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Analysis of Antiretroviral Drugs in Biological Matrices for Therapeutic Drug Monitoring

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ABSTRACT

High-performance liquid chromatographic (HPLC) analysis of antiretroviral (ARV) drugs in biological matrices are reviewed for therapeutic drug monitoring purposes. HPLC is the most often used analysis method of these drugs. Liquid-liquid extraction, solid phase extraction and protein precipitation were used for the prepurification of the biological samples. ARV drugs were detected by various detection methods, including ultraviolet, fluorescence, mass spectrometry (MS) and MS/MS. This review shows that HPLC methods allow quantitative determination of antiretroviral drugs, individually or simultaneously in biological matrices for therapeutic drug monitoring purposes.

Key words: therapeutic drug monitoring, protease inhibitors, nucleoside reverse transcriptase inhibitors, nucleotide reverse transcriptase inhibitors, high performance liquid chromatography

INTRODUCTION

As infection with human immunodeficiency virus (HIV) continues to spread throughout the world, HIV/AIDS has become a major contributor to global morbidity and mortality. At the end of 2003, 40 million people worldwide were estimated to be living with HIV infection[1].

Five drug classes have since been developed for the treatment of infection with HIV: (I) protease inhibitors (PIs), (II) nucleoside reverse transcriptase inhibitors (NRTIs), (III) non-nucleoside reverse transcriptase inhibitors (NNRTIs), (IV) nucleotide reverse transcriptase inhibitors (NtRTIs) and (V) fusion inhibitors (Table 1).

The use of combination regimens of three or more antiretrovirals, often referred to as highly active antiretroviral therapy (HAART) has proven extremely effective in reducing the morbidity and mortality associated with HIV infection[2]. HAART regimens typically are composed of a backbone of 2 NRTIs combined with either a PI (often boosted with low-dose ritonavir) or an NNRTI.

There is increasing evidence that virological treatment failure is at least partially correlated with variations in the pharmacokinetic parameters in drugs[3,4]. These variations may be due to drug-drug interactions, low bioavailability, inter-patient variability in drug disposition and special variations in the activity of metabolic enzymes. Therapeutic drug monitoring (TDM) comprises of determination of a drug's concentration in blood, comparison of the result with a therapeutic range, and adjustment of the applied dose according to the measured concentration. TDM may be the only way to effectively verify compliance, an issue which has been shown to be critical in HIV therapy[5].

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Numerous methods have been published for the analysis of protease inhibitors[6,7] and nucleoside analogues[8] in biological matrices, which have been reviewed. In this literature, the utilization of HPLC methods for quantitative determination of antiretroviral drugs in biological matrices for therapeutic drug monitoring purposes were reviewed.

METABOLISM AND PHARMACOKINETICS

At the present, available antiretroviral drugs include six nucleoside reverse transcriptase inhibitors (zidovudine (AZT), didanosine (ddI), zalcitabine (ddc), stavudine (d4T), lamivudine (3TC) and abacavir (ABC)), three non-nucleoside reverse transcriptase inhibitors (nevirapine (NVP), delavirdine (DLV), efavirenz (EFV)), nucleotide reverse transcriptase inhibitors (tenofovir (TNF)), ten HIV protease inhibitors (saquinavir (SQV), ritonavir (RTV), indinavir (IDV), nelfinavir (NFV), amprenavir (APV), lopinavir (LPV), atazanavir (ATV), emtricitabine (FTC), tipranavir (TPV) and fosamprenavir) and fusion (entry) inhibitors (enfuvirtide (T-20)).

Reverse transcriptase inhibitors act through at least two mechanisms. First, they act as "chain terminators", in other words, they block the elongation of the DNA chain through blockage of further nucleosides. This mechanism is characteristic of the nucleoside analogs, such as AZT, ddI, ddc, d4T, and 3TC, and depends on the intracellular phosphorylation of the drugs to the corresponding triphosphate. Second, they act by competition/binding of the reverse transcriptase in functionally essential sites. Non-nucleoside reverse transcriptase inhibitors act only through this mechanism and not as "chain terminators". Nucleoside analogs in general have good oral bioavailability bind only

Table 1. Antiretroviral drugs

Protease inhibitors (PIs)	Nucleoside reverse transcriptase inhibitors (NRTIs)	Non-nucleoside reverse transcriptase inhibitors (NNRTIs)	Nucleotide reverse transcriptase inhibitors (NtRTIs)	Fusion (entry) inhibitors
Amprenavir	Abacavir	Delavirdine	Tenofovir	Enfuvirtide
Indinavir	Didanosine	Efavirenz		
Nelfinavir	Lamivudine	Nevirapine		
Ritonavir	Stavudine			
Saquinavir	Zalcitabine			
Lopinavir	Zidovudine			
Atazanavir				
Emtricitabine				
Tipranavir				
Fosamprenavir				

minimally to plasma proteins and are excreted through the kidneys. Cerebrospinal fluid to plasma ratios may be variable, ranging from 10% to 80%. They are generally active on HIV-1 and HIV-2. Non-nucleoside reverse transcriptase inhibitors are characterized by an HIV-1 restricted antiviral activity and are generally metabolized by the liver; interactions with other drugs with hepatic metabolism may occur. Their binding to plasma protein can also be higher than that with nucleoside analogs and binding site displacement effects are possible[9].

NtRTIs are very similar to the NRTIs, but are chemically pre-activated to quickly convert to the actual form of drug in the body, allowing the NtRTIs to enter the HIV's DNA more rapidly than the drugs in the NRTI class. Tenofovir is the first drug (and so far, the only one) in the category of nucleotide reverse transcriptase inhibitors (NtRTIs) to be approved by the FDA. While the NRTIs, NtRTIs, NNRTIs, and PIs are all working inside the infected CD4 cell to treat HIV, fusion inhibitors fight HIV outside the CD4 cell by blocking fusion of HIV before the virus enters the cell and begins its replication process[10]. Enfuvirtide was approved by the FDA in March 2003 for use in adults and children with advanced HIV infection.

The HIV-1 protease is a dimer consisting of two 99-amino acids to form the catalytic site[11]. Protease inhibitors act by binding to the catalytic site of the HIV aspatic protease. This enzyme is critical in the post-translational processing of the polyprotein products of gag and *gag-pol* genes into the functional core proteins and viral enzymes, respectively. Its inhibition leads to the release of immature, noninfectious viral particles. Most of the protease inhibitors are compounds that mimic the part of the structure of *gag-pol* protein that is recognized by HIV-protease. PIs as nucleoside analogs, which are active on HIV-1 and HIV-2, have shown antiviral activity in primary human lymphoid and monocytic cell lines and against a variety of viral strains, unlike inhibitors of reverse transcriptase which provides no protection in established *in vitro* infection. Protease inhibitors are active in chronically infected cells. Finally, PIs are active as the administered compound and do not need intracellular phosphorylation[9]. Most of the HIV protease inhibitors have poor systematic bioavailability. APV, IDV, RTV, NFV, SQV and LPV all undergo oxidative metabolism by CYP3A4, and additional CYP isoforms metabolize individual protease inhibitors. Metabolism occurs predominantly in the liver, but metabolism by intestinal epithelial cells may also decrease bioavailability[12].

Selected pharmacokinetic parameters of some ARV drugs are shown in Table 2.

ANALYTICAL METHODS

The acquired immunodeficiency syndrom epidemic is one of the greatest challanges facing the medical community today. Over the past several years, there has been rapid increase in the number of marketed anti-HIV drugs. Currently, there are 21 marketed antiretrovirals with several others expected to reach the marked in the near future.

As antiretroviral regimens become more complicated, there is a growing need for monitoring antiretroviral drug levels in HIV infected patients to maintain concentrations that provide maximal therapeutic effect with the least possible toxicity. TDM may be useful in the management of patients who are prescribed combination therapies with CYP3A4 inhibitors, in patients with severe liver failure, in pregnant women and in children.

The majority of the methods for the quantitation of ARV drugs in biological matrices involves HPLC assay. HPLC methods developed for the analysis of nucleoside analogs[8] and PIs[6] up to the year of 2001 have already been reviewed. This review, therefore, includes only recently developed HPLC methods of NRTIs and PIs established since 2001. The other review study reported by Crommentuyn *et al*.[7] includes HPLC methods based on the bioanalysis of PIs in only Peripheral blood mononuclear cells (PBMC) samples. HPLC methods in this review,

Table 2. Pharmacokinetic parameters of antiretroviral drugs

Agent	Oral bio-availaility (hr)	Plasma $t_{1/2elim}$ (hr)	Elimination	%Plasma protein binding	%Renal excretion of parent drug	T_{max} (hr)	C_{max} (mg/L)	Volume of distribution (V_d) (L/hr•kg)
NRTIs								
Zidovudine	60	0.8-1.9	Hepatic glucuronidation Renal excretion	20-38	15	0.5-1	—	1.6+0.6
Didanosine	40	1.4	Celllular metabolism Renal excretion	<5	20-50	1	2.6	0.8-1.2
Zalcitabine	90	1-2	Renal excretion	<5	70	1	–	0.5
Stavudine	80-90	1.4	Renal excretion	<5	40	0.5-1		0.5
Lamivudine	80	5-7	Renal excretion	<35	70	1-1.5	–	1.3
Abacavir	>70	0.8-1.5	Hepatic glucuronidation and carboxylation	50	<5	–	3.36	–
NNRTIs								
Nevirapine	90	25-30	Hepatic cytochrome P450	60	<3	–	20	–
Delavirdine	85	2-11	Hepatic cytochrome P450	98	<3	–	35	–
Efavirenz	50	40-90	Hepatic cytochrome P450	99	<3	–	6-10	
PIs								
Saquinavir	12	7-12	Hepatic cytochrome P450	98	<3	–	0.04-0.10	10
Ritonavir	65-75	3-5	Hepatic cytochrome P450	98-99	3.5	–	10-12	0.4
Indinavir	60-65	1.5-2	Hepatic cytochrome P450	60	11	–	5-11	–
Nelfinavir	20-80	3.5-5	Hepatic cytochrome P450	98	1-2	–	3-4	2-7
Amprenavir	35-90	7-11	Hepatic cytochrome P450	90	<3	–	3-8	–
Lopinavir	?	6-8	Hepatic cytochrome P450	98-99	<3	–	–	–
NtRTIs								
Tenofovir	25	17	Glomerular filtration and active tubular secretion	0.7	–	–	–	1.3 ± 0.6

include the determination of all classes of ARV drugs in various biological matrices. Reported publications are summarized below and listed in Table 3. Among these methods, those used for biological samples of animals are also reviewed as though applicable to biological samples of humans.

I. *Analysis of Single ARV*

(I) *Protease Inhibitors*

Campanero *et al*.[13] developed HPLC-UV method for measuring SQV in plasma samples of HIV patients. The LOQ was 1 ng mL^{-1} and only 0.5 mL of plasma sample was required for the analysis. Burhenne *et al*.[14] developed a highly sensitive method for analysis of SQV in plasma, saliva or urine samples, consisting of liquid-liquid extraction (LLE) followed by HPLC and tandem mass spectrophotometric (TMS) detection using an electrospray ion source. In this method, the LOQ was 0.05 ng mL^{-1}. The method was reported by Pereira *et al*.[15] for the measurement of APV in seminal plasma using HPLC-TMS, which requires only 100 μL of sample. The method is sensitive and selective. Gunawan *et al*.[16] reported LC/MS/MS assay for quantitation of APV (agenere) in patient serum or plasma. Schuster *et al*.[17] developed HPLC/MS method for the determination of ATV in plasma using automated 96-well solid phase extraction. Jemal *et al*.[18] also developed another HPLC/MS method for analysis of ATV in PBMCs. Colombo *et al*.[19] described HPLC-UV assay for measuring ATV in plasma using solid phase extraction (SPE). Hua *et al*.[20] reported on-line column-switch LC/MS/MS method to measure NFV and its major metabolite (M1) in rat plasma. Herforth *et al*.[21] developed and validated a method for measuring the free fraction of NFV in plasma employing equilibrium dialysis for the separation of free (unbound) drug and LC/TMS for quantitation. NFV is a highly bound HIV protease inhibitor with the fraction bound in plasma greater than 98%. Thus variations in the free fraction may be clinically important when interpreting total drug concentrations. Panchagnula *et al*.[22] published an HPLC method based on UV detection for quantification of IDV from ex-vivo rat intestinal permeability studies, in the presence of propranolol. Jayewardene *et al*.[23] described the method validation of LC/MS/MS assay for IDV. The sample preparation consisted of precipitating plasma proteins with acetonitrile. Darque *et al*.[24] developed HPLC method combining SPE to quantitate the intracellular active 5'-triphosphate (TP) of emtricitabine (FTC) in human PBMCs of patients infected with HIV after various oral

Table 3. HPLC analysis of antiretroviral drugs

Drug, metabolites	Tissue volume (µL)	Pretreatment	Stationary phase	Mobile phase Flow rate (mL/min)	Detection (nm)	Run time (min)	Range	IS	Ref
SQV	P 500	LLE Tert-butyl methyl ether	LiChrospher 60 Select B C8 column	Isocratic elution MeCN/0.05 M ammonium acetate (68:32) FR: 1	UV 238	10	0.0025-5 µg mL^{-1}	Verapamil	13
SQV	P, U, saliva samples 1000	LLE Ethyl acetate	Kromasil C18 column	Isocratic elution 0.1% aqueous acetic acid including 5 mM ammonium acetate (A) and MeCN (B) The eluent (55%A/45%B) FR:0.35	MS/MS	5	0.05-87.6 ng mL^{-1}	$^{2}H_5$-SQV	14
APV	H seminal P 100	PP	Aquasil C18 column	Isocratic elution Mobile phase I: MeCN/water (55:45, v/v) with 0.1% formic acid FR: 0.3 Mobile phase II: MeCN/water (55:45, v/v) FR: 0.3	TMS	4.5	0.01-5 µg mL^{-1}	$^{13}C_6$-APV	15
APV	S, P 500	LLE Diethylether	C18 column	Isocratic elution MeCN/water (1:1, v/v) with 0.1 % formic acid FR: 0.15	TMS	5	0.05-10 µg mL^{-1}	Reserpine	16
ATV	P 250	SPE Oasis HLB	C18 column HDO	Gradient elution A: 5 mM ammonium acetate solution B: MeCN FR: 0.8	TMS	4.5	0.01-1 µg mL^{-1} 0.01-2 µg mL^{-1}	$^{13}C_6$-Atanazavir	17
ATV	Human PBMC	Automated SPE 3M Empose® C2-SD 96 well plates	YMC Basic	Isocratic elution MeCN/MeOH/water (300 mL:300 mL:400 mL) with 250 µL of 88% formic acid FR: 0.25	MS/MS	4	5-2500 fmol/10^6 cells	$^{13}C_6$-Atanazavir	18
ATV	P 600	SPE C18 cartridge	Nucleosil C18 column AB	Gradient elution A: MeCN B: adding 8.5% H_3PO_4 (11.8 mL) and 0.2 g sodium heptane sulfonate to 988.2 mL of H_2O (pH = 5 with NaOH) C: 0.3% ACOH in MeCN FR: 1	UV 201	45	0.25-10 µg mL^{-1}	Clozapine	19
NFV and major metabolite M1	Rat P	na	C8 column	Isocratic elution MeCN:0.05% acetic acid (60:40) FR: 1	MS	5	0.8-400 ng mL^{-1} for NFV, 0.2-80 ng mL^{-1} for M1	Reserpine	20
NFV	P dialysates 50	PP MeCN	Zorbax XDB-C8 column	Gradient elution A: Ammonium formate buffer (10 mmol/L, pH = 4.1) B: MeCN with concentrated formic acid (98%)	TMS	2	1-50 ng mL^{-1} for free drug buffer dialysate 100-5000 ng mL^{-1} for total drug (plasma dialysate)	Methyl-indinavir	21

Table 3. HPLC analysis of antiretroviral drugs

Drug, metabolites	Tissue volume (μL)	Pretreatment	Stationary phase	Mobile phase Flow rate (mL/min)	Detection (nm)	Run time (min)	Range	IS	Ref
IDV, Propranolol	Rat Ex-viva samples	PP MeCN	Hypersil BDS C18 column	Isocratic elution Phosphate buffer/acetonitrile (68:32, v/v) FR: 1	UV 210	12	2-20 μg mL^{-1}	Fluorescein	22
IDV	P 200	PP MeCN	XDB-C8 Column	Gradient elution A:10 mM ammonium formate buffer (pH=4.1) B: MeCN FR: 0.20-1	TMS	5	3-12320 ng mL^{-1}	Methylderivative of IDV	23
FTC	PBMCs	Anion-exchange SPE cartridge	C18 column	Isocratic elution Phosphate buffer (43 mM, pH=7.0)/acetonitrile (93:7, v/v) FR: 1	UV 280	14	na	β-l-FddC	24
AZT, G-AZT	P 500 U	SPE on-line	Nova Pak C18 column	Isocratic elution MeOH-THF-potassium phosphate buffer (0.0025 M, pH = 3.1) (3.7:2.8:93.5, v/v) FR: 1	UV 270	na	0.02-2 μg mL^{-1} for AZT 0.01-2 μg mL^{-1} for G-AZT	IPU	25
AZT and its anabolites	Animal Tissues	Ice-cold trichloroacetic acid	Nova Pak C18 column	Isocratic elution 0.2 M potassium phosphate buffer containing 4 mM TBA (pH = 7.5) /MeCN (97.5:2.5, v/v) FR: 1.5	UV 270	16	na	na	26
AZT	P 200	SPE C18	C18 column	Isocratic elution MeOH/water (80:20) with 5 mM TEM with phosphoric acid (pH = 7) FR: 1 for dose formulation, Ammonium phosphate/MeCN (88:12) adjusted to pH = 7.2 using HCl for P analyses FR: 0.8	UV 254 for dose 267 for P analyses	na	0.1-30 μg mL^{-1} for P	1-(3-azido-2,3-dideoxy B-Dthreopentafuranosyl) thiamine for P	27
DdI	Human body fluids	SPE C18	NovaPak C18	Isocratic elution 5% MeCN in heptafluorobutyric acid (0.1 %, v/v) in water (v/v) FR: 2	UV 252 and 260	8.4 for P and CSF; 19.6 for U	0.212-13.6 μM for P	2'-deoxyguanosine	28
ABC its major metabolites 2269w, 361w	U, CSF	na	Kromasil octadecyl column	Gradient elution Mobile phase A: 25 mM ammonium acetate buffer (pH = 4 with acetic acid)/MeOH (95:5, v/v) Mobile phase B: MeCN FR: 0.7	UV 295	40	0.629-52.1 μg mL^{-1} for U, 0.062-5.13 μg mL^{-1} for cerebrospinal fluid	na	38
NVP	P 250	PP MeCN	(ion-pair) C18 column	Isocratic elution 25 mM phosphate buffer (pH = 5.5)/MeOH/MeCN (7:2:1, v/v/v) containing 25 mM hexane-sulfonic acid FR: 1	UV 282	12	0.052-10.4 μg mL^{-1}	na	39

Table 3. HPLC analysis of antiretroviral drugs

Drug, metabolites	Tissue volume (µL)	Pretreatment	Stationary phase	Mobile phase Flow rate (mL/min)	Detection (nm)	Run time (min)	Range	IS	Ref
NVP	P 150	PP Perchloric acid	Hypersil ODS column	Isocratic elution MeCN/60 mM phosphate buffer (pH=4.5) (30:70, v/v) FR: 1.5	UV 280	6.5	0.1-10 µg mL^{-1}	na	40
NVP	S 200	PP Trichloroacetic acid	Spherisorb C18 column	Isocratic elution 10 mM phosphate buffer (containing 10 mM triethylamine, pH=5) /MeCN (82:18, v/v) FR: 1	UV 240	3	0.1-10 µg mL^{-1}	na	41
NVP	P 50	PP Perchloric acid	Zorbax XDB-C8 column	Gradient elution A: ammonium formate (pH=4.1) B: 0.1 % (v/v) solution of pure formic in HPLC grade MeCN FR: 0.4	MS/MS	5	0.025-1 µg mL^{-1} and 1-10 µg mL^{-1}	IS+	42
DLV	P 50	PP MeCN	C18 column	Isocratic elution MeCN/50 mM sodium dihydrogenphosphate (60:40, v/v) FR: 1	FL Ex: 295 Em: 425	7	0.025-25 µg mL^{-1}	Cisapride	43
DLV	P 200	PP MeCN	Zorbax SB C18 column	Isocratic elution 25 mM Citrate buffer/MeCN (82:18, v/v) FR: 1.5	FL Ex: 300 Em: 425	25	0.050-50 µg mL^{-1}	na	44
EFV	P 300	SPE Oasis	Supelcosil LC8 column	Isocratic elution phosphate buffer (pH: 5.75)/MeCN (55:45, v/v) FR: 1	UV 250	10	0.1-10 µg mL^{-1}	L-737,354	45
EFV	P 250	PP MeCN	Zorbax SB C18 column	Isocratic elution 25 mM phosphate buffer/MeCN (53:47, v/v) FR: 1.5	UV 246	15	0.010-10 µg mL^{-1}	na	46
EFV	P 200	LLE Diethylether	X Terra RP18 column	Isocratic elution MeCN/67 mM potassium dihydrogenphosphate (pH = 7.4) (50:50, v/v) FR: 0.2	UV 246	18	0.025-15 µg mL^{-1}	A-86091	47
EFV	P 100	LLE Diethylether	Inertsil ODS column	Isocratic elution MeCN/water (65:35, v/v) FR: 1.2	UV 247	13	0-9000 ng mL^{-1}	SQV	48
EFV	P 500	LLE Hexane/ methylene chloride	YMC Octyl column S-5 120 A	Isocratic elution MeCN/50 mM phosphate buffer (pH = 3.5) (53:47, v/v) FR: 1	FL Ex: 310 Em: 390	14	0.050-1 µg mL^{-1}	IS+	49

Table 3. HPLC analysis of antiretroviral drugs

Drug, metabolites	Tissue volume (μL)	Pretreatment	Stationary phase	Mobile phase Flow rate (mL/min)	Detection (nm)	Run time (min)	Range	IS	Ref
TNF	P 1000	SPE Supelclean TM LC-18	Symmetry Shield C18 column	Gradient elution A: pH = 6 buffer B: MeCN FR: 1	UV 259	10	0.010-4 μg mL^{-1}	na	50
TNF	P 200	PP Trichloroacetic acid	(ion-pair) Chromspher C8 column	Gradient elution A: buffer (pH = 7) 10 mM sodium phosphate and 5 mM tetrabuthylammonium hydrogen sulfate B: Comprised of this buffer with 50% (v/v) MeCN FR: 1.5	FL Ex: 254 Em: 425	20	0.02-1 μg mL^{-1}	Adefovir	51
TNF	P 200	PP MeOH	C8 plus satisfaction column	Isocratic elution 5 mM (pH = 6) phosphate buffer containing tetrabthuylammonium chloride/MeCN (85:15, v/v) FR: 0.5	FL Ex: 236 Em: 420	12	0.005-1 μg mL^{-1}	Adefovir	52
DDC	Rat MP, AF, PL, FT	PP for MP, AF MeCN SPE for PL, FT Oasis HLB	Spherisorb S 3W silica column	Isocratic elution 10% MeOH in water with 22 mM formic acid FR: 0.5	UV 275	12	0.15-75 μg mL^{-1}	3TC	53
T-20 and its metabolite	P	PP MeCN	C18 column	Gradient elution Mobile phase A: water/acetic acid/TFA (100:0.2:0.02, v/v/v) Mobile phase B: MeCN/MeOH/acetic acid/TFA (85:15:0.2:0.02, v/v/v/v) FR: 0.4-1.0	MS/MS	7.5	10-2000 ng mL^{-1} for T-20	leucine. d_{10} T-20	54
APV, IDV, LPV, NFV,RTV, SQV, M8, EFV	P 1000	SPE, Oasis HLB extraction Cartridges	Novapak C18 column	Gradient elution A: buffer (pH = 5)/MeCN/MEOH (42.5: 28:29.5) B: 75% MeCN C: 25% HPLC grade water FR: 0.45	UV 265 for APV; 210 for all other assay drug	60	0.025-10 μg mL^{-1}	Monohydrate IDV, methyl IDV	55
ABC, ZDV, EFV, NVP, IDV, LPV, NFV	P 500 and 1000	LLE, Ethyl acetate-hexane	LiChroCART C18 column	Isocratic elution 450 mL MeCN/50 mL MeOH in 15 mmol/L phosphate buffer (pH = 7.5) for IDV, LPV, NFV, EFV FR: 1 170 mL MeCN in 15 mmol/L phosphate buffer (pH = 7.5) for ZDV, ABC, NVP FR: 1.5	UV 215 for IDV, LPV, NFV; 254 for EFV; 266 for NVP, AZT, ABC	na	0.2-10 μg mL^{-1}	na	56

Table 3. HPLC analysis of antiretroviral drugs

Drug, metabolites	Tissue volume (µL)	Pretreatment	Stationary phase	Mobile phase Flow rate (mL/min)	Detection (nm)	Run time (min)	Range	IS	Ref
IDV, APV, SQV, NFV, RTV, LPV, NVP, DLV, EFV	Human B, P 550	SPE BOND ELUT C18	Zorbax C18 column	Gradient elution Mobile phase A: 10 mM phosphate monobasic (pH = 4.5) with 150 mL MeOH Mobile phase B: Mixture of mobile phase A (250 mL) with MeCN (600 mL), MeOH (150 mL) and TFA (0.75 mL) FR: 0.9-1.1	UV 220, 0-9 min 210, 9-30 min.	30	0.01-10 µg mL^{-1} for NVP, IDV, SQV; 0.01-5 µg mL^{-1} for EFV, APV; 0.025-5 µg mL^{-1} for DLV, NFV, RTV, LPV	Midazolam	57
APV, EFV, IDV, LPV, NFV, M8, RTV, SQV	Human P 250	LLE, Ethylacetate	Symmetry C8 column	Isocratic elution MeCN/formic acid buffer pH 4.26 to 4.27 (40:60, v/v) FR: 0.75-1.6	UV 215 for IDV, LPV, NFV; 235 for SQV and M8; 265 for APV; 248 for EFV	20	0.1-20 µg mL^{-1} for all analytes except for LPV and RTV, which ranged from 0.2-20 µg mL^{-1}	IS+	58
IDV, APV, SQV, RTV, LPV, NFV, M8, NVP	P 500	LLE, Methyl tert-butyl ether	Omnispher 5 C18 column	Gradient elution A: MeCN and 50 mmol/L potassium phosphate (pH = 5.75) B: 36%-61% MeCN FR: 1.5	UV 215 for PI, 280 for NVP	25	0.05-30 µg mL^{-1} for IDV, NFV, RTV, SQV; 0.07-30 µg mL^{-1} for APV, LPV; 0.05-15 µg mL^{-1} for M8, NVP	na	60
IDV, APV, RTV, LPV, SQV, NFV, M8	P 500	LLE Heptane-ethylacetate	Nova-Pak C18 column	Isocratic elution 140 mL of MeCN, 75 µL of TEM, 160 µL of 5 mM sodiumdhydrogen phosphate (pH:6) FR: 1	UV 210, 0-9.8 min 239, 9.8-15 min	30	230-1130 ng mL^{-1} for IDV, 76-1127 ng mL^{-1} for APV, 51-2025 ng mL^{-1} for RTV, 1743-3667 ng mL^{-1} for LPV, 75-2449 ng mL^{-1} for SQV, 390-2598 ng mL^{-1} for NFV, 195-864 ng mL^{-1} for M8	na	61
LPV, NVP	P 600	SPE C18 cartridges	Nucleosil C18 AB column	Gradient elution A: Pure MeCN B: 11.8 mL H_3PO_4 8.5%/0.2 g sodium heptane sulfonate to 988.2 mL H_2O (pH = 5 with NaOH) C: 0.3% ACOH in MeCN FR: 1	UV 201 for LPV, 282 for NVP	30	0.5-10 µg mL^{-1} for NVP, 0.1-10 µg mL^{-1} for LPV	Clozapine	62
SQV, RTV	P 1000	LLE Ethylacetate/ hexane (50:50, v/v)	C18 column	Isocratic elution MeCN: 70 mM KH_2PO_4 (pH = 5) (46:54, v/v) FR: 1	UV 240 for SQV, 210 for RTV	9	100-2500 ng mL^{-1} for SQV, 200-2500 ng mL^{-1} for RTV	na	64

Table 3. HPLC analysis of antiretroviral drugs

Drug, metabolites	Tissue volume (µL)	Pretreatment	Stationary phase	Mobile phase Flow rate (mL/min)	Detection (nm)	Run time (min)	Range	IS	Ref
IDV, APV, SQV, RTV, NFV	P 500	LLE 0.5 mL of NH_4OH and 5 mL of methyl tert-butyl ether	C18 column	Isocratic elution MeCN and 50 mM KH_2PO_4 (pH:5.6) (43:57, v/v) FR: 1.5	UV 215	40	0.05-20 µg mL^{-1}	na	65
IDV, APV, RTV, SQV, NFV	P 400	SPE Oasis	C18 column	Gradient elution A: 15 mM phosphate buffer (pH=5.75) B: MeCN FR: 1	UV	25	na	Verapamil	66
IDV, RTV, SQV	P 500	LLE methyl tert-butyl ether	C18 column	Isocratic elution 50 mM phosphate buffer (pH=5.6)/MeCN (55:45, v/v) FR: 1.5	UV 215	15	0.1-5 µg mL^{-1}	Propyl-paraben	67
APV, IDV, LPV, NFV, RTV, SQV, EFV, NVP, M8	P 250	LLE Diethylether	X-Terra column	Isocratic elution 58% water (with 3 mmol/L pyrrolidine)/42 % MeCN FR:1	UV 222 for LPV, 240 for RTV, SQV, 260 for the others	30	25-9000 ng mL^{-1}	A86093.0	68
LPV, RTV, EFV	P, S 500	LLE Ethylacetate/ n-hexane (50:50, v/v)	C18 column	Isocratic elution MeCN/MeOH/0.02 M tetra-methylammonium perchlorate (TMAP) in dilute aqueous trifluoroacetic acid (45:5:50, v/v/v) FR: na	UV 205	20	0.060-24.06 µg mL^{-1} for LPV, 0.010-4.16 for RTV, 0.047-37.44 for EFV	IS+	69
ATV, NVP, EFV, IDV, APV, SQV, NFV, RTV, LPV, M8	P 1000	LLE Ethylacetate: n-hexane (9:1, v/v)	C18 column	Gradient elution A: MeCN/0.025 M tetramethyl ammonium perchlorate (0.2% aqueous trifluoroacetic acid (55:45, v/v) B: MeOH/0.025 M tetramethyl ammonium perchlorate (0.2% aqueous trifluoroacetic acid (55:45, v/v) FR: 0.9 for LPV, FR: 1.1 for the others	UV 259 except for LPV (205), NVP (320)	35	0.125-5 µg mL^{-1} for M8; 0.25-10 µg mL^{-1} for IDV, ATZ, NFV, APV, SQV, RTV; 0.5-20 µg mL^{-1} for NVP, LPV, EFV	A 86093	70
ATV, IDV, LPV; NFV, RTV, SQV, M8, EFV	P 500	LLE Diethylether	RP18 column	Gradient elution A: 15 mM phosphate buffer (pH=4.2) B: MeCN FR: 1	UV 210	60	50-1000 ng mL^{-1}	IS+	71

Table 3. HPLC analysis of antiretroviral drugs

Drug, metabolites	Tissue volume (µL)	Pretreatment	Stationary phase	Mobile phase Flow rate (mL/min)	Detection (nm)	Run time (min)	Range	IS	Ref
APV, IDV, LPV, NFV, RTV, SQV, EFV, NVP	P 1000	LLE, Diethylether	Stability C18 column	Gradient elution MeCN/phosphate buffer (50 mM, pH=5.65) A: 36-64% MeCN B: 80% MeCN C: 36% MeCN FR: 1.5	UV 240, 5 min, 215, 22 min, 260, 45 min	45	0.1-10 µg mL^{-1}	JRO51012	72
APV, IDV, RTV, LPV, NFV, SQV, NVP, EFV, DLV	P 1000	PP (MeOH/0.2 M ZnSO4, 7:3 ,v/v) SPE C8	Eclipse XDB-C8 column	Gradient elution A: MeOH B: 2 mM ammonium acetate FR: 0.5	MS	6	20-20000 ng mL^{-1}	Pepstatin A	73
IDV, SQV, NFV, APV	Rat P 100, LDS 150	LLE Ether	QUICKSORB ODS column	Isocratic elution 50% MeCN containing 1 % acetic acid FR:0.2	MS	5	0.005-10 µg mL^{-1} for P, 0.005-2 µg mL^{-1} for LDS	IDV for SQV, NFV, APV; SQV for IDV	74
APV, IDV, LPV, NFV, RTV, SQV, EFV, NVP	P 100 or 1000	SPE C18 Cartridges	Nucleosil C18 column HD	Isocratic elution Eluent A: consisted of MeCN containing 30% MeOH and ammonium carbonate buffer (pH:9.3) (5:95, v/v). Eluent B: consisted of MeCN containing 30% MeOH and ammonium carbonate buffer (pH:9.3) (95:5, v/v). FR:0.2	MS	21	0.01-12 µg mL^{-1} for EFV; 0.2-6 µg mL^{-1} for LPV; 0.94-15.8 µg mL^{-1} for NFV; 0.094-3.49 µg mL^{-1} for SQV; 0.47-11.8 µg mL^{-1} for NVP, RTV	A-86093	75
LPV, RTV	P 50	PP MeCN	Zorbax XDB-C18 column	na	TMS	na	277-16000 ng mL^{-1} for LPV, 102-12100 ng mL^{-1} for RTV	na	76
NFV, IDV, RTV, SQV, APV	P 100	PP MeCN	Zorbax XDB-C8 column	Gradient elution A: 1.28 ammonium formate in 2 L HPLC grade water (pH:4.1) B: 0.1% v/v solution of pure formic in HPLC grade MeCN FR:0.4	MS/MS	3.5	0.005-10 µg mL^{-1} for each analyte	IS+	77
APV, RTV, SQV, LPV, IDV, NFV, M8	P 250	LLE, Hexane-ethylacetate	Symmetry C18 column	Gradient elution A: MeCN B:5 mM acetate buffer (pH:3.5) FR:0.35	TMS	3.4	0.0163-10 µg mL^{-1} for APV; 0.0163-4 µg mL^{-1} for IDV, LPV, NFV, SQV; 0.00819-5 µg mL^{-1} for M8; 0.0512-5 µg mL^{-1} for RTV	A-86093	78
IDV, SQV, APV, NFV, RTV, LPV, M8	P 100	PP (MeOH: MeCN)	Inertsil ODS3 column	Gradient elution A:MeOH-10 mM ammonium acetate buffer pH: 5 (35:65, v/v) B: Eluent A were mixed with 85% MeOH C: Eluent A Flow rate:0.5	MS/MS	5.5	0.01-10 µg mL^{-1} for IDV, SQV; 0.1-10 µg mL^{-1} for APV; 0.05-10 µg mL^{-1} for NFV, RTV; 0.1-20 µg mL^{-1} for LPV, 0.01-5 µg mL^{-1} for M8	SQV d_5, IDV d_6	79

Table 3. HPLC analysis of antiretroviral drugs

Drug, metabolites	Tissue volume (μL)	Pretreatment	Stationary phase	Mobile phase Flow rate (mL/min)	Detection (nm)	Run time (min)	Range	IS	Ref
ATV, Tipranavir	P 50-100	PP (MeOH: MeCN)	Inertsil ODS3 column	Gradient elution A: acetate buffer (pH:3.5) B:MeOH FR:0.5	TMS	5.5	0.05-10 μg mL^{-1} for ATV, 0.1-75 μg mL^{-1} for tipranavir	SQV-d_5	80
AZT, 3TC, AZTG, AMT, AZT-5'-phosphate, 3TC-5'-phosphate	Mice S, spleen, rat S (for validation) 10-100	SPE, Isolute ENV+, Isolute NH_2 Cartridge for nucleoside, nucleotite analogue, respectively	Polaris C8 column	Gradient elution A: 0.1% formic acid B: MeCN FR: 0.2	MS	9	0.1-200 μg mL^{-1} for AZT, 0.1-10 μg mL^{-1} for AZTG, 0.1-5 μg mL^{-1} for AMT, 0.1-20 μg mL^{-1} for 3TC	$^{13}C_{11}{}^{5}N_1d_3$ AZT $^{13}C_2{}^{15}N_3$-3TC	81
AZT, ddI, d4T, NVP, 3TC, ABC, ddc	P 800	SPE BOND ELUT C18	Polarity dC column	Gradient elution Mobile phase A: 10 mM ammonium acetate buffer (pH = 6.5) Mobile phase B: Mixture of mobile phase A (200 mL) and MeCN (500 mL) and MeOH (300 mL) FR: 1.1	UV 269, 0-11 min 250, 11-14 min, 271, 14-24 min, 230, 24-33 min.	30	0.01-10 μg mL^{-1} for all analytes except ddc, which is 0.01-5 μg mL^{-1}	Hexobarbital	82
3TC, ddI, d4T, AZT, ABC	P 500	SPE Oasis	C18 column	Gradient elution Mobile phase A: Acetate buffer/MeCN (95:5, v/v) Mobile phase B: Acetate buffer/MeCN (76:24, v/v) FR: 1	UV 260	25	0.015-5 μg mL^{-1}	na	83
Group A: ddc, 3TC, d4T, ddI, AZT, ziagen Group B: IDV, NFV, SQV, RTV, NVP, DLV, EFV	S 500	SPE Method A: Dual Zone C18 Method B: Ordinary C18	Luna C18 column	Gradient elution Method A: Linear gradient: 5% MeCN/45% MeCN FR: 0.85 Method B: 0.004 M sulfuric acid and MeCN (8% to 63% MeCN in 45 min)	UV Method A: 250 Method B: 265 for 31 min, 240 thereafter	45	10 μg mL^{-1} upper limit of calibration standards for drugs in group B except SQV (5 μg mL^{-1}) 8.5 μg mL^{-1} upper limit of calibration standards for drugs in group A	na	84
AZT, 3TC, NVP	P 1000	SPE Oasis HLB	(ion-pair) Nova-Pak C8	Isocratic elution 20 mM sodium phosphate buffer/MeCN (pH = 3.2)(86:14, v/v) FR: 1	UV 265	15	0.0576-2.88 μg mL^{-1} for AZT, 0.059-17.650 μg mL^{-1} for 3TC, 0.0532-13.300 μg mL^{-1} for NVP	Aprobarbital	85

Table 3. HPLC analysis of antiretroviral drugs

Drug, metabolites	Tissue volume (µL)	Pretreatment	Stationary phase	Mobile phase Flow rate (mL/min)	Detection (nm)	Run time (min)	Range	IS	Ref
ddI, d4T	P, BALF, AC, PBMC, seminal P CSF, tonsil tissue	SPE	C18 colum	For isocratic elution, P, AC, PBMC, CSF, For gradient elution, BALF, semen plasma, tonsil tissue Isocratic elution: MeOH/water (16:84, v/v), 0.05 % trifluoroacetic acid and 1 mM ammonium formate FR: 0.8 Gradient elution: A: MeOH/water (16:84, v/v) containing 0.05 % trifluoroacetic acid and 1 mM ammonium formate B: MeOH/water (80:20, v/v) containing 0.05 % trifluoroacetic acid and 1 mM ammonium formate FR: 0.8	TMS	na	1-400 ng mL^{-1} for seminal plasma, 0.4-200 ng mL^{-1} for BALF and PBMC, 0.4-100 ng mL^{-1} for BALF supernatant, 0.5-100 ng mL^{-1} for CSF, 0.01-0.4 ng mL^{-1} for tonsil tissue	3'-Deoxythymidine	86
AZT, 3TC	S 250	Automated	Aquasil C18 column	Isocratic elution MeCN/water (15:85, v/v) FR: 0.35	TMS	6	0.0025-2.5 µg mL^{-1} for AZT 0.0025-5 µg mL^{-1} for 3TC	Isotope labeled AZT	87
3TC, AZT	Human Seminal Plasma 25	Automated	Aquasil C18 column	Isocratic elution MeCN/water (15:85, v/v) FR: 0.3	TMS	5.5	0.005-5 µg mL^{-1}	Isotopically labeled internal standard	88
3TC, AZT	Human serum	SPE polydivinyl benzene cartridges	C18 column	Isocratic elution 20 mM ammonium acetate/methanol (60:40, v/v) containing 1 % acetic acid FR: 1	TMS	4	10-1500 ng $mL^{-1}$for 3TC, 15-3000 ng $mL^{-1}$for AZT	Didanosine	89
ABC, 3TC, AZT	Serum	Automated for 3TC, ZDV LLE trichloroacetic acid for ABC	C18 column	Isocratic elution 40% MeCN in 25 mM ammonium phosphate and 0.3% triethylamine FR: 1	TMS for zidovudine, 3TC UV for ABC 284 nm	na	0.0025-2.5 µg mL^{-1} for 3TC and ZDV 0.025-10 µg mL^{-1} for ABC	na	90
AZT, 3 TC, d4T	PBMC	SPE Anion exchange and C18 cartridges	C18 column	Isocratic elution 10 mM Amonnium acetate/MeCN (86:14, v/v) FR: 0.05	TMS	6	50-45000 pg	AZDU	91
EFV, AZT, d4T, IDV, ABC, NFV, DLV, SQV, NVP, 3TC, RTV, APV, Zalcitabine, ddI, LPV	S, P 80	PP Acetonitrile On-line extraction with ammonium acetate	LC-18-DB column	Switching valve was activated, the column was flushed with MeOH at a rate of 1 mL min^{-1}	TMS	4.5	2-2000 ng mL^{-1} for d4T, ddI, zalcitabine and AZT,10-10000 ng mL^{-1} for all other drugs	cimetidine	92

Table 3. HPLC analysis of antiretroviral drugs

Drug, metabolites	Tissue volume (μL)	Pretreatment	Stationary phase	Mobile phase Flow rate (mL/min)	Detection (nm)	Run time (min)	Range	IS	Ref
AZT, NVP	P 200	SLEOasis HLB	Zorbax SB-C18 column	Isocratic elution 10 mM Potassium dihydrogen phosphate (pH=6.5)/ MeCN (83:17, v/v) FR: 1	UV 265	35	0.05-5 μg mL^{-1} for AZT, 0.150-10 μg mL^{-1} for NVP	3-isobutyl-1-methyl xanthine	93
NVP, DLV, EFV	Human B, P	SPE AcuuBond ODS	Eclipse XDB C8 column	Gradient elution Mobile phase A: 50 mM sodium phosphate buffer (pH = 4.8) Mobile phase B: Mixture of mobile phase A (200 mL) and MeCN (800 mL) FR: 1.5	UV 220, 0-14 min, 224, 14-20 min, 248, 20-28 min.	25	0.01-50 μg mL^{-1} for NVP, 0.025-25 μg mL^{-1} for DLV, 0.010-10 μg mL^{-1} for EFV	Hexobarbital	94
EFV, NVP	P 100	PP MECN	Zorbax Extend C18 column	Isocratic elution 25 mM triethylamine in water/MeCN (65:35, v/v) pH = 11.7 FR: 0.2	UV 275	9	0.05-15 μg mL^{-1} for EFV, 0.25-15 μg mL^{-1} for NVP	Carbamazepine	95
EFV, Rifampicin	P 200	LLE Ethylacetate/ n-hexane (80:20, v/v)	C18 column	Isocratic elution sodium phosphate buffer 0.01 mol L^{-1} (pH = 5.2)/ MeCN/MeOH (40:45:15, v/v/v) FR: 1	UV 254	15	0.5-8 μg mL^{-1}	IS+	96

ABC=Abacavir; AF=Amniotic fluid; APV=Amprenavir; ARV=Antiretroviral; AUC=Area under the curve; ATV= Atazanavir; AZDU=3'-azido-2',3'-dideoxyuridine; AZT= Zidovudine; B=Blood; BALF= Bronchoalveolar lavage fluid; Cmax=Maximum (peak) concentration; CSF=Cerebrospinal fluid; DDC=2',3'-dideoxycytidine; ddI=Didanosine; DLV=Delavirdine; d4T= Stavudine; EFV=Efavirenz; Em=Wavelength of emission; Ex=Wavelength of excitation; FL=Fluorescence; FR=Flow rate; FTC=Emtricitabine; HAART=Highly active antiretroviral therapy; HIV =Human immunodeficiency virus; HPLC=High performance liquid chromatography; IDV=Indinavir; IS+=Internal standard is available, but name is not reported; LC=Liquid chromatography; LLE=Liquid–liquid extraction; LLQ=Lower limit of quantification; LOD=Limit of detection; LOQ=Limit of quantification; LPV=Lopinavir; M8=Nelfinavir metabolite; MeCN= Acetonitrile; MeOH=Methanol; MP=Maternal plasma; MS =Mass spectrophotometry; na=Not available (or not reported); NFV=Nelfinavir; NNRTI=Non-nucleoside reverse transcriptase inhibitor; NRTI=Nucleoside reverse transcriptase inhibitor; NtRI=Nucleotide reverse transcriptase inhibitor; NVP=Nevirapine; P=Plasma; PBMC= Peripheral blood mononuclear cells; PI=Protease inhibitor; PP =Protein precipitation; RTV=Ritonavir; S=Serum; SPE=Solid-phase extraction; SQV=Saquinavir; T-20= Enfuvirtide; TBA=Tetrabuthylammonium hydrogen sulphate; 3TC= Lamivudine; TDM=Therapeutic drug monitoring; TEM=Trietilamine; TFA=Trifluoroacetic acid; TPV =Tipranavir; TMAP=Tetramethylammonium perchlorate; TMS= Tandem mass spectrophotometry; TNF=Tenofovir; ULQ=Upper limit of quantification; UV=Ultraviolet.

dosing regimens of FTC monotherapy.

(II) *Nucleoside Reverse Transcriptase Inhibitors*

Schrive *et al*.[25] presented HPLC-UV method for the assay of AZT and its metabolites in biological fluids using SPE on-line with chromatographic separation. For the analysis, the ionic strength of the potassium phosphate buffer used as the mobile phase plays an important role. pH of the mobile phase is also very important. Knowledge of the pKa of the compounds allows prediction their behaviour and choosing the optimal pH for the mobile phase. Chow *et al*.[26] aimed to examine the *in vivo* disposition of AZT and AZT anabolites in various target tissues in mice and to investigate the effect of chronic retrovirus infection on the tissue disposition of AZT anabolites using HPLC-UV method. Trang *et al*.[27] determined the bioavailability and pharmacokinetics of AZT in mice following single dose oral and intravenous administration of 15, 30, and 60 mg/kg using HPLC-UV method. Because chronic toxicity studies are normally conducted in laboratory animals following oral administration, it is necessary to investigate the bioavailability of AZT which will be used in the animal model.

Carpen *et al*.[28] developed an ion-paired HPLC method to measure concentrations of ddI in human plasma, urine and cerebrospinal fluid and presented results of a pharmacokinetic study in a child treated both with intravenous and oral ddI. The stability of ddI under a condition similar to the acid encountered in the stomach was also examined. Knupp *et al*.[29] described the validation of HPLC methods for the quantitaion of ddI in plasma and urine samples obtained from AIDS patients. Clark *et al*.[30] suggested HPLC method for the determination of 3'-azido-2',3'-dideoxyuridine (AZDU, nucleoside analog of AZT) in rat maternal plasma, amniotic fluid, fetal and placental tissues. Prior to analysis, tissue samples were homogenized in distilled water, protein was precipitated and purified using SPE and protein in plasma and amniotic fluid samples were precipitated. This method allowed for a pharmacokinetic investigation for the determination of placental transport of AZDU. The HPLC analysis of AZDU and its prodrugs I, II, III and IV in rat plasma for preclinical pharmacokinetic studies were reported by Kong *et al*.[31]. Harker *et al*.[32] developed HPLC method for the determination of 3TC. 3TC was extracted from serum samples using SPE prior to reversed-phase chromatography with UV detection. Morris *et al*.[33] method allowed direct injection of urine (10 μL) using HPLC column switching followed by UV detection for the analysis of 3TC. Alnouti *et al*.[34] introduced an analytical method using HPLC-UV for the quantification of 3TC in plasma, amniotic fluid, placental, and fetal matrices from a pregnant rat. This method utilized liquid-liquid extraction and protein precipitation (PP) for the extraction of 3TC from the four biological matrices. Contreras *et al*.[35] developed HPLC –UV method of d4T in rat plasma incorporating an internal standart, a small sample volume for a short and simple preparation procedure. Sarasa *et al*.[36] described the development and validation of HPLC method for the quantification of d4T in urine and following SPE of 0.2 mL of plasma. Reduced sample volume used was suitable for pharmacokinetic studies in HIV infected pediatric population. Wiesner *et al*.[37] described LC/MS/MS method for the determination of d4T in plasma using SPE extraction with a total turnaround of 4 min between sample injections. Ravitch *et al*.[38] described the development and validation of assay of ABC and its two major metabolites in human urine and cerebrospinal fluid, using HPLC with UV detection.

(III) *Non-Nucleoside Reverse Transcriptase Inhibitors*

Some HPLC methods with UV detection[39-41] or with MS/MS[42] detection have been described for the determination of NVP. The latter is highly selective and required only 50 μL sample, which is advantageous for pediatric samples. HPLC methods have been reported for the determination of DLV in plasma using fluorescence detection[43,44]. Cheng *et al*.[43] used very small volumes of plasma. It was suitable for the study of the pharmacokinetics of DLV in HIV patients, children and small animals. Several HPLC–UV method for quantitation of EFV in plasma have been published[45-48]. These methods used SPE[45], PP[46] or LLE procedure[47,48]. Matthews *et al*.[49] described HPLC method for the determination of EFV in plasma with fluorescence detection following post-column photochemical derivatization. The use of the selective detection technique significantly reduced the possibility of interference from endogenous compounds.

(IV) *Nucleotide Reverse Transcriptase Inhibitors*

Sentenac *et al*.[50] presented HPLC method for TNF in plasma using UV detection. The procedure of Sparidans[51] and Jullien[52] needed derivatisation of TNF by chloroacetaldehyde before separation by HPLC with fluorescence detection. Ding *et al*.[53] reported HPLC-UV method for the measurement of DDC in maternal plasma, amniotic fluid, placental and fetal tissues.

(V) *Fusion (entry) Inhibitors*

Chang *et al*.[54] published a method for measuring a (HIV) cell membrane fusion inhibitor enfuvirtide (T-20) and its metabolite (M-20/Ro 50-6343) in human plasma by gradient HPLC with MS/MS detection.

II. *Simultaneous Analysis of ARVs*

Because of the use of multiple drugs in the same patient, analytical methods are needed for simultaneously determining blood levels for many anti-human immunodeficiency virus drugs.

Poirier *et al*.[55] measured EFV and active metabolite

of NFV, M8 together with six PIs in plasma. Separation was achieved by gradient elution by SPE. Run time might be considered long (60 min). In the method by Donnerer[56], seven drugs, ABC, AZT, EFV, NVP, IDV, LPV and NFV, were analyzed in plasma by HPLC with UV detection. Two different extraction procedures and two different HPLC eluents on a C8 reversed-phase column were used to monitor all seven compounds. Rezk *et al*.[57] reported HPLC-UV analysis for the determination of six PIs and three NNRTIs using SPE extraction procedure. Gradient elution was used. The gradient flow rate was increased with the run time (from 0.9 to 1.1 mL over 30 min) to shorten the analysis time. Keil *et al*.[58] developed a HPLC-UV method for measurement of six PIs, NFV active metabolite of M8 and EFV, depending on careful control of pH of the mobile phase and of the column temperature. The increased flow rate allowed separation of the compounds in a single run. Walson *et al*.[59] published HPLC-UV method for the determination of IDV, SQV, RTV, NFV in children an adults. The procedure of Droste[60] separated six PIs and NVP by HPLC-UV method after liquid –liquid extraction. EFV was not included in this analysis because the recovery of EFV after extraction was only 20% but the active metabolite of NFV (M8) was analyzed simultaneously with PIs. Justesen *et al*.[61] developed HPLC-UV method for the determination of six PIs and active metabolite of NFV (M8) in plasma. Marzolini *et al*.[62] reported HPLC-UV method for the determination of LPV and NVP in biological fluid after SPE adaptated from the methods the analysis of five first marketed PIs and EFV[63]. Albert *et al*.[64] developed two HPLC methods for the identification and quantitation of SQV and RTV in human plasma. The error function of the analytical method was established. Yamada *et al*.[65] presented HPLC method for the simultaneous determination of five HIV protease inhibitors (IDV, APV, RTV, SQV and NFV) in human plasma. Sarasa-Nacenta *et al*.[66] developed HPLC assay for the five protease inhibitors IDV, APV, RTV, SQV and NFV in human plasma. Hsieh *et al*.[67] developed a method for simultaneous determination of plasma concentrations of IDV, RTV and SQV by HPLC. Tribut *et al*.[68] reported HPLC method for the therapeutic drug monitoring of six approved protease inhibitors (APV, IDV, LPV, NFV, RTV, and SQV) and two approved non-nucleoside reverse transcriptase inhibitors (EFV and NVP). Three ultraviolet wavelengths were used for detection with a diode array detector. Usami *et al*.[69] published HPLC method based on UV detection for simultaneous determination of LPV, RTV and EFV to evaluate the efficiency of co-administration of LPV/RTV and EFV in HIV-1 infected patients to prevent treatment failure. Dailly *et al*.[70] reported HPLC-UV assay for TDM of ATV and six PIs (IDV, APV, RTV, SQV, LPV, NFV and the active metabolite of NFV, M8) and two NNRTIs (NVP and EFV). Poirier *et al*.[71] presented simultaneous HPLC determination of ATV with all the other PIs (NFV metabolite M8 included) and the two NNRTIs, EFV and NVP. This HPLC method allowed the analysis of all these drugs at a single ultraviolet wavelength following an one step liquid-liquid extraction procedure. Titier *et al*.[72] developed HPLC method for simultaneous quantitation of PIs (APV, IDV, LPV, NFV, RTV, and SQV) and two NNRTIs (EFV and NVP). It involved a rapid liquid–liquid extraction, the use of a gradient elution on a reversed-phase column, and a sequential ultraviolet detection. Egge-Jacobsen *et al*.[73] separated six PIs and three NNRTIs in 6 min using LC/MS.

Gao *et al*.[74] reported LC-MS method of APV, SQV, IDV and NFV in rat samples. Rentsch *et al*.[75] described HPLC-MS method for determination of six PIs and two NRTIs in plasma. Run time was not as short as that in other HPLC-MS methods. According to the authors, the run time was set at 21 min to decrease the risk of any interference of metabolites or concomitant medications. Alexander *et al*.[76] investigated LPV and RTV pharmacokinetics in a clinical cohort using multiple-drug rescue therapy by HPLC-TMS. A new analysis method using fewer samples for the investigation of the correlation between steady-state plasma-concentrations C, Cmax and AUC was developped in order to obtain these pharmacokinetic parameters easier and make drug monitoring more cost effective and practicle. Chi *et al*.[77] had presented LC/MS/MS method for five PIs in human plasma after protein precipitation with acetonitrile. Run time was 3.5 min. Frerichs *et al*.[78] reported LC/MS/MS method for the determination of six PIs and the active metabolite of NFV (M8) after liquid-liquid extraction. Crommentuyn *et al*.[79] separated six PIs in 5.5 min using LC/MS/MS. The assay was preceded by a simple protein precipitation step and only 100 μL of plasma sample was required. It was suitable for small animals and pediatric studies. Crommentuyn *et al*.[80] developed an assay method for ATV and TPV and the method could be combined with their earlier described LC/MS/MS method for the quantification of the PIs and NFV active metabolite, M8 in human plasma[79].

Williams *et al*.[81] described HPLC/MS method of AZT and 3TC along with several toxicologically significant metabolites in mouse serum and spleen for the study of metabolism, pharmacokinetics, and placental transfer. Rezk *et al*.[82] used optimized SPE extraction procedure for six NRTIs and NVP in human plasma. Verweij-van Wissen *et al*.[83] developed HPLC-UV method for simultaneous determination of NRTIs (3TC, ddI, d4T, AZT and ABC) in plasma. Simon *et al*.[84] presented two HPLC-UV methods which together covered the determination of all three pharmaceutical classes of ARV in serum after SPE procedure. Fan *et al*.[85] developed an isocratic ion-pair HPLC assay with UV detection for the simultaneous determination of AZT, 3TC, and NVP in human plasma. Huang *et al*.[86] developed an LC/MS/MS method for simultaneous determination of ddI and d4T in seven matrices (human plasma, bronchoalveolar lavage fluid, alveolar cells, peripheral blood mononuclear cells, seminal plasma, cerebrospinal fluid, and tonsil tissue). These assays were utilized in a distribution study of ddI and d4T in these compartments in an HIV positive patient receiving

HAART therapy for 6 months. Kenney *et al*.[87] presented HPLC/MS/MS method using an ultrafiltration extraction step for the simultaneous determination of AZT and 3TC in human serum. For the validation process, they used a cross-validation with the established methods such as RIA and HPLC-UV. Pereira *et al*.[88] described HPLC/MS/MS method to measure 3TC and AZT concentrations in human seminal plasma. The procedure required small quantities of seminal plasma (25 μL), no radioactive material, minimal preparation, and short run time. Estrela *et al*.[89] reported SPE-LC/MS/MS method for simultaneous monitoring of concentration profiles of 3TC and AZT in serum. The method was applied to a bioequivalance trial in healthy volunteers and shown to be adequate and reliable. Cremieux *et al*.[90] examined steady-state pharmacokinetics of ABC, 3TC, and AZT using HPLC method. MS/MS detection was used to measure 3TC and AZT in serum. Serum concentration of ABC was measured with UV detection. Moore *et al*.[91] described a validated HPLC/MS/MS method for the simultaneous measurement of intracellular 3TC, d4T, and AZT 5'-triphosphates in PBMCs of HIV patients to investigate the intracellular pharmacokinetics and pharmacodynamics. Volosov *et al*.[92] developed a HPLC-TMS method for the simultaneous measurement of any combination of 15 AIDS drugs in less than 5 min. Marchei *et al*.[93] published a HPLC-UV method for the separation and simultaneous quantitation of AZT and NVP in plasma after SPE. Rezk *et al*.[94] suggested HPLC-UV method for the quantitative determination of NNRTIs in human plasma.

Kappelhoff *et al*.[95] used 100 μL of plasma sample for the measuring of EFV and NVP by HPLC-UV method. Boffito *et al*.[96] developed HPLC assay for the simultaneous determination of a wide range of concentrations of rifampicin and EFV in human plasma to monitor drug levels in HIV patients receiving EFV-containing regimens and rifampicin.

CONCLUSIONS

Therapeutical drug monitoring is a very useful approach in optimising antiretroviral treatment to avoid early virological failure and to preserve other therapeutic options for the future. HPLC methods have been largely applied in pharmacokinetic studies of the ARV drugs for TDM purposes not only in research laboratories but also in hospital laboratories.

Numerous HPLC methods have been developed for the analysis of ARV drugs in biological matrices. C18 columns were most often used to separate ARVs. The majority of the mobile phase consisted of a mixture of acetonitrile and buffer. Isocratic or gradient elution were used depending on the number of ARV drugs to be analyzed. Before injection of samples to the chromatographic system, some purification methods have been used: liquid-liquid extraction, solid-phase extraction and protein precipitation, etc. Generally, SPE is an expensive and time-consuming process in comparison with LLE or PP techniques. Some methods include automation of the extraction procedure which permits the analysis of hundreds of samples in a day without any loss of accuracy or precision. Internal standards leading to more accurate results have not been used in some of the assays. UV detection was mostly preferred for the analysis. However, due to potential for interference and reduced specificity as well as low sensitivity, UV detection is not the most suitable detection method. For the analysis of DLV and TNF, fluorimetric detection was used because it has better sensitivity and selectivity than UV detection. In some methods, a single MS detection has been used. In terms of specifity and selectivity single MS detection is superior to HPLC methods and the required time for analysis is relatively shorter. However, it is not the most suitable detection method because it has a degree of specificity and sensitivity compared to MS/MS. Apparently, MS/MS is the most suitable detection method for determining ARV concentrations in biological matrices.

Several studies suggest that therapeutic drug monitoring of ARV drugs may contribute to the management of treatment toxicities or virologic response in HIV infected patients. Because of the growing number of antiretroviral drugs and drug combinations which can be administered in AIDS patients, developing new HPLC methods allowing the analysis of these drugs in biological matrices may be useful. The developed methods should be convenient for clinical laboratories responsible for the in therapeutic drug monitoring for ARV drugs.

REFERENCES

1. Joint United Nations Programme on HIV/AIDS (UNAIDS). Global Summary of the HIV/AIDS Epidemic, December 2003. Available at: hattp://www.unaids.org/en/ in+focus/topic+areas/estimates+and+projections+-+epidemiology.asp. Accessed January 29, 2004.
2. Palella, F. J. J., Delane, K. M., Moorman, A. C., Aschman, D. J. and Holmberg, S. D. 1998. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. N. Engl. J. Med. 338: 853-860.
3. Burger, D. M., Hoetelmans, R. M. W. and Mulder, J. W. 1998. 12th World AIDS Conference, Geneva, Switzerland, July 42275.
4. Vanhove, G. F., Gries, J. M., Verotta, D., Sheiner, L. B., Coombs, R., Collier, A. C. and Blaschke, T. F. 1997. Exposure-response relationships for saquinavir, zidovudine, and zalcitabine in combination therapy. Antimicrob Agents Chemother. 41: 2433-2438.
5. Kastrissios, H., Suarez, J. R., Hammer, S., Katzenstein, D. and Blaschke, T. F. 1998. The extent of non-adherence in a large AIDS clinical trial using plasma dideoxynucleoside concentrations as a marker. AIDS 12: 2305-2311.

6. Aamnoutse, R. E., Verweij-van Wissen, C. P., Underberg, W. J., Kleinnijenhuis, J., Hekster, Y. A. and Burger, D. M. 2001. High-performance liquid chromatography of HIV protease inhibitors in human biological matrices. J. Chromatogr. B Biomed. Sci. Appl. 764: 363-384.
7. Crommentuyn, K. M. L., Huitema, A. D. R. and Beijnen, J. H. 2005. Bioanalysis of HIV protease inhibitors in samples from sanctuary sites. J. Pharm. Biomed. Anal. (In Press)
8. Pereira, A. S. and Tidwell, R. R. 2001. Separation methods for nucleoside analogues used for treatment of HIV-1 infection. J. Chromatogr. B Biomed. Sci. Appl. 764: 327-347.
9. Armstrong, D. and Cohen, J. 1999. Infectious Diseases. Vol. II. MOSBY An imprint of Harcourt Publishers Ltd. London, U. K.
10. HIV treatment series III. Part four of five sponsored in part by an unrestricted grant from Abbot virology from research to reality: How HIV DrugsWork. Available at http://www. tpan.com/publications/positively_aware/nov_dec_04/ts_how_drugs_work.html. Accessed April 10, 2005
11. Pearl, L. H. and Taylor, W. R. 1987. A structural model for the retroviral proteases. Nature 329: 351-354.
12. Goodman, S. and Gilman, A. 2001. The Pharmacological Basis of Therapeutics. 10th ed. p. 1364. The McGraw Hill Co. Inc. U. S. A.
13. Campanero, M. A., Escolar, M., Arangoa, M. A., Sadaba, B. and Azanza, J. R. 2002. Development of a chromatographic method for the determination of saquinavir in plasma samples of HIV patients. Biomed. Chromatogr. 16: 7-12.
14. Burhenne, J., Riedel, K. D., Martin-Facklam, M., Mikus, G. and Haefeli, W. E. 2003. Highly sensitive determination of saquinavir in biological samples using liquid chromatography–tandem mass spectrometry. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 784: 233-242.
15. Pereira, A. S., Kenney, K. B., Cohen, M. S., Eron, J. J., Tidwell, R. R. and Dunn, J. A. 2002. Determination of amprenavir, a HIV-1 protease inhibitor, in human seminal plasma using high-performance liquid chromatography–tandem mass spectrometry. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 766: 307-317.
16. Gunawan, S., Griswold, M. P. and Kahn, D. G. 2001. Liquid chromatographic–tandem mass spectrometric determination of amprenavir (agenerase) in serum/plasma of human immunodeficiency virus type-1 infected patients receiving combination antiretroviral therapy. J. Chromatogr A. 914: 1-4.
17. Schuster, A., Burzawa, S., Jemal, M., Loizillon, E., Couerbe, P. and Whigan, D. 2003. Quantitative determination of the HIV protease inhibitor atazanavir (BMS-232632) in human plasma by liquid chromatography–tandem mass spectrometry following automated solid-phase extraction. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 788: 377-386.
18. Jemal, M., Rao, S., Gatz, M. and Whigan, D. 2003. Liquid chromatography-tandem mass spectrometric quantitative determination of the HIV protease inhibitor atazanavir (BMS-232632) in human peripheral blood mononuclear cells (PBMC): practical approaches to PBMC preparation and PBMC assay design for high-throughput analysis. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 795: 273-289.
19. Colombo, S., Guignard, N., Marzolini, C., Telenti, A., Biollaz, J. and Decosterd, L. A. 2004. Determination of the new HIV-protease inhibitor atazanavir by liquid chromatography after solid-phase extraction. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 810: 25-34.
20. Hua, Y., Fan-Havard, P. and Chan, K. K. 2004. Subnanogram on-line column-switching liquid chromatographic-tandem mass spectrometric quantification method for nelfinavir and methoxyphenol metabolite M1 in rat plasma. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 809: 1-7.
21. Herforth, C., Stone, J. A., Jayewardene, A. L., Blaschke, T. F., Fang, F., Motoya, T. and Aweeka, F. T. 2002. Determination of nelfinavir free drug concentrations in plasma by equilibrium dialysis and liquid chromatography/tandem mass spectrometry: important factors for method optimization. Eur. J. Pharm. Sci. 15: 185-195.
22. Panchagnula, R., Bansal, T., Varma, M. V. and Kaul, C. L. 2004. Reversed-phase liquid chromatography with ultraviolet detection for simultaneous quantitation of indinavir and propranolol from ex-vivo rat intestinal permeability studies. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 806: 277-282.
23. Jayewardene, A. L, Kearney, B., Stone, J. A., Gambertoglio, J. G. and Aweeka, F. T. 2001. An LC-MS-MS method for the determination of indinavir, an HIV-1 protease inhibitor, in human plasma. J. Pharm. Biomed. Anal. 25: 309-317.
24. Darque, A., Valette, G., Rousseau, F., Wang, L. H., Sommadossi, J. P. and Zhou, X. J. 1999. Quantitation of intracellular triphosphate of emtricitabine in peripheral blood mononuclear cells from human immunodeficiency virus-infected patients. Antimicrob. Agents Chemother. 43: 2245-2250.
25. Schrive, I. and Plasse, J. C. 1994. Quantification of zidovudine and one of its metabolites in plasma and urine by solid-phase extraction and high-performance liquid chromatography. J. Chromatogr. B Biomed. Appl. 657: 233-237.
26. Chow, H. H., Li, P., Brookshier, G. and Tang, Y. 1997. *In vivo* tissue disposition of 3'-azido-3'-deoxythymidine and its anabolites in control and retrovirus-infected mice. Drug Metab. Dispos. 25: 412-422.
27. Trang, J. M., Prejean, J. D., James, R. H., Irwin, R. D., Goehl, T. J. and Page, J. G. 1993. Zidovudine bioavailability and linear pharmacokinetics in female B6C3F1 mice. Drug Metab. Dispos. 21: 189-193.

28. Carpen, M. E., Poplack, D. G., Pizzo, P. A. and Balis, F. M. 1990. High-performance liquid chromatographic method for analysis of 2',3'-dideoxyinosine in human body fluids. J. Chromatogr. 526: 69-75.
29. Knupp, C. A, Stancato, F. A, Papp, E. A. and Barbhaiya, R. H. 1990. Quantitation of didanosine in human plasma and urine by high-performance liquid chromatography. J. Chromatogr. 533: 282-290.
30. Clark, T. N., White C.A., Chu C. K. and Bartlett M. G. 2001. Determination of 3'-azido-2',3'-dideoxyuridine in maternal plasma, amniotic fluid, fetal and placental tissues by high-performance liquid chromatography. J. Chromatogr. B Biomed. Sci. Appl. 755: 165-172.
31. Kong, L., Cooperwood, J. S., Oh, C. H., Tam, B. S., Liu, D. T. and Chan, C. K. 2003. Simultaneous determination of 3'-azido-2',3'-dideoxyuridine and novel prodrugs in rat plasma by liquid chromatography. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 795: 371-376
32. Harker, A. J., Evans, G. L., Hawley, A. E. and Morris, D. M. 1994. High-performance liquid chromatographic assay for 2'-deoxy-3'-thiacytidine in human serum. J. Chromatogr. B Biomed. Appl. 657: 227-232.
33. Morris, D. M. and Selinger, K. 1994. Determination of 2'-deoxy-3'-thiacytidine (3TC) in human urine by liquid chromatography: direct injection with column switching. J. Pharm. Biomed. Anal. 12: 255-264.
34. Alnouti, Y., White, C. A. and Bartlett, M. G. 2004. Determination of lamivudine in plasma, amniotic fluid, and rat tissues by liquid chromatography. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 803: 279-284.
35. Contreras, J., Gonzalez, H. M., Menendez, R. and Lopez, M. 2004. Development and validation of a reversed-phase liquid chromatographic method for analysis of D4T (Stavudine) in rat plasma. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 801: 199-203.
36. Sarasa, M., Riba, N., Zamora, L. and Carne, X. 2000. Determination of stavudine in human plasma and urine by high-performance liquid chromatography using a reduced sample volume. J. Chromatogr. B Biomed. Sci. Appl. 746: 183-189.
37. Wiesner, J. L., Sutherland, F. C., Smit, M. J., van Essen, G. H., Hundt, H. K., Swart, K. J. and Hundt, A. F. 2002. Sensitive and rapid liquid chromatography-tandem mass spectrometry method for the determination of stavudine in human plasma. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 773: 129-134.
38. Ravitch, J. R. and Moseley, C. G. 2001. High-performance liquid chromatographic assay for abacavir and its two major metabolites in human urine and cerebrospinal fluid. J. Chromatogr. B Biomed. Sci. Appl. 762: 165-173.
39. van Heeswijk, R. P., Hoetelmans, R. M., Meenhorst, P. L., Mulder, J. W. and Beijnen, J. H. 1998. Rapid determination of nevirapine in human plasma by ion-pair reversed-phase high-performance liquid chromatography with ultraviolet detection. J. Chromatogr. B Biomed. Sci. Appl. 713: 395-399.
40. Hollanders, R. M., van Ewijk-Beneken Kolmer, E. W., Burger, D. M., Wuis, E. W., Koopmans, P. P. and Hekster, Y. A. 2000. Determination of nevirapine, an HIV-1 non-nucleoside reverse transcriptase inhibitor, in human plasma by reversed-phase high-performance liquid chromatography. J. Chromatogr. B Biomed. Sci. Appl. 744: 65-71.
41. Lopez, R. M., Pou, L., Gomez, M. R., Ruiz, I. and Monterde, J. 2001. Simple and rapid determination of nevirapine in human serum by reversed-phase high-performance liquid chromatography. J. Chromatogr. B Biomed. Sci. Appl. 751: 371-376.
42. Chi, J., Jayewardene, A. L., Stone, J. A. and Aweeka, F. T. 2003. An LC-MS-MS method for the determination of nevirapine, a non-nucleoside reverse transcriptase inhibitor, in human plasma. J. Pharm. Biomed. Anal. 31: 953-959.
43. Cheng, C. L., Chou, C. H. and Hu, O. Y. 2002. Determination of delavirdine in very small volumes of plasma by high-performance liquid chromatography with fluorescence detection. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 769: 297-303.
44. Veldkamp, A. I., van Heeswijk, R. P., Hoetelmans, R. M., Meenhorst, P. L., Mulder, J. W., Lange, J. M. and Beijnen, J. H. 1999. Rapid quantification of delavirdine, a novel non-nucleoside reverse transcriptase inhibitor, in human plasma using isocratic reversed-phase high-performance liquid chromatography with fluorescence detection. J. Chromatogr. B Biomed. Sci. Appl. 727: 151-157.
45. Sarasa-Nacenta, M., Lopez-Pua, Y., Lipez-Cortes, L. F., Mallolas, J., Gatell, J. M. and Carne, X. 2001. Determination of efavirenz in human plasma by high-performance liquid chromatography with ultraviolet detection. J. Chromatogr. B Biomed. Sci. Appl. 763: 53-59.
46. Veldkamp, A. I., van Heeswijk, R. P., Meenhorst, P. L., Mulder, J. W., Lange, J. M., Beijnen, J. H. and Hoetelmans, R. M. 1999. Quantitative determination of efavirenz (DMP 266), a novel non-nucleoside reverse transcriptase inhibitor, in human plasma using isocratic reversed-phase high-performance liquid chromatography with ultraviolet detection. J. Chromatogr. B Biomed. Sci. Appl. 734: 55-61.
47. Langmann, P., Schirmer, D., Vath, T., Zilly, M. and Klinker, H. 2001. High-performance liquid chromatographic method for the determination of HIV-1 non-nucleoside reverse transcriptase inhibitor efavirenz in plasma of patients during highly active antiretroviral therapy. J. Chromatogr. B Biomed. Sci. Appl. 755: 151-156.
48. Villani, P., Pregnolato, M., Banfo, S., Rettani, M., Burroni, D., Seminari, E., Maserati, R. and Regazzi M. B. 1999. High-performance liquid chromatography method for analyzing the antiretroviral agent efavirenz

in human plasma.Ther. Drug Monit. 21: 346-350.

49. Matthews, C. Z., Woolf, E. J., Mazenko, R. S., Haddix-Wiener, H., Chavez-Eng, C. M., Constanzer, M. L., Doss, G. A. and Matuszewski, B. K. 2002. Determination of efavirenz, a selective non-nucleoside reverse transcriptase inhibitor, in human plasma using HPLC with post-column photochemical derivatization and fluorescence detection. J. Pharm. Biomed. Anal. 28: 925-934.
50. Sentenac, S., Fernandez, C., Thuillier Lechat, P. and Aymard, G. 2003. Sensitive determination of tenofovir in human plasma samples using reversed-phase liquid chromatography. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 793: 317-324.
51. Sparidans, R. W., Crommentuyn, K. M., Schellens, J. H. and Beijnen, J. H. 2003. Liquid chromatographic assay for the antiviral nucleotide analogue tenofovir in plasma using derivatization with chloroacetaldehyde. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 791: 227-233.
52. Jullien, V., Treluyer, J. M., Pons, G. and Rey, E. 2003. Determination of tenofovir in human plasma by high-performance liquid chromatography with spectrofluorimetric detection. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 785: 377-381.
53. Ding, Y., Williamson, L. N., White, C. A. and Bartlett, M. G. 2004. Determination of 2',3'-dideoxycytidine in maternal plasma, amniotic fluid, placental and fetal tissues by high-performance liquid chromatography. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 811: 183-189.
54. Chang, D, Kolis, S. J., Linderholm, K. H., Julian, T. F., Nachi, R., Dzerk, A. M., Lin, P. P., Lee, J. W. and Bansal, S. K. 2005. Bioanalytical method development and validation for a large peptide HIV fusion inhibitor (Enfuvirtide, T-20) and its metabolite in human plasma using LC–MS/MS. J. Pharm. Biomed. Anal.. 38: 487-496.
55. Poirier, J. M., Robidou, P. and Jaillon, P. 2002. Simultaneous determination of the six HIV protease inhibitors (amprenavir, indinavir, lopinavir, nelfinavir, ritonavir, and saquinavir) plus M8 nelfinavir metabolite and the nonnucleoside reverse transcription inhibitor efavirenz in human plasma by solid-phase extraction and column liquid chromatography. Ther. Drug Monit. 24: 302-309.
56. Donnerer, J., Kronawetter, M., Kapper, A., Haas, I. and Kessler, H. H. 2003. Therapeutic drug monitoring of the HIV/AIDS drugs abacavir, zidovudine, efavirenz, nevirapine, indinavir, lopinavir, and nelfinavir. Pharmacology 69: 197-204.
57. Rezk, N. L., Tidwell, R. R. and Kashuba, A. D. 2004. High-performance liquid chromatography assay for the quantification of HIV protease inhibitors and non-nucleoside reverse transcriptase inhibitors in human plasma. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 805: 241-247.
58. Keil, K., Frerichs, V. A., DiFrancesco, R. and Morse, G. 2003. Reverse phase high-performance liquid chromatography method for the analysis of amprenavir, efavirenz, indinavir, lopinavir, nelfinavir and its active metabolite (M8), ritonavir, and saquinavir in heparinized human plasma. Ther. Drug Monit. 25: 340-346.
59. Walson, P. D., Cox, S., Utkin, I., Gerber, N., Crim, L., Brady, M. and Koranyi, K. 2003. Clinical use of a simultaneous HPLC assay for indinavir, saquinavir, ritonavir and nelfinavir in children and adults. Ther. Drug Monit. 25: 650-656.
60. Droste, J. A., Verweij-Van Wissen, C. P. and Burger, D. M. 2003. Simultaneous determination of the HIV drugs indinavir, amprenavir, saquinavir, ritonavir, lopinavir, nelfinavir, the nelfinavir hydroxymetabolite M8, and nevirapine in human plasma by reversed-phase high-performance liquid chromatography. Ther. Drug Monit. 25: 393-399.
61. Justesen, U. S., Pedersen, C. and Klitgaard, N. A. 2003. Simultaneous quantitative determination of the HIV protease inhibitors indinavir, amprenavir, ritonavir, lopinavir, saquinavir, nelfinavir and the nelfinavir active metabolite M8 in plasma by liquid chromatography. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 783: 491-500.
62. Marzolini, C., Beguin, A., Telenti, A., Schreyer, A., Buclin, T., Biollaz, J. and Decosterd, L. A. 2002. Determination of lopinavir and nevirapine by high-performance liquid chromatography after solid-phase extraction: application for the assessment of their transplacental passage at delivery. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 774: 127-140.
63. Marzolini, C., Telenti, A., Buclin, T., Biollaz, J. and Decosterd, L. A. 2000. Simultaneous determination of the HIV protease inhibitors indinavir, amprenavir, saquinavir, ritonavir, nelfinavir and the non-nucleoside reverse transcriptase inhibitor efavirenz by high-performance liquid chromatography after solid-phase extraction. J. Chromatogr. B Biomed. Sci. Appl. 740: 43-58.
64. Albert, V., Modamio, P., Lastra, C. F. and Marino, E. L. 2004. Determination of saquinavir and ritonavir in human plasma by reversed-phase high-performance liquid chromatography and the analytical error function. J. Pharm. Biomed. Anal. 36: 835-840.
65. Yamada, H., Kotaki, H., Nakamura, T. and 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: 85-89.
66. Sarasa-Nacenta, M., Lopez-Pua, Y. and Mallolas, J. 2001. Simultaneous determination of the HIV-protease inhibitors indinavir, amprenavir, ritonavir, saquinavir and nelfinavir in human plasma by reversed-phase high-performance liquid chromatography. J. Chromatogr. B Biomed. Sci. Appl. 757: 325-332.
67. Hsieh, S. M., Yu, H. Y. and Chang, S. C. 2004.

Simultaneous determination of indinavir, ritonavir and saquinavir in plasma by high-performance liquid chromatography. J. Formos Med. Assoc. 103: 191-195.

68. Tribut, O., Arvieux, C., Michelet, C., Chapplain, J. M., Allain, H. and Bentue-Ferrer, D. 2002. Simultaneous quantitative assay of six HIV protease inhibitors, one metabolite, and two non-nucleoside reverse transcriptase inhibitors in human plasma by isocratic reversed-phase liquid chromatography. Ther. Drug Monit. 24: 554-562.
69. Usami, Y., Oki, T., Nakai, M., Sagisaka, M. and Kaneda, T. 2003. A simple HPLC method for simultaneous determination of lopinavir, ritonavir and efavirenz. Chem. Pharm. Bull. (Tokyo). 51: 715-718.
70. Dailly, E., Raffi, F. and Jolliet, P. 2004. Determination of atazanavir and other antiretroviral drugs (indinavir, amprenavir, nelfinavir and its active metabolite M8, saquinavir, ritonavir, lopinavir, nevirapine and efavirenz) plasma levels by high performance liquid chromatography with UV detection. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 813: 353-358.
71. Poirier, J. M., Robidou, P. and Jaillon, P. 2005. Simple and simultaneous determination of the HIV-protease inhibitors Amprenavir, Atazanavir, Indinavir, Lopinavir, Nelfinavir, Ritonavir and Saquinavir plus M8 Nelfinavir metabolite and the nonnucleoside reverse transcriptase inhibitors Efavirenz and Nevirapine in human plasma by reversed-phase liquid chromatography. Ther. Drug Monit. 27: 186-192.
72. Titier, K., Lagrange, F., Pehourcq, F., Edno-Mcheik, L., Moore, N. and Molimard, M. 2002. High-performance liquid chromatographic method for the simultaneous determination of the six HIV-protease inhibitors and two non-nucleoside reverse transcriptase inhibitors in human plasma. Ther. Drug Monit. 24: 417-424.
73. Egge-Jacobsen, W., Unger, M., Niemann, C. U., Baluom, M., Hirai, S., Benet, L. Z. and Christians, U. 2004. Automated, fast, and sensitive quantification of drugs in human plasma by LC/LC-MS: quantification of 6 protease inhibitors and 3 nonnucleoside transcriptase inhibitors. Ther. Drug Monit. 26: 546-562.
74. Gao, W., Kishida, T., Kimura, K., Kageyama, M., Sumi, M., Yoshikawa, Y., Shibata, N. and Takada, K. 2002. Sensitive and simultaneous determination of HIV protease inhibitors in rat biological samples by liquid chromatography-mass spectrometry. Biomed. Chromatogr. 16: 267-273.
75. Rentsch, K. M. 2003. Sensitive and specific determination of eight antiretroviral agents in plasma by high-performance liquid chromatography-mass spectrometry. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 788: 339-350.
76. Alexander, C. S., Montaner, J. S., Asselin, J. J., Ting, L., McNabb, K., Harris, M., Guillemi, S. and Harrigan, P. R. 2004. Simplification of therapeutic drug monitoring for twice-daily regimens of lopinavir/ritonavir for HIV infection. Ther. Drug Monit. 26: 516-523.
77. Chi, J., Jayewardene, A. L., Stone, J. A., Motoya, T. and Aweeka, F. T. 2002. Simultaneous determination of five HIV protease inhibitors nelfinavir, indinavir, ritonavir, saquinavir and amprenavir in human plasma by LC/MS/MS. J. Pharm. Biomed. Anal. 30: 675-684.
78. Frerichs, V. A., DiFrancesco, R. and Morse, G. D. 2003. Determination of protease inhibitors using liquid chromatography-tandem mass spectrometry. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 787: 393-403.
79. Crommentuyn, K. M., Rosing, H., Nan-Offeringa, L. G., Hillebrand, M. J., Huitema, A. D. and Beijnen, J. H. 2003. Rapid quantification of HIV protease inhibitors in human plasma by high-performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry. J. Mass Spectrom. 38: 157-166.
80. Crommentuyn, K. M., Rosing, H., Hillebrand, M. J., Huitema, A. D. and Beijnen, J. H. 2004. Simultaneous quantification of the new HIV protease inhibitors atazanavir and tipranavir in human plasma by high-performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 804: 359-367.
81. Williams, L. D., Von Tungeln, L. S., Beland, F. A. and Doerge, D. R. 2003. Liquid chromatographic-mass spectrometric determination of the metabolism and disposition of the anti-retroviral nucleoside analogs zidovudine and lamivudine in C57BL/6N and B6C3F1 mice. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 798: 55-62.
82. Rezk, N. L, Tidwell, R. R. and Kashuba, A. D. 2003. Simultaneous determination of six HIV nucleoside analogue reverse transcriptase inhibitors and nevirapine by liquid chromatography with ultraviolet absorbance detection. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 791: 137-147.
83. Verweij-van Wissen, C. P., Aarnoutse, R. E. and Burger, D. M. 2005. Simultaneous determination of the HIV nucleoside analogue reverse transcriptase inhibitors lamivudine, didanosine, stavudine, zidovudine and abacavir in human plasma by reversed phase high performance liquid chromatography. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 816: 121-129.
84. Simon, V. A., Thiam, M. D. and Lipford, L. C. 2001. Determination of serum levels of thirteen human immunodeficiency virus-suppressing drugs by high-performance liquid chromatography. J. Chromatogr. A 913: 447-453.
85. Fan, B. and Stewart, J. T. 2002. Determination of zidovudine/lamivudine/nevirapine in human plasma using ion-pair HPLC. J. Pharm. Biomed. Anal. 28: 903-908.
86. Huang, Y., Zurlinden, E., Lin, E., Li, X., Tokumoto, J., Golden, J., Murr, A., Engstrom, J. and Conte, J. J. 2004. Liquid chromatographic-tandem mass spectrometric assay for the simultaneous determination of didanosine and stavudine in human plasma, bronchoalveolar lavage fluid, alveolar cells, peripheral blood mononuclear cells, seminal plasma, cerebrospinal fluid and tonsil tissue. J.

Chromatogr. B Analyt. Technol. Biomed. Life Sci. 799: 51-61.

87. Kenney, K. B., Wring, S. A., Carr, R. M., Wells, G. N. and Dunn, J. A. 2000. Simultaneous determination of zidovudine and lamivudine in human serum using HPLC with tandem mass spectrometry. J. Pharm. Biomed. Anal. 22: 967-983.
88. Pereira, A. S., Kenney, K. B., Cohen, M. S., Hall, J. E., Eron, J. J., Tidwell, R. R. and Dunn, J. A. 2000. Simultaneous determination of lamivudine and zidovudine concentrations in human seminal plasma using high-performance liquid chromatography and tandem mass spectrometry. J. Chromatogr. B Biomed. Sci. Appl. 742: 173-183.
89. Estrela Rde, C., Salvadori, M. C. and Suarez-Kurtz, G. 2004. A rapid and sensitive method for simultaneous determination of lamivudine and zidovudine in human serum by on-line solid-phase extraction coupled to liquid chromatography/tandem mass spectrometry detection. Rapid Commun. Mass Spectrom. 18: 1147-1155.
90. Cremieux, A. C., Katlama, C., Gillotin, C., Demarles, D., Yuen, G. J. and Raffi, F. 2001. A comparison of the steady-state pharmacokinetics and safety of abacavir, lamivudine, and zidovudine taken as a triple combination tablet and as abacavir plus a lamivudine-zidovudine double combination tablet by HIV-1-infected adults. Pharmacotherapy 21: 424-430.
91. Moore, J. D., Valette, G., Darque, A., Zhou, X. J. and Sommadossi, J. P. 2000. Simultaneous quantitation of the 5'-triphosphate metabolites of zidovudine, lamivudine, and stavudine in peripheral mononuclear blood cells of HIV infected patients by high-performance liquid chromatography tandem mass spectrometry. J. Am. Soc. Mass Spectrom. 11: 1134-1143.
92. Volosov, A., Alexander, C., Ting, L. and Soldin, S. J. 2002. Simple rapid method for quantification of antiretrovirals by liquid chromatography-tandem mass-spectrometry. Clin. Biochem. 35: 99-103.
93. Marchei, E., Valvo, L., Pacifici, R., Pellegrini, M., Tossini, G. and Zuccaro, P. 2002. Simultaneous determination of zidovudine and nevirapine in human plasma by RP-LC. J. Pharm. Biomed. Anal. 29: 1081-1088.
94. Rezk, N. L., Tidwell, R. R. and Kashuba, A. D. 2002. Simple and rapid quantification of the non-nucleoside reverse transcriptase inhibitors nevirapine, delavirdine, and efavirenz in human blood plasma using high-performance liquid chromatography with ultraviolet absorbance detection. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 774: 79-88.
95. Kappelhoff, B. S., Rosing, H., Huitema, A. D. and Beijnen, J. H. 2003. Simple and rapid method for the simultaneous determination of the non-nucleoside reverse transcriptase inhibitors efavirenz and nevirapine in human plasma using liquid chromatography. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 792: 353-362.
96. Boffito, M., Tija, J., Reynolds, H. E., Hoggard, P. G., Bonora, S., Di Perri, G. and Back, D. J. 2002. Simultaneous determination of rifampicin and efavirenz in plasma. Ther. Drug Monit. 24: 670-674.