial transport of SQV and nanodispersible forms of SQV

The composition of nanodispersed SQV samples is given in table 6.1. Solutions of 10 mg/ml SQV and the nanodispersible SQV samples SQV O5, SQV O6, SQV O7, SQV O9 and SQV 13 were prepared by weighing out and dissolving SQV in DMSO and nanodispersed formulations in distilled water. Nanodispersed samples were prepared by a ratio of 1:5 since the drug proportion in the powder was 20% as outlined in table 6.1. Each experiment was carried out using paired samples of SQV and a nanodispersed SQV sample so as to maintain similar experimental conditions.

TEER was measured prior to transport studies and each monolayer was washed and equilibrated with the transport medium (DMEM without FBS). The medium was removed from all apical (AP) and basolateral (BL) compartments of the transwells and replaced with 2 ml of the transport medium (DMEM alone) and equilibrated for 1 h (37°C, 10% CO_2 incubator), after which the TEER was re-assessed. The medium was then removed from both compartments and replaced with an equal volume of pre-warmed medium containing 20 µg/ml of the SQV sample under investigation. For the AP→BL transport, 2 ml the medium containing the SQV sample was placed on the apical chamber and 2 ml of the medium alone on the basolateral chamber, 2 ml of medium containing the drug were placed on the basolateral and 2 ml of medium on the apical chamber for the BL→AP transport. Transport in each direction was conducted in pentuplicate.

The transwell plates were then incubated (37°C, 10% CO_2 incubator), and 100µl of the samples from the AP and BL compartments were taken at 60, 120, 180 and 240

min and quantified by HPLC. The integrity of the Caco-2 cell monolayers during the experiment was monitored by measuring the TEER at the beginning (0 min) and the end of the experiment (240 min). The results were expressed as apparent permeability *P*app , calculated as described in section 4.2.4.3.

Exp #	Sample	% SQV mesylate	% HPMC	% PVP	% PVA	%Pluronic F127	%Lecithin S75	% Span 80	Initial Sample PS/nm	Type
#23/101/05	**SQV 05**	20	70			10			430	
#23/101/06	**SQV 06**	20				10			117	M
#23/101/07	**SQV 07**	20		70		10			100	M
#23/101/09	**SQV 09**	20	65			10	5		196	
#23/101/13	**SQV 13**	20			65	10		5	336	

Exp # Manufacturer's reference

HPMC Hydroxypropyl methyl cellulose

PVP Polyvinyl-pyrolidone

PVA Polyvinyl alcohol

M Multimodal

PS Particle size

Table 6.1 Properties (chemical compositions and sizes) of the nanodispersible SQV formulations

6.2.4 Cellular accumulation experiments

CEM parental and CEMVBL cells were cultured in 175cm^2 flasks containing RPMI (+ 10% FBS) in a humidified incubator (37 °C, 10% CO_2) as described in section 3.2.5.1. The cells were counted and media containing 10 million cells centrifuged (2000 × *g* for 5 min at 4°C) and the pellet reconstituted to a cell count of one million cells per ml in 10ml of fresh RPMI. This was followed by the addition of Solutions of 100 µl of 1mg/ml of SQV and the nanodispersed SQV samples to yield a drug concentration of 10µg/ml in each flask. Each set of experiments consisted of seven flasks one each for SQV and the five nanodispersed samples. The seventh flask contained 100 µl of 1mg/ml of SQV combined with excipients used for the preparation of the SQV 13 sample in the nanodispersion process that was provided by IOTA. The purpose of carrying out this experiment was to rule out the possibility that the powder may have been responsible for the apparently reduced transport of the sample in the Caco-2 transport experiment (section 6.2.3).

The cells were then incubated at 37°C for 30 min in a shaking water bath and the resulting cell suspensions centrifuged (2000 × *g* for 5 min at 4°C). Aliquots of 100µl of the supernatant fraction were then used to determine the extracellular (EXT) concentration. The excess supernatant fraction was then removed and the resulting cell pellet washed three times in 10ml HBSS and then centrifuged (2000 ×*g* for 5 min). The resulting pellets were reconstituted in 100µl of distilled water and used to determine intracellular (INT) concentrations. Samples were then assayed by HPLC and the data expressed as cellular accumulation ratio (CAR).

6.2.5 Statistical analysis

The results were presented as mean ± standard deviation (SD) of five experiments with 95% confidence intervals for differences between the means where appropriate. The assessment of normality was done using Shapiro – Wilk test and the analysis performed using the unpaired t-test. A two-tailed p value of <0.05 was accepted as being significant.

6.3 Results

6.3.1 Cumulative trans-epithelial transport

The results for the cumulative trans-epithelial transport across the CCM of dissolved SQV and the nanodispersed forms were as illustrated in Figs 6.2, 6.3 and 6.4. As in the previous experiments on SQV (section 4.3.1.1), the cumulative transport of BL→AP efflux of SQV and all the nanodispersed forms exceeded the AP→BL transport, indicating that the nanodispersed formulations maintained the P-gp substrate specificity of SQV. The cumulative AP→BL and BL→AP transport of SQV and the nanodispersed samples provided were similar.

6.3.2 Apparent permeability coefficients, *P*app (efflux ratios)

The apparent permeability coefficients of SQV and the nanodispersed samples (n = 5) are as depicted in Table 6.2. The mean *P*apps for each of the samples and the statistical analysis are outlined in Table 6.3. The mean value of the $P\text{app}_{\text{BL-AP}}$ transport was higher than the $P\text{app}_{\text{AP-BL}}$ value across the spectrum confirming the active efflux transport (Table 6.2). The mean efflux ratio were 2.88 for SQV while the SQV 05 had 1.91, SQV06 2.0, SQV 07 3.12, and 09 2.55. SQV 013 sample had a fourfold increase (13.95) in comparison to SQV (Table 6.3). Apart from one outlier result for sample SQV 13, all the other four values were much higher compared to SQV and other nanodispersed SQV samples, and this coupled with marginally statistically insignificant (p = 0.07) reduction in transport suggests potential for facilitated absorption (Table 6.3).

Sample	Apparent permeability (cm/s)									
	A		B		C		D		E	
	$Papp_{AP-BL}$	$Papp_{BL-AP}$	$Papp_{AP-BL}$	$Papp_{BL-AP}$	$Papp_{AP-BL}$	$Papp_{BL-AP}$	$Papp_{AP-BL}$	$Papp_{BL-AP}$	$Papp_{AP-BL}$	$Papp_{BL-AP}$
SQV	3.64E-09	7.27E-09	2.65E-09	1.26E-08	2.37E-09	1.4E-08	1.52E-08	8.97E-09	8.02E-09	9.32E-09
SQV 05	3.16E-09	1.75E-09	3.14E-09	1.36E-08	5.07E-09	1.36E-08	6.18E-09	7.61E-09	1.01E-08	7.87E-09
SQV 06	1.92E-09	5.18E-09	4.11E-09	9.74E-09	8.69E-09	1.69E-08	3.11E-09	7.24E-09	1.32E-08	8.53E-09
SQV 07	1.95E-09	6.23E-09	1.98E-09	1.39E-08	5.08E-09	1.8E-08	1.4E-08	8.9E-09	1.03E-08	1.24E-08
SQV 09	1.79E-09	2.99E-09	1.38E-09	5.92E-09	3.41E-09	1.16E-08	3.42E-09	7.2E-09	7.25E-09	9.19E-09
SQV 13	6.68E-10	5.92E-09	2.07E-09	1.57E-08	5.72E-10	1.37E-08	2.83E-10	7.88E-09	6.24E-09	9.45E-09

Table 6.2 Apparent permeability for the apical to basal ($Papp_{AP-BL}$) and basolateral to apical ($Papp_{BL-AP}$) transport of SQV and nanodispersible SQ

6.3.2 Accumulation experiments

The intracellular accumulation of SQV in CEMVBL cells was lower than CEM in all the samples (Fig.6.5 and Table 6.4). There was significantly higher accumulation in the CEM cells for three samples in comparison with SQV; 1.52 ± 0.19 versus 1.94 ± 0.16, $p = 0.006$ (SQV 05), versus 1.9 ± 0.29, $p = 0.05$ (SQV 06) and 1.87 ± 0.25, $p = 0.04$ (SQV 09) [Fig. 6.5 and Table 6.5)]. There was no significant difference in the accumulation of SQV and the nanodispersed samples within the CEMVBL samples. The excipients had no effect on the accumulation of SQV; 1.52 ± 0.01 versus 1.52 ± 0.19 [$p = 0.97$] (Table 6.5).

Sample	Mean apparent permeability coefficient, *P*app = $P_{app_{BL-AP}}$:$P_{app_{AP-BL}}$					
	SQV	SQV 05	SQV 06	SQV 07	SQV 09	SQV 13
A	2.00	0.55	2.70	3.19	1.67	8.86
B	4.76	4.33	2.37	7.03	4.30	7.57
C	5.91	2.68	1.95	3.53	3.39	23.93
D	0.59	1.23	2.33	0.64	2.11	27.88
E	1.16	0.78	0.65	1.20	1.27	1.52
Mean	2.88	1.91	2.00	3.12	2.55	13.95
STDEV	2.33	1.58	0.80	2.51	1.26	11.34
		$p = 0.46$	$p = 0.46$	$p = 0.88$	$p = 0.78$	$p = 0.07$

(mean ± s.d, n=5)

Table 6.3 Mean apparent permeability coefficients (Efflux ratios) of the various SQV compositions

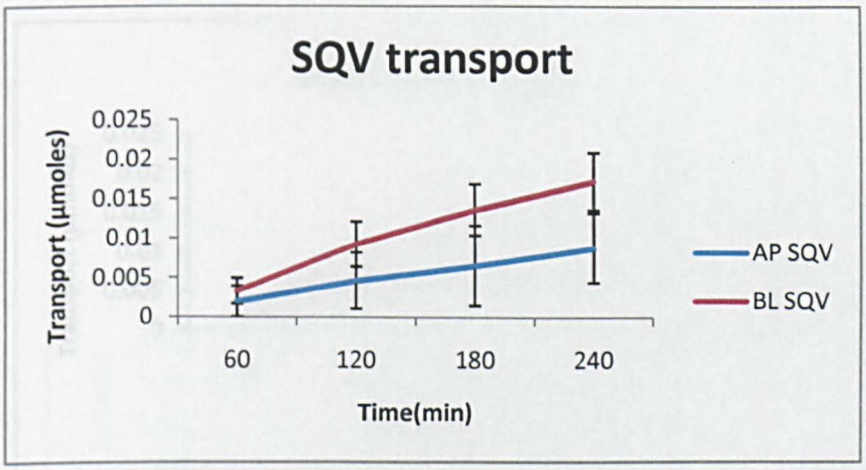
Sample	CEM SQV	VBL SQV	CEM SQV+P	VBL SQV+P	CEM SQV 05	VBL SQV 05	CEM SQV 06	VBL SQV 06
A	1.47	0.97	1.53	1.24	2.22	0.97	2.19	0.95
B	1.34	1.09	1.56	1.45	1.82	0.93	1.49	1.07
C	1.84	1.31	1.48	1.38	1.82	1.32	2.07	1.46
D	1.56	1.40	1.65	1.22	1.90	1.38	2.04	1.63
E	1.41	1.51	1.38	1.39	1.95	1.67	1.70	1.90
Average	1.52	1.26	1.52	1.33	1.94	1.25	1.90	1.40
STDEV	0.19	0.22	0.10	0.10	0.16	0.31	0.29	0.40
				$p = 0.04$		$p = 0.02$		$p = 0.002$

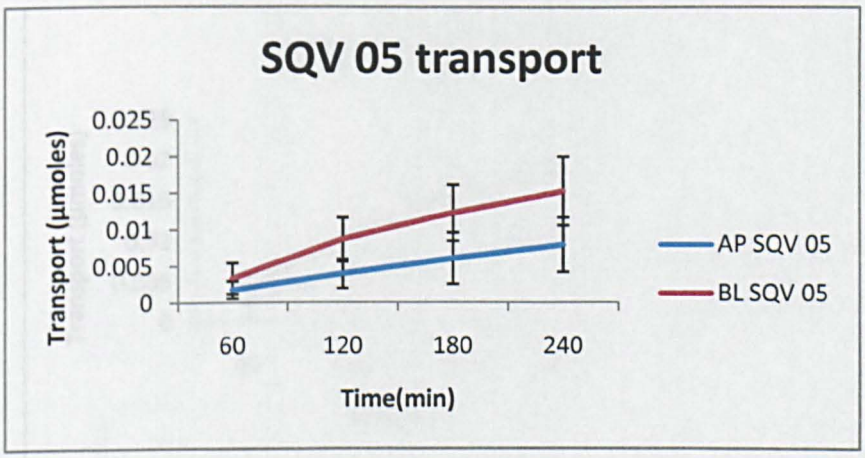
Sample	CEM SQV 07	VBL SQV 07	CEM SQV 09	VBL SQV 09	CEM SQV 13	VBL SQV 13
A	20.92	8.05	19.29	11.18	17.61	9.78
B	14.29	9.82	16.36	8.94	15.61	8.22
C	18.08	12.37	18.62	14.15	15.36	13.50
D	22.12	14.77	22.57	14.77	20.73	14.98
E	15.67	16.94	16.77	13.02	23.93	19.16
Average	18.22	12.39	18.72	12.41	18.65	13.13
STDEV	3.34	3.60	2.47	2.37	3.65	4.34
		$p = 0.02$		$p = 0.003$		$p = 0.004$

Table 6.4 Comparison of intracellular accumulation of the various SQV formulations in CEM and CEM$_{VBL}$ cells.

CEM cells							
Sample	**CEMSQV**	**CEM SQV+P**	**CEM SQV 05**	**CEM SQV 06**	**CEM SQV 07**	**CEM SQV 09**	**CEM SQV 13**
A	1.47	1.53	2.22	2.19	2.09	1.93	1.76
B	1.34	1.56	1.82	1.49	1.43	1.64	1.56
C	1.84	1.48	1.82	2.07	1.81	1.86	1.54
D	1.56	1.65	1.90	2.04	2.21	2.26	2.07
E	1.41	1.38	1.95	1.70	1.57	1.68	2.39
Average	1.52	1.52	1.94	1.90	1.82	1.87	1.87
STDEV	0.19	0.10	0.16	0.29	0.33	0.25	0.37
		$p = 0.97$	$p = 0.006$	$p = 0.05$	$p = 0.12$	$p = 0.04$	$p = 0.1$
CEMVBL cells							
Sample	**VBLSQV**	**VBL SQV+P**	**VBL SQV 05**	**VBL SQV 06**	**VBL SQV 07**	**VBL SQV 09**	**VBL SQV 13**
A	0.97	1.24	0.97	0.95	0.81	1.12	0.98
B	1.09	1.45	0.93	1.07	0.98	0.89	0.82
C	1.31	1.38	1.32	1.46	1.24	1.42	1.35
D	1.40	1.22	1.38	1.63	1.48	1.48	1.50
E	1.51	1.39	1.67	1.90	1.69	1.30	1.92
Average	1.26	1.33	1.25	1.40	1.24	1.24	1.31
STDEV	0.22	0.10	0.31	0.40	0.36	0.24	0.43
		$p = 0.51$	$p = 0.98$	$p = 0.5$	$p = 0.92$	$p = 0.91$	$p = 0.81$

Table 6.5 Intracellular accumulation of SQV, SQV combined with excipients for SQV 13 and the nanodispersible SQV formulations in CEM and CEMVBL cells.





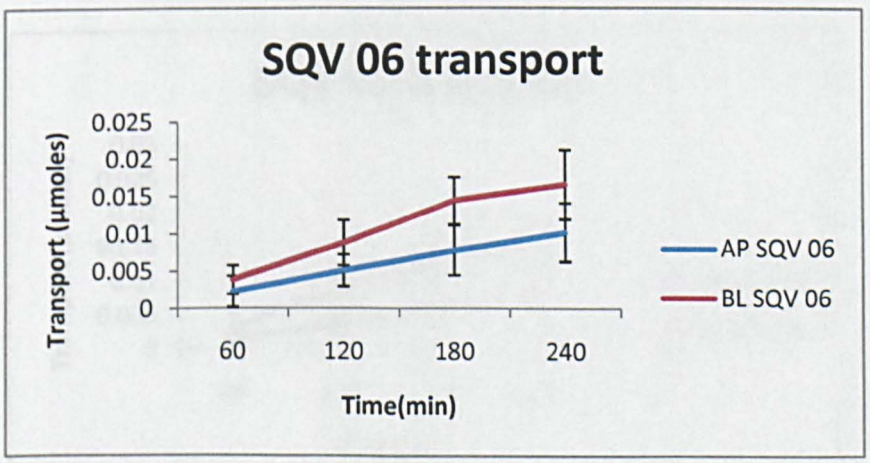
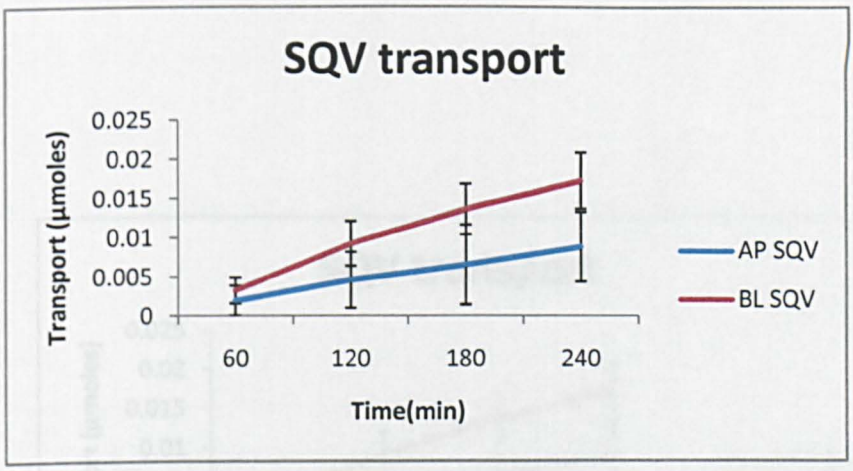
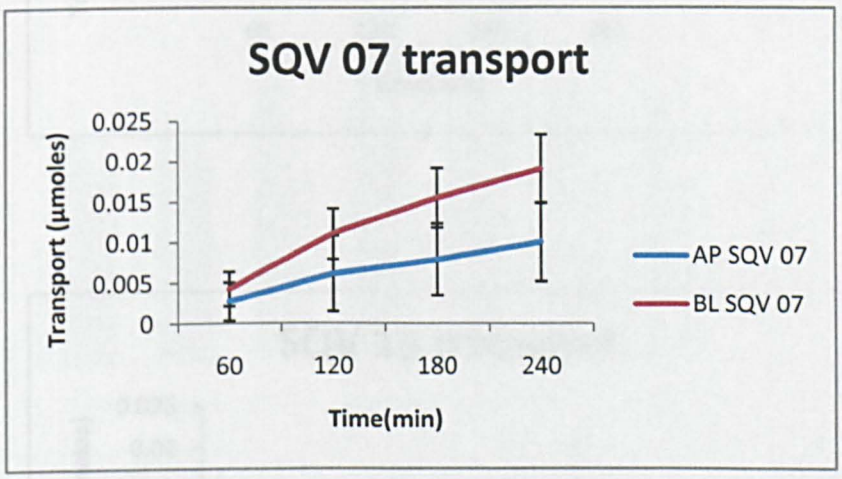


Figure 6.2 Comparison of the cumulative trans-epithelial transport of SQV and nanodispersible forms of SQV 05 and SQV 06 (expressed as mean ± s.d of three individual experiments).





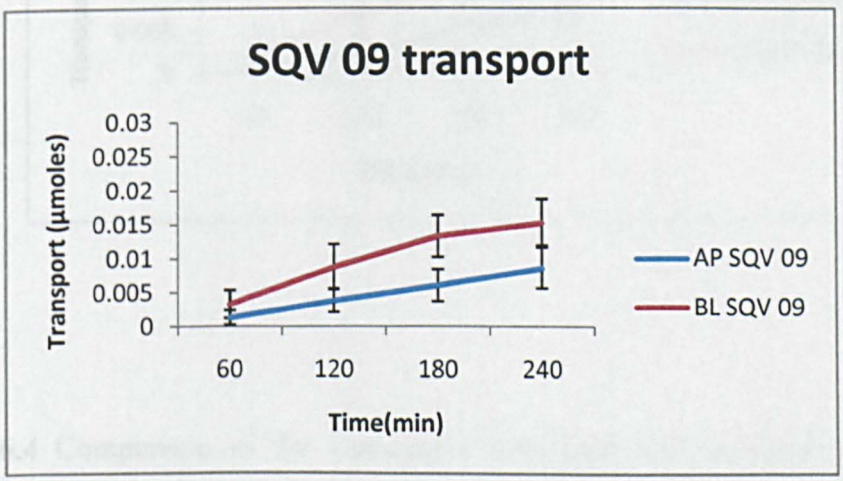


Figure 6.3 Comparison of the cumulative trans-epithelial transport of SQV and nanodispersible forms of SQV 07 and SQV 09 (expressed as mean ± s.d of three individual experiments).

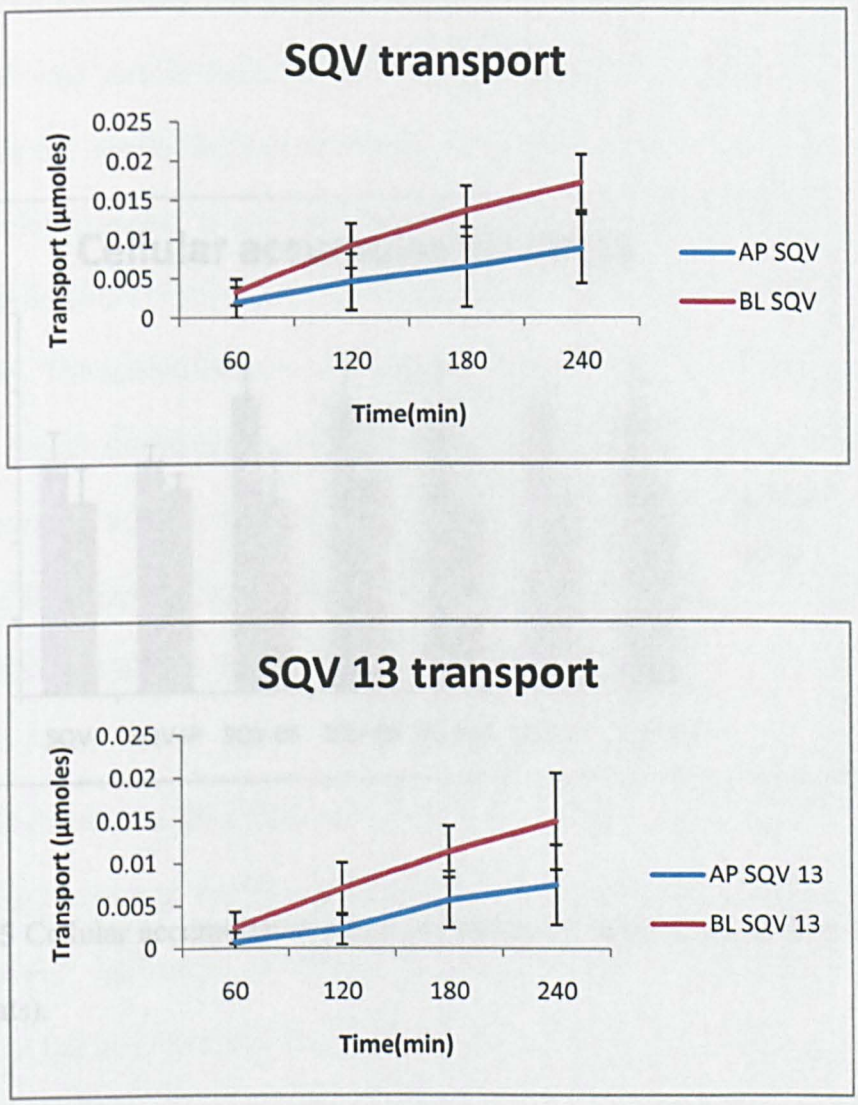


Figure 6.4 Comparison of the cumulative trans-epithelial transport of SQV and nanodispersible forms of SQV 13 (expressed as mean ± s.d of three individual experiments).

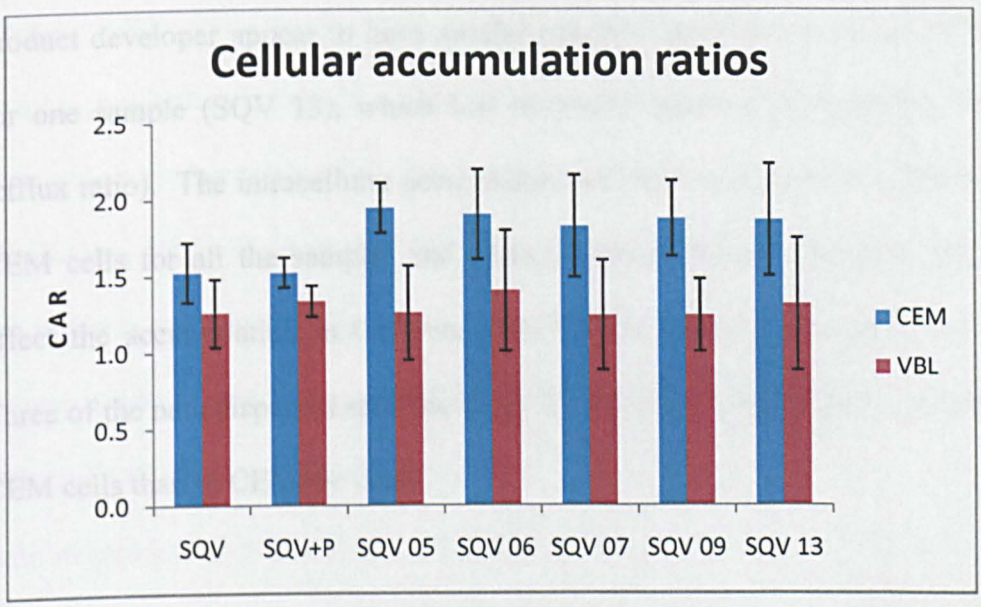


Figure 6.5 Cellular accumulation ratios (expressed as mean ± s.d of five individual experiments).

6.3.4 Discussion

The main aim of this study was to investigate the potential for nanodispersion to improve the efficacy of antiretroviral drugs through increased bioavailability and uptake into target tissues and cells. This involved the comparison of transport between SQV and various formulations of nanodispersed SQV samples supplied by IOTA. From our results, it appears that the samples provided for the study by the product developer appear to have similar transport profiles across the CCM except for one sample (SQV 13), which had increased apparent permeability coefficient (efflux ratio). The intracellular accumulation of SQV was lower in CEMVBL than in CEM cells for all the samples and nanodispersion did not appear to significantly affect the accumulation in CEMVBL cells for the samples provided for the study. Three of the nanodispersed samples (SQV 05, 06 and 13) had higher accumulation in CEM cells than in CEMVBL cells.

The fourfold increase in the efflux ratio of SQV 13 compared to of SQV could be as a result of an increase in the BL→AP transport or a decrease in AP→BL transport. The potentially facilitated absorption resulting to an increase in intracellular concentration led us to hypothesize that it may have been due to inhibition of P-gp. This was later disproved by the reduction in accumulation in the CEMVBL cells. It is noteworthy that the nanodispersed samples were dissolved in water whereas SQV was dissolved in DMSO to improve solubility. This is an important observation as it means that this nanodispersed formulation of SQV has potential for better bioavailability than SQV which has been a major drawback in the efficacy of SQV despite its potency. SQV is a substrate of the multidrug efflux transporter P-gp which

transports drugs out of the cells, and the low oral bioavailability of SQV (4-5%) has been thought to be partly associated with the transporter (Kim *et al.*, 1998a).

SQV has been demonstrated to display a highly potent *in vitro* activity against HIV, especially when in combination with ritonavir (Figgitt *et al.*, 2000). SQV mesylate (Invirase®) was the first PI to be approved in the treatment of HIV/AIDS in 1995 (Baker, 1995), but was discontinued after one year owing to the development of resistance which was largely attributed to its poor bioavailability and replaced by a soft gelatine formulation (Fortovase®) with improved bioavailability in 1997 (Fortovase, 2003). However the soft gelatine formulation was withdrawn in 2006 due to adverse gastrointestinal effects and replaced by the saquinavir boosted with ritonavir which has fewer side effects (Fortovase, 2006; Kurowski *et al.*, 2003). Ritonavir (RTV) is a potent inhibitor of CYP 3A4 and P-gp, and when it is co-administered with SQV, leads to a 20-fold increase in SQV plasma concentrations; hence its use in boosting SQV (Drewe *et al.*, 1999; Hsu *et al.*, 1998b; Veldkamp *et al.*, 2001a).

PIs are substrates for P-gp which decreases their intracellular drug concentration (Kim *et al.*, 1998b; Stormer *et al.*, 2002). A decrease in concentration in the sanctuary sites will result in the development of resistance (Shahiwala *et al.*, 2007). PIs are therefore despite being potent drugs for the management of HIV limited by the efflux transport and poor oral solubility with the resulting decrease in bioavailability. The inhibition of the P-gp mediated efflux would improve their efficacy (Kim *et al.*, 1998b).

The intracellular accumulation was lower in CEMVBL cells than in CEM cells. CEMVBL cells overexpress P-gp (Beck *et al.*, 1979) as discussed earlier in section 3.1 and since SQV is a substrate of P-gp the accumulation there is a net reduction in the intracellular accumulation (Janneh *et al.*, 2005; Srinivas *et al.*, 1998). This indicates that the nanodispersed samples do not circumvent P-gp mediated transport. Nanodispersion did not appear to significantly affect the intracellular accumulation of nanodispersed SQV compared to that of SQV in CEMVBL cells. However in CEM cells the nanodispersions accumulated to a higher degree indicating that at physiologically relevant P-gp density there is enhanced delivery to these cells possibly through other mechanisms such as phagocytosis.

Nanotechnology has been proposed as a potential method to enhance the drug delivery and bioavailability of SQV through increased solubility, improved transport and evasion of the P glycoprotein-mediated drug efflux (Shahiwala *et al.*, 2007). Previous studies using the nanocarrier approach using oil-in-water emulsions have demonstrated enhanced bioavailability and disposition of SQV into the brain in mice after oral administration (Vyas *et al.*, 2008). There have been no reports in the literature to date on nanoengineered SQV, despite the fact that nanodispersible method has more advantages in comparison to nanocarrier system owing to fewer and lower concentrations of excipients. In addition, nanocarriers are generally expensive and less biodegradable which may lead to increased accumulation and toxicity (Lanao *et al.*, 2007; Tosi *et al.*, 2008).

This technology is now considered as a potentially important approach to overcome drug-delivery problems associated with the use of other ARVs through improved bioavailability and subsequent penetration to the sanctuary sites of the HIV virus (Shahiwala *et al.*, 2007). The reported increase of up to eighteen times in the concentrations of AZT bound to nanoparticles in the cells of reticuloendothelial system in comparison to unbound drug demonstrates the suitability of nanoparticles for drug delivery of antiretroviral drugs (Lobenberg *et al.*, 1998).

Nanoengineered drugs achieve enhanced transport through anatomical barriers via several mechanisms including increased solubility and the reversal of the transporter-mediated drug efflux which was discussed earlier in section 6.1.1. This allows for lowering of the drug dosage and reduction of systemic toxicity and the likelihood for development of resistance. In addition nanotechnology has been postulated to be a potential drug-delivery method to the central nervous system whereby currently about 98% of the drugs are unable penetrate due to their inability to cross the blood-brain barrier (Garcia-Garcia *et al.*, 2005; Pardridge, 2003).

The ability of the drugs to penetrate and accumulate in the cellular reservoirs containing HIV (CD+ T lymphocytes and monocytes/macrophages) via endocytosis/phagocytosis is a major step in the treatment of HIV/AIDS (Shahiwala *et al.*, 2007; Vyas *et al.*, 2006). Increased accumulation of nanocarriers in peritoneal and tissue macrophages (e.g. of the liver kidney) *in vivo* has been reported (Wan *et al.*, 2007) and importantly, it has been demonstrated that HIV-infected macrophages have higher phagocytic activity in comparison to uninfected cells (Lanao *et al.*, 2007). In addition the system allows for simultaneous delivery of two or more drugs

which is important as the current management of HIV/AIDS involves combination therapy (Allen *et al.*, 2004a; Temesgen *et al.*, 1997).

In summary if nanodispersion can improve bioavailability of drugs, then it could improve the efficacy of HAART since less dosage of the drugs will be required and therefore reduced toxicity. In addition and more importantly, the drugs will be able to penetrate into the cellular reservoirs of HIV and this will be a major step in the treatment of HIV/AIDS.

Chapter 7

Concluding discussion

Chapter 7

Concluding discussion

The thesis has highlighted drug-drug interactions (DDIs) between antiretrovirals (ARVs) and co-administered drugs in chapter 2. Transporter mediated interactions between ARVs saquinavir (SQV), efavirenz (EFV), nevirapine (NVP) and lopinavir (LPV), and anthelminthics praziqantel (PZQ) and ivermectin (IVM) are described in chapters 3, 4 and 5. The potential of nanodispersion for the improvement of the efficacy of ARVs is discussed in chapter 6.

The prevalence and extent of DDIs between ARVs co-administered drugs has not been properly evaluated, especially in Africa where there is a shortage of resources. Previous studies have mainly been confined to developed countries but no such research has focused on developing countries (Cottle *et al.*, 2009; de Maat *et al.*, 2004; Miller *et al.*, 2007). Identification of such interactions is imperative in the treatment of HIV/AIDS (Robertson *et al.*, 2007). Interactions between ARVs and co-administered drugs have been identified as possible causes of toxicity, treatment failure or resistance in the management of HIV/AIDS (Pontali, 2007). We investigated the prevalence of DDIs between ARVs and co-administered drugs in a large patient cohort in Kenya. In this study, follow up prescriptions for 1040 patients enrolled into AMPATH (Academic Model for Prevention and Treatment of HIV/AIDS) seen over a 22 month period was assessed. DDIs between ARVs and co-prescribed drugs were then determined. Clinically significant DDIs between ARVs and co-administered drugs were common in Kenya, in one out of three patients (34%). Importantly, 32% of these would be predicted to result in lowering of the plasma concentrations of the ARVs, thereby compromising on their efficacy.

Interactions involved mainly ARVs and drugs for treatment of tuberculosis (rifampicin), fungal infections (azoles) and antimalarials (artemether combination therapies). Patients with CD4 counts of less than 79 had a threefold increase in the risk of DDIs comparison to patients with those with higher counts. Similarly, patients in WHO stages 3 and 4 or low weight had higher risk of DDIs as they were most likely on more drugs in line with symptoms associated with the progression of HIV/AIDS (Hare, 2006; WHO, 2005). Strategies should therefore be urgently developed to manage interactions in order to improve the efficacy of ARVs and reduce risk of toxicity and resistance.

P-gp, an efflux transporter, plays an important role in the disposition of drugs and may affect the pharmacokinetics of drugs that are its substrates (Kim *et al.*, 1998b), including their co-administered counterparts (Perloff *et al.*, 2003; Stormer *et al.*, 2002; Thiebaut *et al.*, 1987). PZQ has not been conclusively characterised in relation to P-gp substrate specificity despite its widespread use in the management and control of schistosomiasis (Fenwick *et al.*, 2006; WHO, 2007c). Previous findings indicated that it was an inhibitor without being a substrate (Hayeshi *et al.*, 2006) or it does not interact with efflux transporters (Gonzalez-Esquivel *et al.*, 2005). Several high pressure liquid chromatography (HPLC) methods have been developed to quantify PZQ, but none for simultaneous quantification of PZQ and ARVs (Bonato *et al.*, 2007; Hanpitakpong *et al.*, 2004). We have validated an HPLC method for simultaneous quantification of both PZQ and SQV. This was then used to study the accumulation of PZQ in CEM and CEMVBL cells (the latter overexpress P-gp), in order to determine the substrate specificity for PZQ. SQV was used as a positive control. There was similar accumulation of PZQ in CEM and CEMVBL cells and

PZQ did not significantly affect the accumulation of SQV in either cell line. The study provided evidence that PZQ is neither a substrate nor an inhibitor of P-gp.

PZQ and IVM are among the drugs that may be co-administered with ARVs as a result of co-infection of HIV with schistosomiasis (Chenine *et al.*, 2008; Lloyd-Smith *et al.*, 2008; Secor *et al.*, 2004), or lymphatic filariasis (Nielsen *et al.*, 2006). This may lead to drug transporter or metabolic enzyme-mediated interactions (Baron *et al.*, 2001; Benet *et al.*, 2004; Perloff *et al.*, 2005). To date there is no information in the literature about interactions between the two groups of drugs. The study of the potential interactions between the two groups was conducted by assessing their transport through the Caco-2 cell monolayers (CCM). CCM provide a suitable model for the study of DDIs based on permeability of drugs through the monolayers, as they express transporters and metabolic enzymes (Hidalgo, 2001; Hilgers *et al.*, 1990; Peters *et al.*, 1989). The impact of PZQ and IVM on the transport of ARVs along the CCM was investigated. Furthermore, the influence of the ARVs on the transport of PZQ and IVM was also studied. IVM decreased the basolateral (BL) to apical (AP) [BL→AP] transport of SQV by threefold, suggesting inhibition. It facilitated the BL movement of LPV by eightfold, while LPV increased the transport of IVM in the opposite direction. NVP inhibited the transport of IVM. These studies showed that IVM interacts with SQV, NVP and LPV. Further studies are now required to determine the clinical significance *in vivo*.

The ability of PZQ and IVM to induce ABCB, and CYP3A4 and CYP2B6 genes in Caco-2 cells was also investigated. Previous studies on PZQ indicated that it did not induce CYP1A1 (Bapiro *et al.*, 2002), but the information for IVM is conflicting. In

one study it was reported that it induced CYP enzymes including CYP1A, CYP2B and CYP3A sub-families in mouflon sheep (Skalova *et al.*, 2001), whereas a related study concluded that it did not induce CYP1A1 or CYP1A2 (Bapiro *et al.*, 2002). Results presented in this thesis show that neither drug induces any of these genes, in comparison to rifampicin, and inducer of ABCB1 and CYP3A4 and phenobarbital an inducer of CYP2B6.

One of the main reasons why HAART does not completely eradicate HIV is the inability of the drugs to reach sanctuary sites of the virus such as macrophages (Vyas *et al.*, 2006). This may result in relapses and increased likelihood of resistance. Nanodispersion technology is a new promising technology that may assist in delivering ARVs to these sites (Emerich *et al.*, 2006). Previous studies have demonstrated that this technology may improve bioavailability (Kidane *et al.*, 2005; Zhang *et al.*, 2008b), and circumvent the transporter mediated efflux of drugs (Vauthier *et al.*, 2003). Most protease inhibitors (PIs) are substrates of both drug transporters and metabolic enzymes which may affect their efficacy. SQV in particular has low oral bioavailability and is a substrate for P-gp (Kim *et al.*, 1998a). The transport of nanoengineered SQV samples across the CCM was compared to normal SQV, and nanodispersed samples appeared to have the same transport profiles as that of normal SQV. From the accumulation studies however, three out of five of the samples accumulated to a higher degree in CEM cells indicating that there is enhanced delivery to these cells at physiologically relevant P-gp density. Clearly this is an interesting area and at the moment there is ongoing research at the University of Liverpool aimed at nano-enhancing ARVs.

In conclusion further research should be conducted on the clinical implications of the interactions between IVM and ARVs. The research should be extended to interactions between IVM and PZQ with other co-administered drugs, with a view to prevention of resistance. This is especially important as the two drugs are the mainstay in the mass treatment and control of two diseases that afflict millions of people in the developing world, yet very few studies have been conducted to understand their pharmacokinetics. Schistosomiasis is endemic in 74 countries with 200 million infected and 280,000 deaths annually (Savioli *et al.*, 2004a; Savioli *et al.*, 2004b; Steinmann *et al.*, 2006; WHO, 2007c). Lymphatic filariasis affects over 120 million and over 1 billion are at risk in more than 80 countries (WHO, 2000b). The most worrying issue is that the emergence of resistance to the drugs has now been reported (Osei-Atweneboana *et al.*, 2007), and the modulation of P-gp has been considered as a possible strategy to reverse the resistance (Lespine *et al.*, 2008). The extent and severity of DDIs between ARVs and co-administered drugs has been addressed. There should be more effort to reduce DDIs to maximize the benefits of HAART and reduce resistance and risk of harm. Finally, further research should be conducted on nanotechnology as a drug delivery system for ARVs.

References

Abdulla, MH, Lim, KC, Sajid, M, McKerrow, JH, Caffrey, CR (2007) Schistosomiasis mansoni: novel chemotherapy using a cysteine protease inhibitor. *PLoS Med* **4**(1): e14.

Acosta, EP, Kakuda, TN, Brundage, RC, Anderson, PL, Fletcher, CV (2000) Pharmacodynamics of human immunodeficiency virus type 1 protease inhibitors. *Clin Infect Dis* **30 Suppl 2:** S151-159.

Adams, M, Sharmeen, L, Kimpton, J, Romeo, JM, Garcia, JV, Peterlin, BM, Groudine, M, Emerman, M (1994) Cellular latency in human immunodeficiency virus-infected individuals with high CD4 levels can be detected by the presence of promoter-proximal transcripts. *Proc Natl Acad Sci U S A* **91**(9): 3862-3866.

Adkins, JC, Noble, S (1998) Efavirenz. *Drugs* **56**(6): 1055-1064; discussion 1065-1056.

Adson, A, Burton, PS, Raub, TJ, Barsuhn, CL, Audus, KL, Ho, NF (1995) Passive diffusion of weak organic electrolytes across Caco-2 cell monolayers: uncoupling the contributions of hydrodynamic, transcellular, and paracellular barriers. *J Pharm Sci* **84**(10): 1197-1204.

Agarwal, S, Pal, D, Mitra, AK (2007) Both P-gp and MRP2 mediate transport of Lopinavir, a protease inhibitor. *Int J Pharm* **339**(1-2): 139-147.

Alleman, MM, Twum-Danso, NA, Thylefors, BI (2006) The Mectizan Donation Program - highlights from 2005. *Filaria J* **5:** 11.

Allen, TM, Cullis, PR (2004a) Drug delivery systems: entering the mainstream. *Science* **303**(5665): 1818-1822.

Allen, TM, Martin, FJ (2004b) Advantages of liposomal delivery systems for anthracyclines. *Semin Oncol* **31**(6 Suppl 13): 5-15.

Alonso, MJ (2004) Nanomedicines for overcoming biological barriers. *Biomed Pharmacother* **58**(3): 168-172.

Alsenz, J, Haenel, E (2003) Development of a 7-day, 96-well Caco-2 permeability assay with high-throughput direct UV compound analysis. *Pharm Res* **20**(12): 1961-1969.

Alsenz, J, Steffen, H, Alex, R (1998) Active apical secretory efflux of the HIV protease inhibitors saquinavir and ritonavir in Caco-2 cell monolayers. *Pharm Res* **15**(3): 423-428.

Alvarez-Requejo, A, Carvajal, A, Begaud, B, Moride, Y, Vega, T, Arias, LH (1998) Under-reporting of adverse drug reactions. Estimate based on a spontaneous reporting scheme and a sentinel system. *Eur J Clin Pharmacol* **54**(6): 483-488.

Alvinerie, M, Dupuy, J, Kiki-Mvouaka, S, Sutra, JF, Lespine, A (2008) Ketoconazole increases the plasma levels of ivermectin in sheep. *Vet Parasitol* **157**(1-2): 117-122.

Anderle, P, Niederer, E, Rubas, W, Hilgendorf, C, Spahn-Langguth, H, Wunderli-Allenspach, H, Merkle, HP, Langguth, P (1998) P-Glycoprotein (P-gp) mediated efflux in Caco-2 cell monolayers: the influence of culturing conditions and drug exposure on P-gp expression levels. *J Pharm Sci* **87**(6): 757-762.

Angelucci, F, Basso, A, Bellelli, A, Brunori, M, Pica Mattoccia, L, Valle, C (2007) The anti-schistosomal drug praziquantel is an adenosine antagonist. *Parasitology* **134**(Pt 9): 1215-1221.

Anzenbacher, P, Anzenbacherova, E (2001) Cytochromes P450 and metabolism of xenobiotics. *Cell Mol Life Sci* **58**(5-6): 737-747.

Artursson, P (1990) Epithelial transport of drugs in cell culture. I: A model for studying the passive diffusion of drugs over intestinal absorptive (Caco-2) cells. *J Pharm Sci* **79**(6): 476-482.

Artursson, P, Karlsson, J (1991) Correlation between oral drug absorption in humans and apparent drug permeability coefficients in human intestinal epithelial (Caco-2) cells. *Biochem Biophys Res Commun* **175**(3): 880-885.

Artursson, P, Palm, K, Luthman, K (2001) Caco-2 monolayers in experimental and theoretical predictions of drug transport. *Adv Drug Deliv Rev* **46**(1-3): 27-43.

Avihingsanon A, ea (2007) 14th Conference on Retroviruses and Opportunistic Infections, Los Angeles, February 2007, abstract 576.

Awadzi, K, Edwards, G, Opoku, NO, Ardrey, AE, Favager, S, Addy, ET, Attah, SK, Yamuah, LK, Quartey, BT (2004) The safety, tolerability and pharmacokinetics of levamisole alone, levamisole plus ivermectin, and levamisole plus albendazole, and their efficacy against Onchocerca volvulus. *Ann Trop Med Parasitol* **98**(6): 595-614.

Ayrton, A, Morgan, P (2001) Role of transport proteins in drug absorption, distribution and excretion. *Xenobiotica* **31**(8-9): 469-497.

Bacchetti, P, Moss, AR (1989) Incubation period of AIDS in San Francisco. *Nature* **338**(6212): 251-253.

Back, D, Gibbons, S, Khoo, S (2003) Pharmacokinetic drug interactions with nevirapine. *J Acquir Immune Defic Syndr* **34 Suppl 1**: S8-14.

Baker, R (1995) FDA approves 3TC and saquinavir. Food and Drug Administration. *BETA*: 5, 9.

Bakker-Woudenberg, IA, Storm, G, Woodle, MC (1994) Liposomes in the treatment of infections. *J Drug Target* **2**(5): 363-371.

Ballent, M, Lifschitz, A, Virkel, G, Sallovitz, J, Lanusse, C (2006) Modulation of the P-glycoprotein-mediated intestinal secretion of ivermectin: in vitro and in vivo assessments. *Drug Metab Dispos* **34**(3): 457-463.

Balzarini, J (2004) Current status of the non-nucleoside reverse transcriptase inhibitors of human immunodeficiency virus type 1. *Curr Top Med Chem* **4**(9): 921-944.

Bapiro, TE, Andersson, TB, Otter, C, Hasler, JA, Masimirembwa, CM (2002) Cytochrome P450 1A1/2 induction by antiparasitic drugs: dose-dependent increase in ethoxyresorufin O-deethylase activity and mRNA caused by quinine, primaquine and albendazole in HepG2 cells. *Eur J Clin Pharmacol* **58**(8): 537-542.

Bardsley-Elliot, A, Perry, CM (2000) Nevirapine: a review of its use in the prevention and treatment of paediatric HIV infection. *Paediatr Drugs* **2**(5): 373-407.

Baron, JM, Goh, LB, Yao, D, Wolf, CR, Friedberg, T (2001) Modulation of P450 CYP3A4-dependent metabolism by P-glycoprotein: implications for P450 phenotyping. *J Pharmacol Exp Ther* **296**(2): 351-358.

Baroni, CD, Pezzella, F, Pezzella, M, Macchi, B, Vitolo, D, Uccini, S, Ruco, LP (1988) Expression of HIV in lymph node cells of LAS patients. Immunohistology, in situ hybridization, and identification of target cells. *Am J Pathol* **133**(3): 498-506.

Barre-Sinoussi, F, Chermann, JC, Rey, F, Nugeyre, MT, Chamaret, S, Gruest, J, Dauguet, C, Axler-Blin, C, Vezinet-Brun, F, Rouzioux, C, Rozenbaum, W, Montagnier, L (1983) Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science* **220**(4599): 868-871.

Barre-Sinoussi, F, Chermann, JC, Rey, F, Nugeyre, MT, Chamaret, S, Gruest, J, Dauguet, C, Axler-Blin, C, Vezinet-Brun, F, Rouzioux, C, Rozenbaum, W, Montagnier, L (2004) Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). 1983. *Rev Invest Clin* **56**(2): 126-129.

Barry, M, Mulcahy, F, Merry, C, Gibbons, S, Back, D (1999) Pharmacokinetics and potential interactions amongst antiretroviral agents used to treat patients with HIV infection. *Clin Pharmacokinet* **36**(4): 289-304.

Beach, JW (1998) Chemotherapeutic agents for human immunodeficiency virus infection: mechanism of action, pharmacokinetics, metabolism, and adverse reactions. *Clin Ther* **20**(1): 2-25; discussion 1.

Beck, WT, Mueller, TJ, Tanzer, LR (1979) Altered surface membrane glycoproteins in Vinca alkaloid-resistant human leukemic lymphoblasts. *Cancer Res* **39**(6 Pt 1): 2070-2076.

Benet, LZ, Cummins, CL, Wu, CY (2004) Unmasking the dynamic interplay between efflux transporters and metabolic enzymes. *Int J Pharm* **277**(1-2): 3-9.

Bennett, A, Guyatt, H (2000) Reducing intestinal nematode infection: efficacy of albendazole and mebendazole. *Parasitol Today* **16**(2): 71-74.

Berger, EA, Murphy, PM, Farber, JM (1999) Chemokine receptors as HIV-1 coreceptors: roles in viral entry, tropism, and disease. *Annu Rev Immunol* **17**: 657-700.

Berrey, MM, Schacker, T, Collier, AC, Shea, T, Brodie, SJ, Mayers, D, Coombs, R, Krieger, J, Chun, TW, Fauci, A, Self, SG, Corey, L (2001) Treatment of primary human immunodeficiency virus type 1 infection with potent antiretroviral therapy reduces frequency of rapid progression to AIDS. *J Infect Dis* **183**(10): 1466-1475.

Berruet, N, Sentenac, S, Auchere, D, Gimenez, F, Farinotti, R, Fernandez, C (2005) Effect of efavirenz on intestinal p-glycoprotein and hepatic p450 function in rats. *J Pharm Pharm Sci* **8**(2): 226-234.

Bertz, RJ, Granneman, GR (1997) Use of in vitro and in vivo data to estimate the likelihood of metabolic pharmacokinetic interactions. *Clin Pharmacokinet* **32**(3): 210-258.

BHIVA (2009) BHIVA treatment guidelines for TB/HIV co-infection (2009).

Biberfeld, P, Chayt, KJ, Marselle, LM, Biberfeld, G, Gallo, RC, Harper, ME (1986) HTLV-III expression in infected lymph nodes and relevance to pathogenesis of lymphadenopathy. *Am J Pathol* **125**(3): 436-442.

Biswas, P, Tambussi, G, Lazzarin, A (2007) Access denied? The status of co-receptor inhibition to counter HIV entry. *Expert Opin Pharmacother* **8**(7): 923-933.

Bonato, PS, de Oliveira, AR, de Santana, FJ, Fernandes, BJ, Lanchote, VL, Gonzalez, AE, Garcia, HH, Takayanagui, OM (2007) Simultaneous determination of albendazole metabolites, praziquantel and its metabolite in plasma by high-performance liquid chromatography-electrospray mass spectrometry. *J Pharm Biomed Anal* **44**(2): 558-563.

Borrow, P, Lewicki, H, Hahn, BH, Shaw, GM, Oldstone, MB (1994) Virus-specific CD8+ cytotoxic T-lymphocyte activity associated with control of viremia in primary human immunodeficiency virus type 1 infection. *J Virol* **68**(9): 6103-6110.

Bouley, M, Briere, C, Padoin, C, Petitjean, O, Tod, M (2001) Sensitive and rapid method for the simultaneous quantification of the HIV-protease inhibitors indinavir, nelfinavir, ritonavir, and saquinavir in human plasma by reversed-phase liquid chromatography. *Ther Drug Monit* **23**(1): 56-60.

Bravo, SA, Nielsen, CU, Amstrup, J, Frokjaer, S, Brodin, B (2004) In-depth evaluation of Gly-Sar transport parameters as a function of culture time in the Caco-2 cell model. *Eur J Pharm Sci* **21**(1): 77-86.

Brayden, DJ, Griffin, J (2008) Avermectin transepithelial transport in MDR1- and MRP-transfected canine kidney monolayers. *Vet Res Commun* **32**(1): 93-106.

Brindley, PJ, Strand, M, Norden, AP, Sher, A (1989) Role of host antibody in the chemotherapeutic action of praziquantel against Schistosoma mansoni: identification of target antigens. *Mol Biochem Parasitol* **34**(2): 99-108.

Brinkman, K, Huysmans, F, Burger, DM (1998a) Pharmacokinetic interaction between saquinavir and cyclosporine. *Ann Intern Med* **129**(11): 914-915.

Brinkman, K, ter Hofstede, HJ, Burger, DM, Smeitink, JA, Koopmans, PP (1998b) Adverse effects of reverse transcriptase inhibitors: mitochondrial toxicity as common pathway. *AIDS* **12**(14): 1735-1744.

Bukrinsky, MI, Stanwick, TL, Dempsey, MP, Stevenson, M (1991) Quiescent T lymphocytes as an inducible virus reservoir in HIV-1 infection. *Science* **254**(5030): 423-427.

Busch, MP, Lee, LL, Satten, GA, Henrard, DR, Farzadegan, H, Nelson, KE, Read, S, Dodd, RY, Petersen, LR (1995) Time course of detection of viral and serologic markers preceding human immunodeficiency virus type 1 seroconversion: implications for screening of blood and tissue donors. *Transfusion* **35**(2): 91-97.

Calcagno, AM, Ludwig, JA, Fostel, JM, Gottesman, MM, Ambudkar, SV (2006) Comparison of drug transporter levels in normal colon, colon cancer, and Caco-2 cells: impact on drug disposition and discovery. *Mol Pharm* **3**(1): 87-93.

Carr, A, Miller, J, Law, M, Cooper, DA (2000) A syndrome of lipoatrophy, lactic acidaemia and liver dysfunction associated with HIV nucleoside analogue therapy: contribution to protease inhibitor-related lipodystrophy syndrome. *AIDS* **14**(3): F25-32.

Castor, TP (2005) Phospholipid nanosomes. *Curr Drug Deliv* **2**(4): 329-340.

CDC (1992) 1993 revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. *MMWR Recomm Rep* **41**(RR-17): 1-19.

CDC (1993) From the Centers for Disease Control and prevention. 1993 revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. *JAMA* **269**(4): 460.

CDC (1982) MMWR Weekly 'Current Trends Update on Acquired Immune Deficiency Syndrome (AIDS)- United States', September 24, 31(37); 507-508, 513-514,.

Chan, DC, Kim, PS (1998) HIV entry and its inhibition. *Cell* **93**(5): 681-684.

Chapman, T, McGavin, J, Noble, S (2003) Tenofovir disoproxil fumarate. *Drugs* **63**(15): 1597-1608.

Chenine, AL, Shai-Kobiler, E, Steele, LN, Ong, H, Augostini, P, Song, R, Lee, SJ, Autissier, P, Ruprecht, RM, Secor, WE (2008) Acute Schistosoma mansoni Infection Increases Susceptibility to Systemic SHIV Clade C Infection in Rhesus Macaques after Mucosal Virus Exposure. *PLoS Negl Trop Dis* **2**(7): e265.

Chiou, WL, Chung, SM, Wu, TC (2000) Potential role of P-glycoprotein in affecting hepatic metabolism of drugs. *Pharm Res* **17**(8): 903-905.

Cimoch, PJ, Lavelle, J, Pollard, R, Griffy, KG, Wong, R, Tarnowski, TL, Casserella, S, Jung, D (1998) Pharmacokinetics of oral ganciclovir alone and in combination with zidovudine, didanosine, and probenecid in HIV-infected subjects. *J Acquir Immune Defic Syndr Hum Retrovirol* **17**(3): 227-234.

Cioli, D, Pica-Mattoccia, L (2003) Praziquantel. *Parasitol Res* **90 Supp 1:** S3-9.

Cioli, D, Pica-Mattoccia, L, Archer, S (1993) Drug resistance in schistosomes. *Parasitol Today* **9**(5): 162-166.

Clark, SJ, Saag, MS, Decker, WD, Campbell-Hill, S, Roberson, JL, Veldkamp, PJ, Kappes, JC, Hahn, BH, Shaw, GM (1991) High titers of cytopathic virus in plasma of patients with symptomatic primary HIV-1 infection. *N Engl J Med* **324**(14): 954-960.

Clarke, SM, Mulcahy, FM, Tjia, J, Reynolds, HE, Gibbons, SE, Barry, MG, Back, DJ (2001) Pharmacokinetic interactions of nevirapine and methadone and guidelines for use of nevirapine to treat injection drug users. *Clin Infect Dis* **33**(9): 1595-1597.

Claydon, EJ, Bennett, J, Gor, D, Forster, SM (1991) Transient elevation of serum HIV antigen levels associated with intercurrent infection. *AIDS* **5**(1): 113-114.

Cobo, F, Yarnoz, C, Sesma, B, Fraile, P, Aizcorbe, M, Trujillo, R, Diaz-de-Liano, A, Ciga, MA (1998) Albendazole plus praziquantel versus albendazole alone as a pre-operative treatment in intra-abdominal hydatisosis caused by Echinococcus granulosus. *Trop Med Int Health* **3**(6): 462-466.

Coffin, JM (1995) HIV population dynamics in vivo: implications for genetic variation, pathogenesis, and therapy. *Science* **267**(5197): 483-489.

Cohen, J (1993) AIDS research. Keystone's blunt message: 'it's the virus, stupid'. *Science* **260**(5106): 292-293.

Collett, A, Tanianis-Hughes, J, Hallifax, D, Warhurst, G (2004a) Predicting P-glycoprotein effects on oral absorption: correlation of transport in Caco-2 with drug pharmacokinetics in wild-type and mdr1a(-/-) mice in vivo. *Pharm Res* **21**(5): 819-826.

Collett, A, Tanianis-Hughes, J, Warhurst, G (2004b) Rapid induction of P-glycoprotein expression by high permeability compounds in colonic cells in vitro: a possible source of transporter mediated drug interactions? *Biochem Pharmacol* **68**(4): 783-790.

Collier, AC, Coombs, RW, Schoenfeld, DA, Bassett, RL, Timpone, J, Baruch, A, Jones, M, Facey, K, Whitacre, C, McAuliffe, VJ, Friedman, HM, Merigan, TC, Reichman, RC, Hooper, C, Corey, L (1996) Treatment of human immunodeficiency virus infection with saquinavir, zidovudine, and zalcitabine. AIDS Clinical Trials Group. *N Engl J Med* **334**(16): 1011-1017.

Cooper, DA, Gold, J, Maclean, P, Donovan, B, Finlayson, R, Barnes, TG, Michelmore, HM, Brooke, P, Penny, R (1985) Acute AIDS retrovirus infection. Definition of a clinical illness associated with seroconversion. *Lancet* **1**(8428): 537-540.

Cordon-Cardo, C, O'Brien, JP, Casals, D, Rittman-Grauer, L, Biedler, JL, Melamed, MR, Bertino, JR (1989) Multidrug-resistance gene (P-glycoprotein) is expressed by endothelial cells at blood-brain barrier sites. *Proc Natl Acad Sci U S A* **86**(2): 695-698.

Costa, SF, Weiss, LM (2000) Drug treatment of microsporidiosis. *Drug Resist Updat* **3**(6): 384-399.

Cottle, LE, Evans-Jones J.G, and Khoo S.H (2009) Physician awareness of antiretroviral drug interactions. BHIVA 2009, Abstr P138.

Cox, FE (2000) Elimination of lymphatic filariasis as a public health problem. *Parasitol Today* **16**(4): 135.

Craig, JC, Duncan, IB, Hockley, D, Grief, C, Roberts, NA, Mills, JS (1991) Antiviral properties of Ro 31-8959, an inhibitor of human immunodeficiency virus (HIV) proteinase. *Antiviral Res* **16**(4): 295-305.

Cully, DF, Vassilatis, DK, Liu, KK, Paress, PS, Van der Ploeg, LH, Schaeffer, JM, Arena, JP (1994) Cloning of an avermectin-sensitive glutamate-gated chloride channel from Caenorhabditis elegans. *Nature* **371**(6499): 707-711.

Cummins, CL, Jacobsen, W, Christians, U, Benet, LZ (2004) CYP3A4-transfected Caco-2 cells as a tool for understanding biochemical absorption barriers: studies with sirolimus and midazolam. *J Pharmacol Exp Ther* **308**(1): 143-155.

D'Souza, VM, Shertzer, HG, Menon, AG, Pauletti, GM (2003) High glucose concentration in isotonic media alters caco-2 cell permeability. *AAPS PharmSci* **5**(3): E24.

Daar, ES, Moudgil, T, Meyer, RD, Ho, DD (1991) Transient high levels of viremia in patients with primary human immunodeficiency virus type 1 infection. *N Engl J Med* **324**(14): 961-964.

Dalgleish, AG, Beverley, PC, Clapham, PR, Crawford, DH, Greaves, MF, Weiss, RA (1984) The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. *Nature* **312**(5996): 763-767.

Dayan, AD (2003) Albendazole, mebendazole and praziquantel. Review of non-clinical toxicity and pharmacokinetics. *Acta Trop* **86**(2-3): 141-159.

de Maat, MM, de Boer, A, Koks, CH, Mulder, JW, Meenhorst, PL, van Gorp, EC, Mairuhu, AT, Huitema, AD, Beijnen, JH (2004) Evaluation of clinical pharmacist interventions on drug interactions in outpatient pharmaceutical HIV-care. *J Clin Pharm Ther* **29**(2): 121-130.

de Maat, MM, Ekhart, GC, Huitema, AD, Koks, CH, Mulder, JW, Beijnen, JH (2003) Drug interactions between antiretroviral drugs and comedicated agents. *Clin Pharmacokinet* **42**(3): 223-282.

Deeks, SG, Volberding, PA (1997) HIV-1 protease inhibitors. *AIDS Clin Rev*: 145-185.

Dickinson, L, Robinson, L, Tjia, J, Khoo, S, Back, D (2005) Simultaneous determination of HIV protease inhibitors amprenavir, atazanavir, indinavir, lopinavir, nelfinavir, ritonavir and saquinavir in human plasma by high-performance liquid chromatography-tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* **829**(1-2): 82-90.

Didier, A, Loor, F (1996) The abamectin derivative ivermectin is a potent P-glycoprotein inhibitor. *Anticancer Drugs* **7**(7): 745-751.

Dieleman, JP, Gyssens, IC, van der Ende, ME, de Marie, S, Burger, DM (1999) Urological complaints in relation to indinavir plasma concentrations in HIV-infected patients. *AIDS* **13**(4): 473-478.

Dieterich, DT, Robinson, PA, Love, J, Stern, JO (2004) Drug-induced liver injury associated with the use of nonnucleoside reverse-transcriptase inhibitors. *Clin Infect Dis* **38 Suppl 2:** S80-89.

Doenhoff, MJ, Cioli, D, Utzinger, J (2008) Praziquantel: mechanisms of action, resistance and new derivatives for schistosomiasis. *Curr Opin Infect Dis* **21**(6): 659-667.

Dollery, CT (1999) Albendazole. In: Therapeutic Drugs, vol.1, second ed. Churchill Livingstone, Edinburgh, pp. A51 - A53.

Dresser, GK, Spence, JD, Bailey, DG (2000) Pharmacokinetic-pharmacodynamic consequences and clinical relevance of cytochrome P450 3A4 inhibition. *Clin Pharmacokinet* **38**(1): 41-57.

Dresser, MJ, Leabman, MK, Giacomini, KM (2001) Transporters involved in the elimination of drugs in the kidney: organic anion transporters and organic cation transporters. *J Pharm Sci* **90**(4): 397-421.

Drewe, J, Gutmann, H, Fricker, G, Torok, M, Beglinger, C, Huwyler, J (1999) HIV protease inhibitor ritonavir: a more potent inhibitor of P-glycoprotein than the cyclosporine analog SDZ PSC 833. *Biochem Pharmacol* **57**(10): 1147-1152.

Duncan, R, Vicent, MJ, Greco, F, Nicholson, RI (2005) Polymer-drug conjugates: towards a novel approach for the treatment of endrocine-related cancer. *Endocr Relat Cancer* **12 Suppl 1:** S189-199.

Dybul, M, Fauci, AS, Bartlett, JG, Kaplan, JE, Pau, AK (2002) Guidelines for using antiretroviral agents among HIV-infected adults and adolescents. *Ann Intern Med* **137**(5 Pt 2): 381-433.

Eagling, VA, Back, DJ, Barry, MG (1997) Differential inhibition of cytochrome P450 isoforms by the protease inhibitors, ritonavir, saquinavir and indinavir. *Br J Clin Pharmacol* **44**(2): 190-194.

Ecker, G, Huber, M, Schmid, D, Chiba, P (1999) The importance of a nitrogen atom in modulators of multidrug resistance. *Mol Pharmacol* **56**(4): 791-796.

Edwards, G (2003) Ivermectin: does P-glycoprotein play a role in neurotoxicity? *Filaria J* **2 Suppl 1:** S8.

el Amri, HS, Fargetton, X, Delatour, P, Batt, AM (1987) Sulphoxidation of albendazole by the FAD-containing and cytochrome P-450 dependent mono-oxygenases from pig liver microsomes. *Xenobiotica* **17**(10): 1159-1168.

El Maghraby, GM, Barry, BW, Williams, AC (2008) Liposomes and skin: from drug delivery to model membranes. *Eur J Pharm Sci* **34**(4-5): 203-222.

Elliott, DE (1996) Schistosomiasis. Pathophysiology, diagnosis, and treatment. *Gastroenterol Clin North Am* **25**(3): 599-625.

Emerich, DF, Thanos, CG (2006) The pinpoint promise of nanoparticle-based drug delivery and molecular diagnosis. *Biomol Eng* **23**(4): 171-184.

Endicott, JA, Ling, V (1989) The biochemistry of P-glycoprotein-mediated multidrug resistance. *Annu Rev Biochem* **58:** 137-171.

Engman, HA, Lennernas, H, Taipalensuu, J, Otter, C, Leidvik, B, Artursson, P (2001) CYP3A4, CYP3A5, and MDR1 in human small and large intestinal cell lines suitable for drug transport studies. *J Pharm Sci* **90**(11): 1736-1751.

Epivir (2008) Summary of Product Characteristics, Glaxo Group Ltd, September 2008.

Erickson, DA, Mather, G, Trager, WF, Levy, RH, Keirns, JJ (1999) Characterization of the in vitro biotransformation of the HIV-1 reverse transcriptase inhibitor nevirapine by human hepatic cytochromes P-450. *Drug Metab Dispos* **27**(12): 1488-1495.

Ernest, CS, 2nd, Hall, SD, Jones, DR (2005) Mechanism-based inactivation of CYP3A by HIV protease inhibitors. *J Pharmacol Exp Ther* **312**(2): 583-591.

Fang, AF, Damle, BD, LaBadie, RR, Crownover, PH, Hewlett, D, Jr., Glue, PW (2008) Significant decrease in nelfinavir systemic exposure after omeprazole coadministration in healthy subjects. *Pharmacotherapy* **28**(1): 42-50.

Faucette, SR, Wang, H, Hamilton, GA, Jolley, SL, Gilbert, D, Lindley, C, Yan, B, Negishi, M, LeCluyse, EL (2004) Regulation of CYP2B6 in primary human hepatocytes by prototypical inducers. *Drug Metab Dispos* **32**(3): 348-358.

Fauci, AS (1996) Host factors in the pathogenesis of HIV disease. *Antibiot Chemother* **48**: 4-12.

Fauci, AS (1993) Immunopathogenesis of HIV infection. *J Acquir Immune Defic Syndr* **6**(6): 655-662.

Fenwick, A, Webster, JP (2006) Schistosomiasis: challenges for control, treatment and drug resistance. *Curr Opin Infect Dis* **19**(6): 577-582.

Ferrari, ML, Coelho, PM, Antunes, CM, Tavares, CA, da Cunha, AS (2003) Efficacy of oxamniquine and praziquantel in the treatment of Schistosoma mansoni infection: a controlled trial. *Bull World Health Organ* **81**(3): 190-196.

Figgitt, DP, Plosker, GL (2000) Saquinavir soft-gel capsule: an updated review of its use in the management of HIV infection. *Drugs* **60**(2): 481-516.

Fink, DPA (1989) Pharmacokinetics of ivermectin in animals and humans. In W.C. Campbell (ed.), Ivermectin and abamectin: Springer, New York, 1989, pp. 113-130.

Flexner, C (2000) Dual protease inhibitor therapy in HIV-infected patients: pharmacologic rationale and clinical benefits. *Annu Rev Pharmacol Toxicol* **40**: 649-674.

Flexner, C (1998) HIV-protease inhibitors. *N Engl J Med* **338**(18): 1281-1292.

Foley, GE, Lazarus, H, Farber, S, Uzman, BG, Boone, BA, McCarthy, RE (1965) Continuous Culture of Human Lymphoblasts from Peripheral Blood of a Child with Acute Leukemia. *Cancer* **18**: 522-529.

Ford, J, Boffito, M, Wildfire, A, Hill, A, Back, D, Khoo, S, Nelson, M, Moyle, G, Gazzard, B, Pozniak, A (2004) Intracellular and plasma pharmacokinetics of saquinavir-ritonavir, administered at 1,600/100 milligrams once daily in human immunodeficiency virus-infected patients. *Antimicrob Agents Chemother* **48**(7): 2388-2393.

Fortovase (2006) Discontinuation of Fortovase, Roche Laboratories Inc., New Jersey , U.S.A. February 15, 2006.

Fortovase (2003) FortovaseTM (saquinavir) soft gelatin capsules. Summary of product Characteristics, F-Hoffman La Roche Ltd, Basel, Switzerland (December 2003).

Fromenty, B, Pessayre, D (1997) Impaired mitochondrial function in microvesicular steatosis. Effects of drugs, ethanol, hormones and cytokines. *J Hepatol* **26 Suppl 2:** 43-53.

Fuhr, U (1998) Drug interactions with grapefruit juice. Extent, probable mechanism and clinical relevance. *Drug Saf* **18**(4): 251-272.

Fung, HB, Stone, EA, Piacenti, FJ (2002) Tenofovir disoproxil fumarate: a nucleotide reverse transcriptase inhibitor for the treatment of HIV infection. *Clin Ther* **24**(10): 1515-1548.

Gallagher, M, Malhotra, I, Mungai, PL, Wamachi, AN, Kioko, JM, Ouma, JH, Muchiri, E, King, CL (2005) The effects of maternal helminth and malaria infections on mother-to-child HIV transmission. *AIDS* **19**(16): 1849-1855.

Gallant, JE (2004) Protease-inhibitor boosting in the treatment-experienced patient. *AIDS Rev* **6**(4): 226-233.

Garcia-Garcia, E, Andrieux, K, Gil, S, Couvreur, P (2005) Colloidal carriers and blood-brain barrier (BBB) translocation: a way to deliver drugs to the brain? *Int J Pharm* **298**(2): 274-292.

German, P, Parikh, S, Lawrence, J, Dorsey, G, Rosenthal, PJ, Havlir, D, Charlebois, E, Hanpithakpong, W, Lindegardh, N, Aweeka, FT (2009) Lopinavir/ritonavir affects pharmacokinetic exposure of artemether/lumefantrine in HIV-uninfected healthy volunteers. *J Acquir Immune Defic Syndr* **51**(4): 424-429.

Gimenez, F, Fernandez, C, Mabondzo, A (2004) Transport of HIV protease inhibitors through the blood-brain barrier and interactions with the efflux proteins, P-glycoprotein and multidrug resistance proteins. *J Acquir Immune Defic Syndr* **36**(2): 649-658.

Giorgi, M, Salvatori, AP, Soldani, G, Giusiani, M, Longo, V, Gervasi, PG, Mengozzi, G (2001) Pharmacokinetics and microsomal oxidation of praziquantel and its effects on the P450 system in three-month-old lambs infested by Fasciola hepatica. *J Vet Pharmacol Ther* **24**(4): 251-259.

Gonzalez-Esquivel, D, Rivera, J, Castro, N, Yepez-Mulia, L, Jung Cook, H (2005) In vitro characterization of some biopharmaceutical properties of praziquantel. *Int J Pharm* **295**(1-2): 93-99.

Gonzalez Canga, A, Sahagun Prieto, AM, Diez Liebana, MJ, Fernandez Martinez, N, Sierra Vega, M, Garcia Vieitez, JJ (2008) The pharmacokinetics and interactions of ivermectin in humans--a mini-review. *AAPS J* **10**(1): 42-46.

Goto, M, Masuda, S, Saito, H, Inui, K (2003) Decreased expression of P-glycoprotein during differentiation in the human intestinal cell line Caco-2. *Biochem Pharmacol* **66**(1): 163-170.

Gottesman, MM, Pastan, I (1993) Biochemistry of multidrug resistance mediated by the multidrug transporter. *Annu Rev Biochem* **62:** 385-427.

Greenberg, RM (2005a) Are Ca2+ channels targets of praziquantel action? *Int J Parasitol* **35**(1): 1-9.

Greenberg, RM (2005b) Ca2+ signalling, voltage-gated Ca2+ channels and praziquantel in flatworm neuromusculature. *Parasitology* **131 Suppl:** S97-108.

Gres, MC, Julian, B, Bourrie, M, Meunier, V, Roques, C, Berger, M, Boulenc, X, Berger, Y, Fabre, G (1998) Correlation between oral drug absorption in humans, and apparent drug permeability in TC-7 cells, a human epithelial intestinal cell line: comparison with the parental Caco-2 cell line. *Pharm Res* **15**(5): 726-733.

Grim, SA, Romanelli, F (2003) Tenofovir disoproxil fumarate. *Ann Pharmacother* **37**(6): 849-859.

Grub, S, Bryson, H, Goggin, T, Ludin, E, Jorga, K (2001) The interaction of saquinavir (soft gelatin capsule) with ketoconazole, erythromycin and rifampicin: comparison of the effect in healthy volunteers and in HIV-infected patients. *Eur J Clin Pharmacol* **57**(2): 115-121.

Gryseels, B, Polman, K, Clerinx, J, Kestens, L (2006) Human schistosomiasis. *Lancet* **368**(9541): 1106-1118.

Guengerich, FP (1999) Cytochrome P-450 3A4: regulation and role in drug metabolism. *Annu Rev Pharmacol Toxicol* **39:** 1-17.

Guengerich, FP, Brian, WR, Iwasaki, M, Sari, MA, Baarnhielm, C, Berntsson, P (1991) Oxidation of dihydropyridine calcium channel blockers and analogues by human liver cytochrome P-450 IIIA4. *J Med Chem* **34**(6): 1838-1844.

Gupta, B, Levchenko, TS, Torchilin, VP (2005) Intracellular delivery of large molecules and small particles by cell-penetrating proteins and peptides. *Adv Drug Deliv Rev* **57**(4): 637-651.

Gupta, KK (1993) Acute immunosuppression with HIV seroconversion. *N Engl J Med* **328**(4): 288-289.

Haase, AT (1986a) Pathogenesis of lentivirus infections. *Nature* **322**(6075): 130-136.

Haase, AT (1986b) The pathogenesis of slow virus infections: molecular analyses. *J Infect Dis* **153**(3): 441-447.

Hamers, FF, Downs, AM (2003) HIV in central and eastern Europe. *Lancet* **361**(9362): 1035-1044.

Hanpitakpong, W, Banmairuroi, V, Kamanikom, B, Choemung, A, Na-Bangchang, K (2004) A high-performance liquid chromatographic method for determination of praziquantel in plasma. *J Pharm Biomed Anal* **36**(4): 871-876.

Hare, CB (2006) Clinical Overview of HIV Disease , HIV InSite Knowledge Base Chapter, January 2006, University of California San Francisco.

Havlir, DV, Gilbert, PB, Bennett, K, Collier, AC, Hirsch, MS, Tebas, P, Adams, EM, Wheat, LJ, Goodwin, D, Schnittman, S, Holohan, MK, Richman, DD (2001) Effects of treatment intensification with hydroxyurea in HIV-infected patients with virologic suppression. *AIDS* **15**(11): 1379-1388.

Hayeshi, R, Masimirembwa, C, Mukanganyama, S, Ungell, AL (2006) The potential inhibitory effect of antiparasitic drugs and natural products on P-glycoprotein mediated efflux. *Eur J Pharm Sci* **29**(1): 70-81.

Hazenberg, MD, Hamann, D, Schuitemaker, H, Miedema, F (2000) T cell depletion in HIV-1 infection: how CD4+ T cells go out of stock. *Nat Immunol* **1**(4): 285-289.

Hedaya, MA, Elmquist, WF, Sawchuk, RJ (1990) Probenecid inhibits the metabolic and renal clearances of zidovudine (AZT) in human volunteers. *Pharm Res* **7**(4): 411-417.

Hidalgo, IJ (2001) Assessing the absorption of new pharmaceuticals. *Curr Top Med Chem* **1**(5): 385-401.

Hidalgo, IJ, Borchardt, RT (1990) Transport of bile acids in a human intestinal epithelial cell line, Caco-2. *Biochim Biophys Acta* **1035**(1): 97-103.

Hidalgo, IJ, Raub, TJ, Borchardt, RT (1989) Characterization of the human colon carcinoma cell line (Caco-2) as a model system for intestinal epithelial permeability. *Gastroenterology* **96**(3): 736-749.

Higgins, CF, Gottesman, MM (1992) Is the multidrug transporter a flippase? *Trends Biochem Sci* **17**(1): 18-21.

Hilgers, AR, Conradi, RA, Burton, PS (1990) Caco-2 cell monolayers as a model for drug transport across the intestinal mucosa. *Pharm Res* **7**(9): 902-910.

Hirohashi, T, Suzuki, H, Chu, XY, Tamai, I, Tsuji, A, Sugiyama, Y (2000) Function and expression of multidrug resistance-associated protein family in human colon adenocarcinoma cells (Caco-2). *J Pharmacol Exp Ther* **292**(1): 265-270.

HIVID (2000) Hoffman-La Roche, 2000.

Ho, DD, Neumann, AU, Perelson, AS, Chen, W, Leonard, JM, Markowitz, M (1995) Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. *Nature* **373**(6510): 123-126.

Ho, DD, Pomerantz, RJ, Kaplan, JC (1987) Pathogenesis of infection with human immunodeficiency virus. *N Engl J Med* **317**(5): 278-286.

Ho, RH, Kim, RB (2005) Transporters and drug therapy: implications for drug disposition and disease. *Clin Pharmacol Ther* **78**(3): 260-277.

Hoerauf, A, Mand, S, Adjei, O, Fleischer, B, Buttner, DW (2001) Depletion of wolbachia endobacteria in Onchocerca volvulus by doxycycline and microfilaridermia after ivermectin treatment. *Lancet* **357**(9266): 1415-1416.

Hoetelmans, RM, Meenhorst, PL, Mulder, JW, Burger, DM, Koks, CH, Beijnen, JH (1997) Clinical pharmacology of HIV protease inhibitors: focus on saquinavir, indinavir, and ritonavir. *Pharm World Sci* **19**(4): 159-175.

Hoggard, PG, Owen, A (2003) The mechanisms that control intracellular penetration of the HIV protease inhibitors. *J Antimicrob Chemother* **51**(3): 493-496.

Hoggard, PG, Sales, SD, Kewn, S, Sunderland, D, Khoo, SH, Hart, CA, Back, DJ (2000) Correlation between intracellular pharmacological activation of nucleoside analogues and HIV suppression in vitro. *Antivir Chem Chemother* **11**(6): 353-358.

Holland, DT, DiFrancesco, R, Connor, JD, Morse, GD (2006) Quality assurance program for pharmacokinetic assay of antiretrovirals: ACTG proficiency testing for pediatric and adult pharmacology support laboratories, 2003 to 2004: a requirement for therapeutic drug monitoring. *Ther Drug Monit* **28**(3): 367-374.

Horn, D, Rieger, J (2001) Organic Nanoparticles in the Aqueous Phase-Theory, Experiment, and Use. *Angew Chem Int Ed Engl* **40**(23): 4330-4361.

Hotez, PJ, Molyneux, DH, Fenwick, A, Kumaresan, J, Sachs, SE, Sachs, JD, Savioli, L (2007) Control of neglected tropical diseases. *N Engl J Med* **357**(10): 1018-1027.

Hsu, A, Granneman, GR, Bertz, RJ (1998a) Ritonavir. Clinical pharmacokinetics and interactions with other anti-HIV agents. *Clin Pharmacokinet* **35**(4): 275-291.

Hsu, A, Granneman, GR, Cao, G, Carothers, L, el-Shourbagy, T, Baroldi, P, Erdman, K, Brown, F, Sun, E, Leonard, JM (1998b) Pharmacokinetic interactions between two human immunodeficiency virus protease inhibitors, ritonavir and saquinavir. *Clin Pharmacol Ther* **63**(4): 453-464.

Huang, L, Wring, SA, Woolley, JL, Brouwer, KR, Serabjit-Singh, C, Polli, JW (2001) Induction of P-glycoprotein and cytochrome P450 3A by HIV protease inhibitors. *Drug Metab Dispos* **29**(5): 754-760.

Huang, R, Murry, DJ, Kolwankar, D, Hall, SD, Foster, DR (2006) Vincristine transcriptional regulation of efflux drug transporters in carcinoma cell lines. *Biochem Pharmacol* **71**(12): 1695-1704.

Hubatsch, I, Ragnarsson, EG, Artursson, P (2007) Determination of drug permeability and prediction of drug absorption in Caco-2 monolayers. *Nat Protoc* **2**(9): 2111-2119.

Huisman, MT, Smit, JW, Schinkel, AH (2000) Significance of P-glycoprotein for the pharmacology and clinical use of HIV protease inhibitors. *AIDS* **14**(3): 237-242.

Hunter, J, Jepson, MA, Tsuruo, T, Simmons, NL, Hirst, BH (1993) Functional expression of P-glycoprotein in apical membranes of human intestinal Caco-2 cells. Kinetics of vinblastine secretion and interaction with modulators. *J Biol Chem* **268**(20): 14991-14997.

Ikezoe, T, Hisatake, Y, Takeuchi, T, Ohtsuki, Y, Yang, Y, Said, JW, Taguchi, H, Koeffler, HP (2004) HIV-1 protease inhibitor, ritonavir: a potent inhibitor of CYP3A4, enhanced the anticancer effects of docetaxel in androgen-independent prostate cancer cells in vitro and in vivo. *Cancer Res* **64**(20): 7426-7431.

Inui, K, Yamamoto, M. and Saito, H (1991) Organic cation transport in the renal brush-border and basolateral membranes. In Hatano, M. (ed): Nephrology, Tokyo, Springer-Verlag Tokyo, 1991, pp.1391-1398.

IOTA IOTA NanoSolutions Limited, MerseyBIO, Crown Street, Liverpool, L69 7ZB.

Jacobo-Molina, A, Ding, J, Nanni, RG, Clark, AD, Jr., Lu, X, Tantillo, C, Williams, RL, Kamer, G, Ferris, AL, Clark, P, et al. (1993) Crystal structure of human immunodeficiency virus type 1 reverse transcriptase complexed with double-stranded DNA at 3.0 A resolution shows bent DNA. *Proc Natl Acad Sci U S A* **90**(13): 6320-6324.

James, CE, Davey, MW (2008) Increased expression of ABC transport proteins is associated with ivermectin resistance in the model nematode Caenorhabditis elegans. *Int J Parasitol.*

Janneh, O, Hartkoorn, RC, Jones, E, Owen, A, Ward, SA, Davey, R, Back, DJ, Khoo, SH (2008) Cultured CD4T cells and primary human lymphocytes express hOATPs: intracellular accumulation of saquinavir and lopinavir. *Br J Pharmacol* **155**(6): 875-883.

Janneh, O, Jones, E, Chandler, B, Owen, A, Khoo, SH (2007) Inhibition of P-glycoprotein and multidrug resistance-associated proteins modulates the intracellular concentration of lopinavir in cultured CD4 T cells and primary human lymphocytes. *J Antimicrob Chemother* **60**(5): 987-993.

Janneh, O, Owen, A, Chandler, B, Hartkoorn, RC, Hart, CA, Bray, PG, Ward, SA, Back, DJ, Khoo, SH (2005) Modulation of the intracellular accumulation of saquinavir in peripheral blood mononuclear cells by inhibitors of MRP1, MRP2, P-gp and BCRP. *AIDS* **19**(18): 2097-2102.

Jones, K, Bray, PG, Khoo, SH, Davey, RA, Meaden, ER, Ward, SA, Back, DJ (2001a) P-Glycoprotein and transporter MRP1 reduce HIV protease inhibitor uptake in CD4 cells: potential for accelerated viral drug resistance? *AIDS* **15**(11): 1353-1358.

Jones, K, Hoggard, PG, Sales, SD, Khoo, S, Davey, R, Back, DJ (2001b) Differences in the intracellular accumulation of HIV protease inhibitors in vitro and the effect of active transport. *AIDS* **15**(6): 675-681.

Jung, H, Medina, R, Castro, N, Corona, T, Sotelo, J (1997) Pharmacokinetic study of praziquantel administered alone and in combination with cimetidine in a single-day therapeutic regimen. *Antimicrob Agents Chemother* **41**(6): 1256-1259.

Kakuda, TN (2000) Pharmacology of nucleoside and nucleotide reverse transcriptase inhibitor-induced mitochondrial toxicity. *Clin Ther* **22**(6): 685-708.

Kallestrup, P, Zinyama, R, Gomo, E, Butterworth, AE, van Dam, GJ, Erikstrup, C, Ullum, H (2005) Schistosomiasis and HIV-1 infection in rural Zimbabwe: implications of coinfection for excretion of eggs. *J Infect Dis* **191**(8): 1311-1320.

Kashuba, AD (2005) Drug-drug interactions and the pharmacotherapy of HIV infection. *Top HIV Med* **13**(2): 64-69.

Kattan, J, Droz, JP, Couvreur, P, Marino, JP, Boutan-Laroze, A, Rougier, P, Brault, P, Vranckx, H, Grognet, JM, Morge, X, et al. (1992) Phase I clinical trial and pharmacokinetic evaluation of doxorubicin carried by polyisohexylcyanoacrylate nanoparticles. *Invest New Drugs* **10**(3): 191-199.

Kaufman, J, Jing, J (2002) China and AIDS--the time to act is now. *Science* **296**(5577): 2339-2340.

Kesisoglou, F, Panmai, S, Wu, Y (2007) Nanosizing--oral formulation development and biopharmaceutical evaluation. *Adv Drug Deliv Rev* **59**(7): 631-644.

Khoo, SH, Hoggard, PG, Williams, I, Meaden, ER, Newton, P, Wilkins, EG, Smith, A, Tjia, JF, Lloyd, J, Jones, K, Beeching, N, Carey, P, Peters, B, Back, DJ (2002) Intracellular accumulation of human immunodeficiency virus protease inhibitors. *Antimicrob Agents Chemother* **46**(10): 3228-3235.

Kidane, A, Bhatt, PP (2005) Recent advances in small molecule drug delivery. *Curr Opin Chem Biol* **9**(4): 347-351.

Kim, AE, Dintaman, JM, Waddell, DS, Silverman, JA (1998a) Saquinavir, an HIV protease inhibitor, is transported by P-glycoprotein. *J Pharmacol Exp Ther* **286**(3): 1439-1445.

Kim, RB (2003) Organic anion-transporting polypeptide (OATP) transporter family and drug disposition. *Eur J Clin Invest* **33 Suppl 2:** 1-5.

Kim, RB, Fromm, MF, Wandel, C, Leake, B, Wood, AJ, Roden, DM, Wilkinson, GR (1998b) The drug transporter P-glycoprotein limits oral absorption and brain entry of HIV-1 protease inhibitors. *J Clin Invest* **101**(2): 289-294.

Kim, RB, Wandel, C, Leake, B, Cvetkovic, M, Fromm, MF, Dempsey, PJ, Roden, MM, Belas, F, Chaudhary, AK, Roden, DM, Wood, AJ, Wilkinson, GR (1999) Interrelationship between substrates and inhibitors of human CYP3A and P-glycoprotein. *Pharm Res* **16**(3): 408-414.

King, CH, Dickman, K, Tisch, DJ (2005) Reassessment of the cost of chronic helmintic infection: a meta-analysis of disability-related outcomes in endemic schistosomiasis. *Lancet* **365**(9470): 1561-1569.

Kjetland, EF, Ndhlovu, PD, Gomo, E, Mduluza, T, Midzi, N, Gwanzura, L, Mason, PR, Sandvik, L, Friis, H, Gundersen, SG (2006) Association between genital schistosomiasis and HIV in rural Zimbabwean women. *AIDS* **20**(4): 593-600.

Klimecki, WT, Futscher, BW, Grogan, TM, Dalton, WS (1994) P-glycoprotein expression and function in circulating blood cells from normal volunteers. *Blood* **83**(9): 2451-2458.

Kohl, NE, Emini, EA, Schleif, WA, Davis, LJ, Heimbach, JC, Dixon, RA, Scolnick, EM, Sigal, IS (1988) Active human immunodeficiency virus protease is required for viral infectivity. *Proc Natl Acad Sci U S A* **85**(13): 4686-4690.

Kohn, AB, Anderson, PA, Roberts-Misterly, JM, Greenberg, RM (2001) Schistosome calcium channel beta subunits. Unusual modulatory effects and potential role in the action of the antischistosomal drug praziquantel. *J Biol Chem* **276**(40): 36873-36876.

Kolars, JC, Schmiedlin-Ren, P, Schuetz, JD, Fang, C, Watkins, PB (1992) Identification of rifampin-inducible P450IIIA4 (CYP3A4) in human small bowel enterocytes. *J Clin Invest* **90**(5): 1871-1878.

Kostrubsky, VE, Lewis, LD, Strom, SC, Wood, SG, Schuetz, EG, Schuetz, JD, Sinclair, PR, Wrighton, SA, Sinclair, JF (1998) Induction of cytochrome P4503A by taxol in primary cultures of human hepatocytes. *Arch Biochem Biophys* **355**(2): 131-136.

Koup, RA, Safrit, JT, Cao, Y, Andrews, CA, McLeod, G, Borkowsky, W, Farthing, C, Ho, DD (1994) Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome. *J Virol* **68**(7): 4650-4655.

Kramer, RA, Schaber, MD, Skalka, AM, Ganguly, K, Wong-Staal, F, Reddy, EP (1986) HTLV-III gag protein is processed in yeast cells by the virus pol-protease. *Science* **231**(4745): 1580-1584.

Krishna, DR, Klotz, U (1993) Determination of ivermectin in human plasma by high-performance liquid chromatography. *Arzneimittelforschung* **43**(5): 609-611.

Kumar, GN, Rodrigues, AD, Buko, AM, Denissen, JF (1996) Cytochrome P450-mediated metabolism of the HIV-1 protease inhibitor ritonavir (ABT-538) in human liver microsomes. *J Pharmacol Exp Ther* **277**(1): 423-431.

Kupferschmidt, HH, Fattinger, KE, Ha, HR, Follath, F, Krahenbuhl, S (1998) Grapefruit juice enhances the bioavailability of the HIV protease inhibitor saquinavir in man. *Br J Clin Pharmacol* **45**(4): 355-359.

Kurowski, M, Sternfeld, T, Sawyer, A, Hill, A, Mocklinghoff, C (2003) Pharmacokinetic and tolerability profile of twice-daily saquinavir hard gelatin capsules and saquinavir soft gelatin capsules boosted with ritonavir in healthy volunteers. *HIV Med* **4**(2): 94-100.

Kwong, PD, Wyatt, R, Robinson, J, Sweet, RW, Sodroski, J, Hendrickson, WA (1998) Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody. *Nature* **393**(6686): 648-659.

la Porte, CJ, Colbers, EP, Bertz, R, Voncken, DS, Wikstrom, K, Boeree, MJ, Koopmans, PP, Hekster, YA, Burger, DM (2004) Pharmacokinetics of adjusted-dose lopinavir-ritonavir combined with rifampin in healthy volunteers. *Antimicrob Agents Chemother* **48**(5): 1553-1560.

Lalezari, JP, Eron, JJ, Carlson, M, Cohen, C, DeJesus, E, Arduino, RC, Gallant, JE, Volberding, P, Murphy, RL, Valentine, F, Nelson, EL, Sista, PR, Dusek, A, Kilby, JM (2003) A phase II clinical study of the long-term safety and antiviral activity of enfuvirtide-based antiretroviral therapy. *AIDS* **17**(5): 691-698.

Lamson M, RP, Lamson M, Gigliotti M, Myers MW. (1998) The pharmacokinetic interactions of nevirapine and ketoconazole . International Conference on AIDS. Int Conf AIDS. 1998; 12: 55 (abstract no. 12218).

Lanao, JM, Briones, E, Colino, CI (2007) Recent advances in delivery systems for anti-HIV1 therapy. *J Drug Target* **15**(1): 21-36.

Lanusse, C, Lifschitz, A, Virkel, G, Alvarez, L, Sanchez, S, Sutra, JF, Galtier, P, Alvinerie, M (1997) Comparative plasma disposition kinetics of ivermectin, moxidectin and doramectin in cattle. *J Vet Pharmacol Ther* **20**(2): 91-99.

Ledergerber, B, Egger, M, Opravil, M, Telenti, A, Hirschel, B, Battegay, M, Vernazza, P, Sudre, P, Flepp, M, Furrer, H, Francioli, P, Weber, R (1999) Clinical progression and virological failure on highly active antiretroviral therapy in HIV-1 patients: a prospective cohort study. Swiss HIV Cohort Study. *Lancet* **353**(9156): 863-868.

Lee, BL, Wong, D, Benowitz, NL, Sullam, PM (1993) Altered patterns of drug metabolism in patients with acquired immunodeficiency syndrome. *Clin Pharmacol Ther* **53**(5): 529-535.

Lee, CG, Gottesman, MM, Cardarelli, CO, Ramachandra, M, Jeang, KT, Ambudkar, SV, Pastan, I, Dey, S (1998) HIV-1 protease inhibitors are substrates for the MDR1 multidrug transporter. *Biochemistry* **37**(11): 3594-3601.

Legen, I, Salobir, M, Kerc, J (2005) Comparison of different intestinal epithelia as models for absorption enhancement studies. *Int J Pharm* **291**(1-2): 183-188.

Lennernas, H (1998) Human intestinal permeability. *J Pharm Sci* **87**(4): 403-410.

Lespine, A, Alvinerie, M, Vercruysse, J, Prichard, RK, Geldhof, P (2008) ABC transporter modulation: a strategy to enhance the activity of macrocyclic lactone anthelmintics. *Trends Parasitol* **24**(7): 293-298.

Lespine, A, Dupuy, J, Orlowski, S, Nagy, T, Glavinas, H, Krajcsi, P, Alvinerie, M (2006) Interaction of ivermectin with multidrug resistance proteins (MRP1, 2 and 3). *Chem Biol Interact* **159**(3): 169-179.

Lindley, D (1987) Merck's new drug free to WHO for river blindness programme. *Nature* **329**(6142): 752.

Liptrott, NJ, Penny, M, Bray, PG, Sathish, J, Khoo, SH, Back, DJ, Owen, A (2009) The impact of cytokines on the expression of drug transporters, cytochrome P450 enzymes and chemokine receptors in human PBMC. *Br J Pharmacol* **156**(3): 497-508.

Liu, P, Wang, B, Weili Qiao, JL (2008) Multi-anticancer drugs encapsulated in the micelle: a novel chemotherapy to cancer. *Med Hypotheses* **71**(3): 379-381.

Lloyd-Smith, JO, Poss, M, Grenfell, BT (2008) HIV-1/parasite co-infection and the emergence of new parasite strains. *Parasitology* **135**(7): 795-806.

Lobenberg, R, Araujo, L, von Briesen, H, Rodgers, E, Kreuter, J (1998) Body distribution of azidothymidine bound to hexyl-cyanoacrylate nanoparticles after i.v. injection to rats. *J Control Release* **50**(1-3): 21-30.

Lobenberg, R, Kreuter, J (1996) Macrophage targeting of azidothymidine: a promising strategy for AIDS therapy. *AIDS Res Hum Retroviruses* **12**(18): 1709-1715.

Longini, IM, Jr., Clark, WS, Gardner, LI, Brundage, JF (1991) The dynamics of CD4+ T-lymphocyte decline in HIV-infected individuals: a Markov modeling approach. *J Acquir Immune Defic Syndr* **4**(11): 1141-1147.

Lori, F, Malykh, AG, Foli, A, Maserati, R, De Antoni, A, Minoli, L, Padrini, D, Degli Antoni, A, Barchi, E, Jessen, H, Wainberg, MA, Gallo, RC, Lisziewicz, J (1997) Combination of a drug targeting the cell with a drug targeting the virus controls human immunodeficiency virus type 1 resistance. *AIDS Res Hum Retroviruses* **13**(16): 1403-1409.

Maggi, E, Mazzetti, M, Ravina, A, Annunziato, F, de Carli, M, Piccinni, MP, Manetti, R, Carbonari, M, Pesce, AM, del Prete, G, et al. (1994) Ability of HIV to promote a TH1 to TH0 shift and to replicate preferentially in TH2 and TH0 cells. *Science* **265**(5169): 244-248.

Malingre, MM, Beijnen, JH, Schellens, JH (2001) Oral delivery of taxanes. *Invest New Drugs* **19**(2): 155-162.

Marzolini, C, Gibbons, S, Elzi, L, Khoo, S, Ledergerber, B, Battegay, M, and, Back, D (2009) Prevalence of potential drug–drug interactions in the Swiss HIV Cohort Study, BHIVA 2009. Abstr P141.

Marzolini, C, Paus, E, Buclin, T, Kim, RB (2004) Polymorphisms in human MDR1 (P-glycoprotein): recent advances and clinical relevance. *Clin Pharmacol Ther* **75**(1): 13-33.

Marzolini, C, Telenti, A, Decosterd, LA, Greub, G, Biollaz, J, Buclin, T (2001) Efavirenz plasma levels can predict treatment failure and central nervous system side effects in HIV-1-infected patients. *AIDS* **15**(1): 71-75.

Masimirembwa, CM, Hasler, JA (1994a) Characterisation of praziquantel metabolism by rat liver microsomes using cytochrome P450 inhibitors. *Biochem Pharmacol* **48**(9): 1779-1783.

Masimirembwa, CM, Naik, YS, Hasler, JA (1994b) The effect of chloroquine on the pharmacokinetics and metabolism of praziquantel in rats and in humans. *Biopharm Drug Dispos* **15**(1): 33-43.

Matheny, CJ, Lamb, MW, Brouwer, KR, Pollack, GM (2001) Pharmacokinetic and pharmacodynamic implications of P-glycoprotein modulation. *Pharmacotherapy* **21**(7): 778-796.

Matteelli, A, Regazzi, M, Villani, P, De Iaco, G, Cusato, M, Carvalho, AC, Caligaris, S, Tomasoni, L, Manfrin, M, Capone, S, Carosi, G (2007) Multiple-dose pharmacokinetics of efavirenz with and without the use of rifampicin in HIV-positive patients. *Curr HIV Res* **5**(3): 349-353.

Maurel, P (1996) The CYP3A family. In Cytochrome P450: Metabolic and Toxicological Aspects (C. Ioannides, Ed.), pp. 241–270. CRC Press, (1996). Boca Raton, FL.

McDonald, CK, Kuritzkes, DR (1997) Human immunodeficiency virus type 1 protease inhibitors. *Arch Intern Med* **157**(9): 951-959.

McKellar, QA, Benchaoui, HA (1996) Avermectins and milbemycins. *J Vet Pharmacol Ther* **19**(5): 331-351.

Mealey, KL, Bentjen, SA, Gay, JM, Cantor, GH (2001) Ivermectin sensitivity in collies is associated with a deletion mutation of the mdr1 gene. *Pharmacogenetics* **11**(8): 727-733.

medicine.iupui.edu/kenya/index.html.

Meek, TD, Dreyer, GB (1990) HIV-1 protease as a potential target for anti-AIDS therapy. *Ann N Y Acad Sci* **616:** 41-53.

Mehlhorn, H, Becker, B, Andrews, P, Thomas, H, Frenkel, JK (1981) In vivo and in vitro experiments on the effects of praziquantel on Schistosoma mansoni. A light and electron microscopic study. *Arzneimittelforschung* **31**(3a): 544-554.

Mellors, JW, Kingsley, LA, Rinaldo, CR, Jr., Todd, JA, Hoo, BS, Kokka, RP, Gupta, P (1995) Quantitation of HIV-1 RNA in plasma predicts outcome after seroconversion. *Ann Intern Med* **122**(8): 573-579.

Mellors, JW, Munoz, A, Giorgi, JV, Margolick, JB, Tassoni, CJ, Gupta, P, Kingsley, LA, Todd, JA, Saah, AJ, Detels, R, Phair, JP, Rinaldo, CR, Jr. (1997) Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection. *Ann Intern Med* **126**(12): 946-954.

Merino, G, Alvarez, AI, Prieto, JG, Kim, RB (2002) The anthelminthic agent albendazole does not interact with p-glycoprotein. *Drug Metab Dispos* **30**(4): 365-369.

Merino, G, Jonker, JW, Wagenaar, E, Pulido, MM, Molina, AJ, Alvarez, AI, Schinkel, AH (2005) Transport of anthelmintic benzimidazole drugs by breast cancer resistance protein (BCRP/ABCG2). *Drug Metab Dispos* **33**(5): 614-618.

Merisko-Liversidge, E, Liversidge, GG, Cooper, ER (2003) Nanosizing: a formulation approach for poorly-water-soluble compounds. *Eur J Pharm Sci* **18**(2): 113-120.

Merson, MH (2006) The HIV-AIDS pandemic at 25--the global response. *N Engl J Med* **354**(23): 2414-2417.

Messerli, SM, Kasinathan, RS, Morgan, W, Spranger, S, Greenberg, RM (2009) Schistosoma mansoni P-glycoprotein levels increase in response to praziquantel exposure and correlate with reduced praziquantel susceptibility. *Mol Biochem Parasitol.*

Metwally, A, Bennett, JL, Botros, S, Ebeid, F (1995) Effect of cimetidine, bicarbonate and glucose on the bioavailability of different formulations of praziquantel. *Arzneimittelforschung* **45**(4): 516-518.

Miller, CD, El-Kholi, R, Faragon, JJ, Lodise, TP (2007) Prevalence and risk factors for clinically significant drug interactions with antiretroviral therapy. *Pharmacotherapy* **27**(10): 1379-1386.

Miller, MD, Farnet, CM, Bushman, FD (1997) Human immunodeficiency virus type 1 preintegration complexes: studies of organization and composition. *J Virol* **71**(7): 5382-5390.

Mitsuya, H, Broder, S (1986) Inhibition of the in vitro infectivity and cytopathic effect of human T-lymphotrophic virus type III/lymphadenopathy-associated virus (HTLV-III/LAV) by 2',3'-dideoxynucleosides. *Proc Natl Acad Sci U S A* **83**(6): 1911-1915.

MOH-Kenya (2005) Ministry of Health Kenya (2005). AIDS in Kenya, 7th ed. Nairobi, National AIDS and STI Control Programme (NASCOP).

Molyneux, DH, Bradley, M, Hoerauf, A, Kyelem, D, Taylor, MJ (2003) Mass drug treatment for lymphatic filariasis and onchocerciasis. *Trends Parasitol* **19**(11): 516-522.

Montessori, V, Press, N, Harris, M, Akagi, L, Montaner, JS (2004) Adverse effects of antiretroviral therapy for HIV infection. *CMAJ* **170**(2): 229-238.

Mosmann, T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* **65**(1-2): 55-63.

Moss, AR, Bacchetti, P (1989) Natural history of HIV infection. *AIDS* **3**(2): 55-61.

Moss, AR, Bacchetti, P, Osmond, D, Krampf, W, Chaisson, RE, Stites, D, Wilber, J, Allain, JP, Carlson, J (1988) Seropositivity for HIV and the development of AIDS or AIDS related condition: three year follow up of the San Francisco General Hospital cohort. *Br Med J (Clin Res Ed)* **296**(6624): 745-750.

Mouly, S, Lown, KS, Kornhauser, D, Joseph, JL, Fiske, WD, Benedek, IH, Watkins, PB (2002) Hepatic but not intestinal CYP3A4 displays dose-dependent induction by efavirenz in humans. *Clin Pharmacol Ther* **72**(1): 1-9.

Moyle, GJ, Back, D (2001) Principles and practice of HIV-protease inhibitor pharmacoenhancement. *HIV Med* **2**(2): 105-113.

Muesing, MA, Smith, DH, Cabradilla, CD, Benton, CV, Lasky, LA, Capon, DJ (1985) Nucleic acid structure and expression of the human AIDS/lymphadenopathy retrovirus. *Nature* **313**(6002): 450-458.

Murer, H, Gmaj, P, Steiger, B, Hagenbuch, B (1989) Transport studies with renal proximal tubular and small intestinal brush border and basolateral membrane vesicles: vesicle heterogeneity, coexistence of transport system. *Methods Enzymol* **172**: 346-364.

Murphy, RL, Sommadossi, JP, Lamson, M, Hall, DB, Myers, M, Dusek, A (1999) Antiviral effect and pharmacokinetic interaction between nevirapine and indinavir in persons infected with human immunodeficiency virus type 1. *J Infect Dis* **179**(5): 1116-1123.

NACC-Kenya (2007) National AIDS Control Council Kenya (2007). National HIV prevalence in Kenya. Nairobi.

NASCOP (2005) Guidelines to Antiretroviral Drug Therapy in Kenya. 3rd ed. Nairobi, Kenya: Republic of Kenya, Ministry of Health

Nielsen, NO, Simonsen, PE, Magnussen, P, Magesa, S, Friis, H (2006) Cross-sectional relationship between HIV, lymphatic filariasis and other parasitic infections in adults in coastal northeastern Tanzania. *Trans R Soc Trop Med Hyg* **100**(6): 543-550.

Nishimura, M, Ueda, N, Naito, S (2003) Effects of dimethyl sulfoxide on the gene induction of cytochrome P450 isoforms, UGT-dependent glucuronosyl transferase isoforms, and ABCB1 in primary culture of human hepatocytes. *Biol Pharm Bull* **26**(7): 1052-1056.

Nishimura, M, Yoshitsugu, H, Naito, S, Hiraoka, I (2002) Evaluation of gene induction of drug-metabolizing enzymes and transporters in primary culture of human hepatocytes using high-sensitivity real-time reverse transcription PCR. *Yakugaku Zasshi* **122**(5): 339-361.

O'Brien, TR, Blattner, WA, Waters, D, Eyster, E, Hilgartner, MW, Cohen, AR, Luban, N, Hatzakis, A, Aledort, LM, Rosenberg, PS, Miley, WJ, Kroner, BL, Goedert, JJ (1996) Serum HIV-1 RNA levels and time to development of AIDS in the Multicenter Hemophilia Cohort Study. *JAMA* **276**(2): 105-110.

Obaru, K, Mitsuya, H (1998) [Anti-HIV drugs and drug delivery system]. *Nippon Rinsho* **56**(3): 769-775.

Ohkura, T, Shin, YS, Wakamiya, N, Iwa, N, Kurimura, T (1997) Detection of proviruses and viral RNA in the early stages of feline immunodeficiency virus infection in cats: a possible model of the early stage of HIV infection. *Exp Anim* **46**(1): 31-39.

Okamura, N, Hirai, M, Tanigawara, Y, Tanaka, K, Yasuhara, M, Ueda, K, Komano, T, Hori, R (1993) Digoxin-cyclosporin A interaction: modulation of the multidrug transporter P-glycoprotein in the kidney. *J Pharmacol Exp Ther* **266**(3): 1614-1619.

Okano, T, Inui, K, Maegawa, H, Takano, M, Hori, R (1986) H+ coupled uphill transport of aminocephalosporins via the dipeptide transport system in rabbit intestinal brush-border membranes. *J Biol Chem* **261**(30): 14130-14134.

Olsen, L, Ingvast-Larsson, C, Larsson, P, Brostrom, H, Bondesson, U, Sundqvist, M, Tjalve, H (2006) Fexofenadine in horses: pharmacokinetics, pharmacodynamics and effect of ivermectin pretreatment. *J Vet Pharmacol Ther* **29**(2): 129-135.

Osei-Atweneboana, MY, Eng, JK, Boakye, DA, Gyapong, JO, Prichard, RK (2007) Prevalence and intensity of Onchocerca volvulus infection and efficacy of ivermectin

in endemic communities in Ghana: a two-phase epidemiological study. *Lancet* **369**(9578): 2021-2029.

Osmond, D, Charlebois, E, Lang, W, Shiboski, S, Moss, A (1994) Changes in AIDS survival time in two San Francisco cohorts of homosexual men, 1983 to 1993. *JAMA* **271**(14): 1083-1087.

Ottesen, EA, Hooper, PJ, Bradley, M, Biswas, G (2008) The global programme to eliminate lymphatic filariasis: health impact after 8 years. *PLoS Negl Trop Dis* **2**(10): e317.

Owen, A, Chandler, B, Back, DJ, Khoo, SH (2004a) Expression of pregnane-X-receptor transcript in peripheral blood mononuclear cells and correlation with MDR1 mRNA. *Antivir Ther* **9**(5): 819-821.

Owen, A, Chandler, B, Bray, PG, Ward, SA, Hart, CA, Back, DJ, Khoo, SH (2004b) Functional correlation of P-glycoprotein expression and genotype with expression of the human immunodeficiency virus type 1 coreceptor CXCR4. *J Virol* **78**(21): 12022-12029.

Owen, A, Khoo, SH (2004c) Intracellular pharmacokinetics of antiretroviral agents. *J HIV Ther* **9**(4): 97-101.

Paine, MF, Hart, HL, Ludington, SS, Haining, RL, Rettie, AE, Zeldin, DC (2006) The human intestinal cytochrome P450 "pie". *Drug Metab Dispos* **34**(5): 880-886.

Pal, D, Mitra, AK (2006) MDR- and CYP3A4-mediated drug-drug interactions. *J Neuroimmune Pharmacol* **1**(3): 323-339.

Palella, FJ, Jr., Delaney, KM, Moorman, AC, Loveless, MO, Fuhrer, J, Satten, GA, Aschman, DJ, Holmberg, SD (1998) Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. *N Engl J Med* **338**(13): 853-860.

Pantaleo, G, Demarest, JF, Schacker, T, Vaccarezza, M, Cohen, OJ, Daucher, M, Graziosi, C, Schnittman, SS, Quinn, TC, Shaw, GM, Perrin, L, Tambussi, G, Lazzarin, A, Sekaly, RP, Soudeyns, H, Corey, L, Fauci, AS (1997) The qualitative nature of the primary immune response to HIV infection is a prognosticator of disease progression independent of the initial level of plasma viremia. *Proc Natl Acad Sci U S A* **94**(1): 254-258.

Pantaleo, G, Graziosi, C, Fauci, AS (1993) New concepts in the immunopathogenesis of human immunodeficiency virus infection. *N Engl J Med* **328**(5): 327-335.

Pardridge, WM (2003) Blood-brain barrier drug targeting: the future of brain drug development. *Mol Interv* **3**(2): 90-105, 151.

Patel, J, Mitra, AK (2001) Strategies to overcome simultaneous P-glycoprotein mediated efflux and CYP3A4 mediated metabolism of drugs. *Pharmacogenomics* **2**(4): 401-415.

Pedersen, C, Dickmeiss, E, Gaub, J, Ryder, LP, Platz, P, Lindhardt, BO, Lundgren, JD (1990) T-cell subset alterations and lymphocyte responsiveness to mitogens and antigen during severe primary infection with HIV: a case series of seven consecutive HIV seroconverters. *AIDS* **4**(6): 523-526.

Perloff, ES, Duan, SX, Skolnik, PR, Greenblatt, DJ, von Moltke, LL (2005) Atazanavir: effects on P-glycoprotein transport and CYP3A metabolism in vitro. *Drug Metab Dispos* **33**(6): 764-770.

Perloff, MD, Stormer, E, von Moltke, LL, Greenblatt, DJ (2003) Rapid assessment of P-glycoprotein inhibition and induction in vitro. *Pharm Res* **20**(8): 1177-1183.

Perumal, OP, Inapagolla, R, Kannan, S, Kannan, RM (2008) The effect of surface functionality on cellular trafficking of dendrimers. *Biomaterials* **29**(24-25): 3469-3476.

Peters, WH, Roelofs, HM (1989) Time-dependent activity and expression of glutathione S-transferases in the human colon adenocarcinoma cell line Caco-2. *Biochem J* **264**(2): 613-616.

Pfrunder, A, Gutmann, H, Beglinger, C, Drewe, J (2003) Gene expression of CYP3A4, ABC-transporters (MDR1 and MRP1-MRP5) and hPXR in three different human colon carcinoma cell lines. *J Pharm Pharmacol* **55**(1): 59-66.

Phillips, AN (1992) CD4 lymphocyte depletion prior to the development of AIDS. *AIDS* **6**(7): 735-736.

Picker, LJ, Hagen, SI, Lum, R, Reed-Inderbitzin, EF, Daly, LM, Sylwester, AW, Walker, JM, Siess, DC, Piatak, M, Jr., Wang, C, Allison, DB, Maino, VC, Lifson, JD, Kodama, T, Axthelm, MK (2004) Insufficient production and tissue delivery of CD4+ memory T cells in rapidly progressive simian immunodeficiency virus infection. *J Exp Med* **200**(10): 1299-1314.

Piscitelli, SC, Burstein, AH, Welden, N, Gallicano, KD, Falloon, J (2002) The effect of garlic supplements on the pharmacokinetics of saquinavir. *Clin Infect Dis* **34**(2): 234-238.

Piscitelli, SC, Gallicano, KD (2001) Interactions among drugs for HIV and opportunistic infections. *N Engl J Med* **344**(13): 984-996.

Polk, RE, Brophy, DF, Israel, DS, Patron, R, Sadler, BM, Chittick, GE, Symonds, WT, Lou, Y, Kristoff, D, Stein, DS (2001) Pharmacokinetic Interaction between amprenavir and rifabutin or rifampin in healthy males. *Antimicrob Agents Chemother* **45**(2): 502-508.

Pontali, E (2007) Interactions of antiretroviral drugs with anti-infectives and other antiretrovirals. *Chemotherapy* **53**(1): 26-29.

Pouliot, JF, L'Heureux, F, Liu, Z, Prichard, RK, Georges, E (1997) Reversal of P-glycoprotein-associated multidrug resistance by ivermectin. *Biochem Pharmacol* **53**(1): 17-25.

Profit, L, Eagling, VA, Back, DJ (1999) Modulation of P-glycoprotein function in human lymphocytes and Caco-2 cell monolayers by HIV-1 protease inhibitors. *AIDS* **13**(13): 1623-1627.

Pugach, P, Ketas, TJ, Michael, E, Moore, JP (2008) Neutralizing antibody and anti-retroviral drug sensitivities of HIV-1 isolates resistant to small molecule CCR5 inhibitors. *Virology* **377**(2): 401-407.

Rabinow, BE (2004) Nanosuspensions in drug delivery. *Nat Rev Drug Discov* **3**(9): 785-796.

Raffanti, S, Haas, DW (2001) Antiretroviral agents.Goodman and Gilman's The pharmacological basis of therapeutics,Joel G. Hardman.Lee E. Limbird.McGraw Hill .10th edition.2001:1349-1373.

Rainey, PM, Friedland, G, McCance-Katz, EF, Andrews, L, Mitchell, SM, Charles, C, Jatlow, P (2000) Interaction of methadone with didanosine and stavudine. *J Acquir Immune Defic Syndr* **24**(3): 241-248.

Ramachandran, G, Hemanthkumar, AK, Rajasekaran, S, Padmapriyadarsini, C, Narendran, G, Sukumar, B, Sathishnarayan, S, Raja, K, Kumaraswami, V, Swaminathan, S (2006) Increasing nevirapine dose can overcome reduced bioavailability due to rifampicin coadministration. *J Acquir Immune Defic Syndr* **42**(1): 36-41.

Rawden, HC, Kokwaro, GO, Ward, SA, Edwards, G (2000) Relative contribution of cytochromes P-450 and flavin-containing monoxygenases to the metabolism of albendazole by human liver microsomes. *Br J Clin Pharmacol* **49**(4): 313-322.

Ray, AS, Olson, L, Fridland, A (2004) Role of purine nucleoside phosphorylase in interactions between 2',3'-dideoxyinosine and allopurinol, ganciclovir, or tenofovir. *Antimicrob Agents Chemother* **48**(4): 1089-1095.

Rencurel, F, Stenhouse, A, Hawley, SA, Friedberg, T, Hardie, DG, Sutherland, C, Wolf, CR (2005) AMP-activated protein kinase mediates phenobarbital induction of CYP2B gene expression in hepatocytes and a newly derived human hepatoma cell line. *J Biol Chem* **280**(6): 4367-4373.

Rescriptor (2006) Summary of product characteristics, Pfizer Inc, June 2006.

Retrovir (2007) Summary of Product Characteristics, GlaxoSmithKline UK, October 2007.

Richman, DD, Havlir, D, Corbeil, J, Looney, D, Ignacio, C, Spector, SA, Sullivan, J, Cheeseman, S, Barringer, K, Pauletti, D, et al. (1994) Nevirapine resistance mutations of human immunodeficiency virus type 1 selected during therapy. *J Virol* **68**(3): 1660-1666.

Ridtitid, W, Ratsamemonthon, K, Mahatthanatrakul, W, Wongnawa, M (2007) Pharmacokinetic interaction between ketoconazole and praziquantel in healthy volunteers. *J Clin Pharm Ther* **32**(6): 585-593.

Ridtitid, W, Wongnawa, M, Mahatthanatrakul, W, Punyo, J, Sunbhanich, M (2002) Rifampin markedly decreases plasma concentrations of praziquantel in healthy volunteers. *Clin Pharmacol Ther* **72**(5): 505-513.

Robbins, BL, Srinivas, RV, Kim, C, Bischofberger, N, Fridland, A (1998) Anti-human immunodeficiency virus activity and cellular metabolism of a potential prodrug of the acyclic nucleoside phosphonate 9-R-(2-phosphonomethoxypropyl)adenine (PMPA), Bis(isopropyloxymethylcarbonyl)PMPA. *Antimicrob Agents Chemother* **42**(3): 612-617.

Robertson, SM, Penzak, SR, Pau, A (2007) Drug interactions in the management of HIV infection: an update. *Expert Opin Pharmacother* **8**(17): 2947-2963.

Roos, MT, de Leeuw, NA, Claessen, FA, Huisman, HG, Kootstra, NA, Meyaard, L, Schellekens, PT, Schuitemaker, H, Miedema, F (1994) Viro-immunological studies in acute HIV-1 infection. *AIDS* **8**(11): 1533-1538.

Rosenberg, ES, Billingsley, JM, Caliendo, AM, Boswell, SL, Sax, PE, Kalams, SA, Walker, BD (1997) Vigorous HIV-1-specific CD4+ T cell responses associated with control of viremia. *Science* **278**(5342): 1447-1450.

Sadurni, N, Solans, C, Azemar, N, Garcia-Celma, MJ (2005) Studies on the formation of O/W nano-emulsions, by low-energy emulsification methods, suitable for pharmaceutical applications. *Eur J Pharm Sci* **26**(5): 438-445.

Salzwedel, K, Martin, DE, Sakalian, M (2007) Maturation inhibitors: a new therapeutic class targets the virus structure. *AIDS Rev* **9**(3): 162-172.

Sambuy, Y, De Angelis, I, Ranaldi, G, Scarino, ML, Stammati, A, Zucco, F (2005) The Caco-2 cell line as a model of the intestinal barrier: influence of cell and culture-related factors on Caco-2 cell functional characteristics. *Cell Biol Toxicol* **21**(1): 1-26.

Sanvicens, N, Marco, MP (2008) Multifunctional nanoparticles--properties and prospects for their use in human medicine. *Trends Biotechnol* **26**(8): 425-433.

Satlin, LM, Amin, V, Wolkoff, AW (1997) Organic anion transporting polypeptide mediates organic anion/HCO3- exchange. *J Biol Chem* **272**(42): 26340-26345.

Savioli, L, Albonico, M (2004a) Soil-transmitted helminthiasis. *Nat Rev Microbiol* **2**(8): 618-619.

Savioli, L, Albonico, M, Engels, D, Montresor, A (2004b) Progress in the prevention and control of schistosomiasis and soil-transmitted helminthiasis. *Parasitol Int* **53**(2): 103-113.

Sayana, S, Khanlou, H (2008) Raltegravir: the first in a new class of integrase inhibitors for the treatment of HIV. *Expert Rev Anti Infect Ther* **6**(4): 419-426.

Sayed, AA, Simeonov, A, Thomas, CJ, Inglese, J, Austin, CP, Williams, DL (2008) Identification of oxadiazoles as new drug leads for the control of schistosomiasis. *Nat Med* **14**(4): 407-412.

Scarlatti, G, Tresoldi, E, Bjorndal, A, Fredriksson, R, Colognesi, C, Deng, HK, Malnati, MS, Plebani, A, Siccardi, AG, Littman, DR, Fenyo, EM, Lusso, P (1997) In vivo evolution of HIV-1 co-receptor usage and sensitivity to chemokine-mediated suppression. *Nat Med* **3**(11): 1259-1265.

Schacker, T (1996) Primary HIV infection. *STEP Perspect* **8**(1): 12-14.

Schinkel, AH (1997) The physiological function of drug-transporting P-glycoproteins. *Semin Cancer Biol* **8**(3): 161-170.

Schinkel, AH, Smit, JJ, van Tellingen, O, Beijnen, JH, Wagenaar, E, van Deemter, L, Mol, CA, van der Valk, MA, Robanus-Maandag, EC, te Riele, HP, et al. (1994) Disruption of the mouse mdr1a P-glycoprotein gene leads to a deficiency in the blood-brain barrier and to increased sensitivity to drugs. *Cell* **77**(4): 491-502.

Schmittgen, TD, Livak, KJ (2008) Analyzing real-time PCR data by the comparative C(T) method. *Nat Protoc* **3**(6): 1101-1108.

Schuetz, EG, Schuetz, JD, Strom, SC, Thompson, MT, Fisher, RA, Molowa, DT, Li, D, Guzelian, PS (1993) Regulation of human liver cytochromes P-450 in family 3A in primary and continuous culture of human hepatocytes. *Hepatology* **18**(5): 1254-1262.

Scott, EW, McKellar, QA (1992) The distribution and some pharmacokinetic parameters of ivermectin in pigs. *Vet Res Commun* **16**(2): 139-146.

Scott, LJ, Perry, CM (2000) Delavirdine: a review of its use in HIV infection. *Drugs* **60**(6): 1411-1444.

Secor, WE (2006) Interactions between schistosomiasis and infection with HIV-1. *Parasite Immunol* **28**(11): 597-603.

Secor, WE, Karanja, DM, Colley, DG (2004) Interactions between schistosomiasis and human immunodeficiency virus in Western Kenya. *Mem Inst Oswaldo Cruz* **99**(5 Suppl 1): 93-95.

Shah, LK, Amiji, MM (2006) Intracellular delivery of saquinavir in biodegradable polymeric nanoparticles for HIV/AIDS. *Pharm Res* **23**(11): 2638-2645.

Shah, S, Shah, S, McGowan, J, Opulski, B, A Lieblein, A, Saperstein, A (2007) Identification of Drug Interactions Involving ART in New York City HIV Specialty Clinics, CROI 2007, Abstr 573.

Shahiwala, A, Amiji, MM (2007) Nanotechnology-based delivery systems in HIV/AIDS therapy. *Future HIV Ther* **1**(1): 49-59.

Shen, DD, Kunze, KL, Thummel, KE (1997) Enzyme-catalyzed processes of first-pass hepatic and intestinal drug extraction. *Adv Drug Deliv Rev* **27**(2-3): 99-127.

Shimada, T, Yamazaki, H, Mimura, M, Inui, Y, Guengerich, FP (1994) Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. *J Pharmacol Exp Ther* **270**(1): 414-423.

Shirasaka, Y, Kawasaki, M, Sakane, T, Omatsu, H, Moriya, Y, Nakamura, T, Sakaeda, T, Okumura, K, Langguth, P, Yamashita, S (2006) Induction of human P-glycoprotein in Caco-2 cells: development of a highly sensitive assay system for P-glycoprotein-mediated drug transport. *Drug Metab Pharmacokinet* **21**(5): 414-423.

Signoretti, C, Romagnoli, G, Turriziani, O, Antonelli, G, Dianzani, F, Cianfriglia, M (1997) Induction of the multidrug-transporter P-glycoprotein by 3'-azido-3'-deoxythymidine (AZT) treatment in tumor cell lines. *J Exp Clin Cancer Res* **16**(1): 29-32.

Simpson, DM, Tagliati, M (1995) Nucleoside analogue-associated peripheral neuropathy in human immunodeficiency virus infection. *J Acquir Immune Defic Syndr Hum Retrovirol* **9**(2): 153-161.

Skalova, L, Szotakova, B, Machala, M, Neca, J, Soucek, P, Havlasova, J, Wsol, V, Kridova, L, Kvasnickova, E, Lamka, J (2001) Effect of ivermectin on activities of cytochrome P450 isoenzymes in mouflon (Ovis musimon) and fallow deer (Dama dama). *Chem Biol Interact* **137**(2): 155-167.

Slain, D, Pakyz, A, Israel, DS, Monroe, S, Polk, RE (2000) Variability in activity of hepatic CYP3A4 in patients infected with HIV. *Pharmacotherapy* **20**(8): 898-907.

Smith, C, Smith, H, Seaton, RA, Fox, R (2008) Seroprevalence of schistosomiasis in African patients infected with HIV. *HIV Med.*

Smith, PF, DiCenzo, R, Morse, GD (2001) Clinical pharmacokinetics of non-nucleoside reverse transcriptase inhibitors. *Clin Pharmacokinet* **40**(12): 893-905.

Sommadossi, JP (1999) HIV protease inhibitors: pharmacologic and metabolic distinctions. *AIDS* **13 Suppl 1**: S29-40.

Somogyi, A (1996) Renal transport of drugs: specificity and molecular mechanisms. *Clin Exp Pharmacol Physiol* **23**(10-11): 986-989.

Souhaili-El Amri, H, Mothe, O, Totis, M, Masson, C, Batt, AM, Delatour, P, Siest, G (1988) Albendazole sulfonation by rat liver cytochrome P-450c. *J Pharmacol Exp Ther* **246**(2): 758-764.

Sparreboom, A, van Asperen, J, Mayer, U, Schinkel, AH, Smit, JW, Meijer, DK, Borst, P, Nooijen, WJ, Beijnen, JH, van Tellingen, O (1997) Limited oral bioavailability and active epithelial excretion of paclitaxel (Taxol) caused by P-glycoprotein in the intestine. *Proc Natl Acad Sci U S A* **94**(5): 2031-2035.

Spiegel, H, Herbst, H, Niedobitek, G, Foss, HD, Stein, H (1992) Follicular dendritic cells are a major reservoir for human immunodeficiency virus type 1 in lymphoid tissues facilitating infection of CD4+ T-helper cells. *Am J Pathol* **140**(1): 15-22.

Srinivas, RV, Middlemas, D, Flynn, P, Fridland, A (1998) Human immunodeficiency virus protease inhibitors serve as substrates for multidrug transporter proteins MDR1 and MRP1 but retain antiviral efficacy in cell lines expressing these transporters. *Antimicrob Agents Chemother* **42**(12): 3157-3162.

Starr-Spires, LD, Collman, RG (2002) HIV-1 entry and entry inhibitors as therapeutic agents. *Clin Lab Med* **22**(3): 681-701.

Steel, JW (1993) Pharmacokinetics and metabolism of avermectins in livestock. *Vet Parasitol* **48**(1-4): 45-57.

Stein, DS, Moore, KH (2001) Phosphorylation of nucleoside analog antiretrovirals: a review for clinicians. *Pharmacotherapy* **21**(1): 11-34.

Steinmann, P, Keiser, J, Bos, R, Tanner, M, Utzinger, J (2006) Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk. *Lancet Infect Dis* **6**(7): 411-425.

Stelma, FF, Talla, I, Sow, S, Kongs, A, Niang, M, Polman, K, Deelder, AM, Gryseels, B (1995) Efficacy and side effects of praziquantel in an epidemic focus of Schistosoma mansoni. *Am J Trop Med Hyg* **53**(2): 167-170.

Storch, CH, Theile, D, Lindenmaier, H, Haefeli, WE, Weiss, J (2007) Comparison of the inhibitory activity of anti-HIV drugs on P-glycoprotein. *Biochem Pharmacol* **73**(10): 1573-1581.

Stormer, E, von Moltke, LL, Perloff, MD, Greenblatt, DJ (2002) Differential modulation of P-glycoprotein expression and activity by non-nucleoside HIV-1 reverse transcriptase inhibitors in cell culture. *Pharm Res* **19**(7): 1038-1045.

Sulzbacher, A, Jarosch, A, Schuler, R, Acerbi, D, Ventura, P, Puccini, P, Woodcock, BG (1998) Validation of a Caco-2 cell monolayer culture for drug transport studies. *Int J Clin Pharmacol Ther* **36**(2): 86-89.

Sustiva (2007) Sustiva Summary of Product Characteristics, Bristol-Myers Squibb Pharmaceuticals Ltd, January 2007.

Tadros, T, Izquierdo, P, Esquena, J, Solans, C (2004) Formation and stability of nano-emulsions. *Adv Colloid Interface Sci* **108-109:** 303-318.

Tatro(ed) (2008) Drug interaction Facts - the authority of drug interactions. Facts and Comparisons, St. Louis, MO.

Temesgen, Z, Wright, AJ (1997) Recent advances in the management of human immunodeficiency virus infection. *Mayo Clin Proc* **72**(9): 854-858; quiz 859.

Thiebaut, F, Tsuruo, T, Hamada, H, Gottesman, MM, Pastan, I, Willingham, MC (1987) Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. *Proc Natl Acad Sci U S A* **84**(21): 7735-7738.

Thummel, KE, Wilkinson, GR (1998) In vitro and in vivo drug interactions involving human CYP3A. *Annu Rev Pharmacol Toxicol* **38:** 389-430.

Tosi, G, Costantino, L, Ruozi, B, Forni, F, Vandelli, MA (2008) Polymeric nanoparticles for the drug delivery to the central nervous system. *Expert Opin Drug Deliv* **5**(2): 155-174.

Ueda, K, Cardarelli, C, Gottesman, MM, Pastan, I (1987) Expression of a full-length cDNA for the human "MDR1" gene confers resistance to colchicine, doxorubicin, and vinblastine. *Proc Natl Acad Sci U S A* **84**(9): 3004-3008.

UNAIDS (2006) Progress on global access to HIV antiretroviral therapy: a report on "3 by 5" and beyond, 2006. Geneva, Switzerland: World Health Organization, Joint United Nations Programme on HIV/AIDS (UNAIDS); 2006.

UNAIDS/WHO (2008) UNAIDS/WHO 2008 report on the Global AIDS pandemic.

Utzinger, J, Keiser, J (2004) Schistosomiasis and soil-transmitted helminthiasis: common drugs for treatment and control. *Expert Opin Pharmacother* **5**(2): 263-285.

van den Berg, H, Gerritsen, EJ, van Tol, MJ, Dooren, LJ, Vossen, JM (1994) Ten years after acquiring an HIV-1 infection: a study in a cohort of eleven neonates infected by aliquots from a single plasma donation. *Acta Paediatr* **83**(2): 173-178.

van der Werf, MJ, de Vlas, SJ, Brooker, S, Looman, CW, Nagelkerke, NJ, Habbema, JD, Engels, D (2003) Quantification of clinical morbidity associated with schistosome infection in sub-Saharan Africa. *Acta Trop* **86**(2-3): 125-139.

van Heeswijk, RP, Veldkamp, A, Mulder, JW, Meenhorst, PL, Lange, JM, Beijnen, JH, Hoetelmans, RM (2001) Combination of protease inhibitors for the treatment of HIV-1-infected patients: a review of pharmacokinetics and clinical experience. *Antivir Ther* **6**(4): 201-229.

van Helvoort, A, Smith, AJ, Sprong, H, Fritzsche, I, Schinkel, AH, Borst, P, van Meer, G (1996) MDR1 P-glycoprotein is a lipid translocase of broad specificity, while MDR3 P-glycoprotein specifically translocates phosphatidylcholine. *Cell* **87**(3): 507-517.

van Herwaarden, AE, van Waterschoot, RA, Schinkel, AH (2009) How important is intestinal cytochrome P450 3A metabolism? *Trends Pharmacol Sci* **30**(5): 223-227.

van Leth, F, Phanuphak, P, Ruxrungtham, K, Baraldi, E, Miller, S, Gazzard, B, Cahn, P, Lalloo, UG, van der Westhuizen, IP, Malan, DR, Johnson, MA, Santos, BR, Mulcahy, F, Wood, R, Levi, GC, Reboredo, G, Squires, K, Cassetti, I, Petit, D, Raffi, F, Katlama, C, Murphy, RL, Horban, A, Dam, JP, Hassink, E, van Leeuwen, R, Robinson, P, Wit, FW, Lange, JM (2004) Comparison of first-line antiretroviral therapy with regimens including nevirapine, efavirenz, or both drugs, plus stavudine and lamivudine: a randomised open-label trial, the 2NN Study. *Lancet* **363**(9417): 1253-1263.

van Vlerken, LE, Amiji, MM (2006) Multi-functional polymeric nanoparticles for tumour-targeted drug delivery. *Expert Opin Drug Deliv* **3**(2): 205-216.

Vanhems, P, Dassa, C, Lambert, J, Cooper, DA, Perrin, L, Vizzard, J, Hirschel, B, Kinloch-de Loes, S, Carr, A, Allard, R (1999) Comprehensive classification of symptoms and signs reported among 218 patients with acute HIV-1 infection. *J Acquir Immune Defic Syndr* **21**(2): 99-106.

Vauthier, C, Dubernet, C, Chauvierre, C, Brigger, I, Couvreur, P (2003) Drug delivery to resistant tumors: the potential of poly(alkyl cyanoacrylate) nanoparticles. *J Control Release* **93**(2): 151-160.

Veldkamp, AI, van Heeswijk, RP, Mulder, JW, Meenhorst, PL, Schreij, G, van der Geest, S, Lange, JM, Beijnen, JH, Hoetelmans, RM (2001a) Steady-state pharmacokinetics of twice-daily dosing of saquinavir plus ritonavir in HIV-1-infected individuals. *J Acquir Immune Defic Syndr* **27**(4): 344-349.

Veldkamp, AI, Weverling, GJ, Lange, JM, Montaner, JS, Reiss, P, Cooper, DA, Vella, S, Hall, D, Beijnen, JH, Hoetelmans, RM (2001b) High exposure to nevirapine in plasma is associated with an improved virological response in HIV-1-infected individuals. *AIDS* **15**(9): 1089-1095.

Vento, S, Di Perri, G, Garofano, T, Concia, E, Bassetti, D (1993) Pneumocystis carinii pneumonia during primary HIV-1 infection. *Lancet* **342**(8862): 24-25.

Vergis, EN, Mellors, JW (2000) Natural history of HIV-1 infection. *Infect Dis Clin North Am* **14**(4): 809-825, v-vi.

Vermeir, M, Annaert, P, Mamidi, RN, Roymans, D, Meuldermans, W, Mannens, G (2005) Cell-based models to study hepatic drug metabolism and enzyme induction in humans. *Expert Opin Drug Metab Toxicol* **1**(1): 75-90.

Videx (2008) Summary of Product Characteristics, Bristol-Myers Squibb Pharmaceuticals Ltd, August 2008.

Villani, P, Feroggio, M, Gianelli, L, Bartoli, A, Montagna, M, Maserati, R, Regazzi, MB (2001) Antiretrovirals: simultaneous determination of five protease inhibitors and three nonnucleoside transcriptase inhibitors in human plasma by a rapid high-performance liquid chromatography--mass spectrometry assay. *Ther Drug Monit* **23**(4): 380-388.

Vittinghoff, E, Scheer, S, O'Malley, P, Colfax, G, Holmberg, SD, Buchbinder, SP (1999) Combination antiretroviral therapy and recent declines in AIDS incidence and mortality. *J Infect Dis* **179**(3): 717-720.

Voinea, M, Simionescu, M (2002) Designing of 'intelligent' liposomes for efficient delivery of drugs. *J Cell Mol Med* **6**(4): 465-474.

Volberding, PA, Deeks, SG (1998) Antiretroviral therapy for HIV infection: promises and problems. *JAMA* **279**(17): 1343-1344.

von Moltke, LL, Greenblatt, DJ, Granda, BW, Giancarlo, GM, Duan, SX, Daily, JP, Harmatz, JS, Shader, RI (2001) Inhibition of human cytochrome P450 isoforms by nonnucleoside reverse transcriptase inhibitors. *J Clin Pharmacol* **41**(1): 85-91.

Vyas, TK, Shah, L, Amiji, MM (2006) Nanoparticulate drug carriers for delivery of HIV/AIDS therapy to viral reservoir sites. *Expert Opin Drug Deliv* **3**(5): 613-628.

Vyas, TK, Shahiwala, A, Amiji, MM (2008) Improved oral bioavailability and brain transport of Saquinavir upon administration in novel nanoemulsion formulations. *Int J Pharm* **347**(1-2): 93-101.

Wacher, VJ, Wu, CY, Benet, LZ (1995) Overlapping substrate specificities and tissue distribution of cytochrome P450 3A and P-glycoprotein: implications for drug delivery and activity in cancer chemotherapy. *Mol Carcinog* **13**(3): 129-134.

Wainberg, MA, Friedland, G (1998) Public health implications of antiretroviral therapy and HIV drug resistance. *JAMA* **279**(24): 1977-1983.

Wakeham, K, Ggayi, AB, V Watson, V, Parkes, R, H Grosskurth, H, Kamali, A, Khoo, S, and Lalloo, D (2009) Co-administration of fluconazole increases nevirapine concentrations in HIV-infected Ugandans, CROI, Montreal, 2009 Abstr no.700.

Wan, L, Pooyan, S, Hu, P, Leibowitz, MJ, Stein, S, Sinko, PJ (2007) Peritoneal macrophage uptake, pharmacokinetics and biodistribution of macrophage-targeted PEG-fMLF (N-formyl-methionyl-leucyl-phenylalanine) nanocarriers for improving HIV drug delivery. *Pharm Res* **24**(11): 2110-2119.

Wang, H, Tompkins, LM (2008) CYP2B6: new insights into a historically overlooked cytochrome P450 isozyme. *Curr Drug Metab* **9**(7): 598-610.

Watkins, PB, Wrighton, SA, Schuetz, EG, Molowa, DT, Guzelian, PS (1987) Identification of glucocorticoid-inducible cytochromes P-450 in the intestinal mucosa of rats and man. *J Clin Invest* **80**(4): 1029-1036.

Weiss, J, Rose, J, Storch, CH, Ketabi-Kiyanvash, N, Sauer, A, Haefeli, WE, Efferth, T (2007a) Modulation of human BCRP (ABCG2) activity by anti-HIV drugs. *J Antimicrob Chemother* **59**(2): 238-245.

Weiss, J, Theile, D, Ketabi-Kiyanvash, N, Lindenmaier, H, Haefeli, WE (2007b) Inhibition of MRP1/ABCC1, MRP2/ABCC2, and MRP3/ABCC3 by nucleoside, nucleotide, and non-nucleoside reverse transcriptase inhibitors. *Drug Metab Dispos* **35**(3): 340-344.

Weiss, J, Weis, N, Ketabi-Kiyanvash, N, Storch, CH, Haefeli, WE (2008) Comparison of the induction of P-glycoprotein activity by nucleotide, nucleoside, and non-nucleoside reverse transcriptase inhibitors. *Eur J Pharmacol* **579**(1-3): 104-109.

WHO (2005) Interim WHO Clinical Staging of HIV/AIDS and HIV/AIDS Case Definitions for Surveillance . 2005. (www.who.int/hiv/pub/guidelines/casedefinitions/en/index.html.)

WHO (2007a) WHO Case Definitions of HIV for Surveillance and Revised Clinical Staging and Immunological Classification of HIV-Related Disease In Adults and Children.

WHO (2000a) WHO fact sheet no. 102, Revised September 2000.

WHO (2000b) WHO fact sheet no. 102, Revised September 2000.

WHO (2007b) WHO Schistosomiasis :Fact sheet N°115 (July 2007).

WHO (2007c) WHO Schistosomiasis :Fact sheet N°115 (updated on January 28 and 2007 July 2007).

WHO (2004) World Health Report . Changing history: Deaths and burden of disease by cause, sex and mortality stratum in WHO Regions,a estimates for 2002 (Annex Table 2 and 3).

Williams, D, Feely, J (1999) Underreporting of adverse drug reactions: attitudes of Irish doctors. *Ir J Med Sci* **168**(4): 257-261.

Wong, HL, Bendayan, R, Rauth, AM, Xue, HY, Babakhanian, K, Wu, XY (2006) A mechanistic study of enhanced doxorubicin uptake and retention in multidrug resistant breast cancer cells using a polymer-lipid hybrid nanoparticle system. *J Pharmacol Exp Ther* **317**(3): 1372-1381.

Wu, MH, Wei, CC, Xu, ZY, Yuan, HC, Lian, WN, Yang, QJ, Chen, M, Jiang, QW, Wang, CZ, Zhang, SJ, et al. (1991) Comparison of the therapeutic efficacy and side

effects of a single dose of levo-praziquantel with mixed isomer praziquantel in 278 cases of schistosomiasis japonica. *Am J Trop Med Hyg* **45**(3): 345-349.

www.avert.org.

www.hiv-druginteractions.org.

Yamada, H, Kotaki, H, Nakamura, T, Iwamoto, A (2001) Simultaneous determination of the HIV protease inhibitors indinavir, amprenavir, saquinavir, ritonavir and nelfinavir in human plasma by high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* **755**(1-2): 85-89.

Yates, DM, Portillo, V, Wolstenholme, AJ (2003) The avermectin receptors of Haemonchus contortus and Caenorhabditis elegans. *Int J Parasitol* **33**(11): 1183-1193.

Yeni, PG, Hammer, SM, Carpenter, CC, Cooper, DA, Fischl, MA, Gatell, JM, Gazzard, BG, Hirsch, MS, Jacobsen, DM, Katzenstein, DA, Montaner, JS, Richman, DD, Saag, MS, Schechter, M, Schooley, RT, Thompson, MA, Vella, S, Volberding, PA (2002) Antiretroviral treatment for adult HIV infection in 2002: updated recommendations of the International AIDS Society-USA Panel. *JAMA* **288**(2): 222-235.

Youdim, KA, Avdeef, A, Abbott, NJ (2003) In vitro trans-monolayer permeability calculations: often forgotten assumptions. *Drug Discov Today* **8**(21): 997-1003.

Young, B (2005) Review: mixing new cocktails: drug interactions in antiretroviral regimens. *AIDS Patient Care STDS* **19**(5): 286-297.

Zack, JA, Arrigo, SJ, Weitsman, SR, Go, AS, Haislip, A, Chen, IS (1990) HIV-1 entry into quiescent primary lymphocytes: molecular analysis reveals a labile, latent viral structure. *Cell* **61**(2): 213-222.

Zala, C, Salomon, H, Ochoa, C, Kijak, G, Federico, A, Perez, H, Montaner, JS, Cahn, P (2002) Higher rate of toxicity with no increased efficacy when hydroxyurea is added to a regimen of stavudine plus didanosine and nevirapine in primary HIV infection. *J Acquir Immune Defic Syndr* **29**(4): 368-373.

Zapor, MJ, Cozza, KL, Wynn, GH, Wortmann, GW, Armstrong, SC (2004) Antiretrovirals, Part II: focus on non-protease inhibitor antiretrovirals (NRTIs, NNRTIs, and fusion inhibitors). *Psychosomatics* **45**(6): 524-535.

Zeng, Z, Andrew, NW, Arison, BH, Luffer-Atlas, D, Wang, RW (1998) Identification of cytochrome P4503A4 as the major enzyme responsible for the metabolism of ivermectin by human liver microsomes. *Xenobiotica* **28**(3): 313-321.

Zerit (2007) Summary of Product Characteristics, Bristol-Myers Squibb Pharmaceuticals Ltd, January 2007.

Zhang, H, Wang, D, Butler, R, Campbell, NL, Long, J, Tan, B, Duncalf, DJ, Foster, AJ, Hopkinson, A, Taylor, D, Angus, D, Cooper, AI, Rannard, SP (2008a) Formation and enhanced biocidal activity of water-dispersable organic nanoparticles. *Nat Nanotechnol* **3**(8): 506-511.

Zhang, L, Gu, FX, Chan, JM, Wang, AZ, Langer, RS, Farokhzad, OC (2008b) Nanoparticles in medicine: therapeutic applications and developments. *Clin Pharmacol Ther* **83**(5): 761-769.

Zhang, Y, Guo, X, Lin, ET, Benet, LZ (1998) Overlapping substrate specificities of cytochrome P450 3A and P-glycoprotein for a novel cysteine protease inhibitor. *Drug Metab Dispos* **26**(4): 360-366.

Zhang, YJ, Quan, YZ (1997) [Involvement of cytochrome P4503A in the monohydroxylation of ring A of praziquantel in rat liver microsomes]. *Yao Xue Xue Bao* **32**(1): 5-10.

Zimmerman, C, Klein, KC, Kiser, PK, Singh, AR, Firestein, BL, Riba, SC, Lingappa, JR (2002) Identification of a host protein essential for assembly of immature HIV-1 capsids. *Nature* **415**(6867): 88-92.