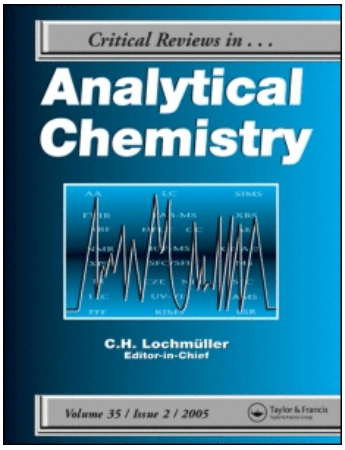

Critical Reviews in Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713400837

High-Performance Liquid Chromatography Methods to Simultaneous Determination of Anti-Retroviral Drugs in Biological Matrices

Cafer Saka[a]
[a] School of Health, Sırt University, Sırt, Turkey

To cite this Article Saka, Cafer(2009) 'High-Performance Liquid Chromatography Methods to Simultaneous Determination of Anti-Retroviral Drugs in Biological Matrices', Critical Reviews in Analytical Chemistry, 39: 2, 108 — 125
To link to this Article: DOI: 10.1080/10408340902820759
URL: http://dx.doi.org/10.1080/10408340902820759

Critical Reviews in Analytical Chemistry, 39:108–125, 2009

ISSN: 1040-8347 print / 1547-6510 online
DOI: 10.1080/10408340902820759

High-Performance Liquid Chromatography Methods to Simultaneous Determination of Anti-Retroviral Drugs in Biological Matrices

Cafer Saka
School of Health, Sıırt University, Sıırt, Turkey

Acquired immune deficiency syndrome or acquired immunodeficiency syndrome (AIDS or Aids) is symptoms and infections resulting from the damage to the human immune system. The purpose of this paper is to review the literature for high performance liquid chromatography methods to simultaneous determination of anti-retroviral drugs in biological matrices covering the articles mainly from 2001 to 2009. The biological matrix samples include various biological matrices such as human plasma, serum, breast milk, saliva, cerebrospinal fluid and seminal fluid. The review covers high-performance liquid chromatography coupled with ultraviolet detection and reversed-phase high-performance liquid chromatography coupled with tandem mass spectrometry.

Keywords Review, AIDS, anti-retroviral drugs, high-performance liquid chromatography, ultra-violet detection, tandem mass spectrometry

INTRODUCTON

Acquired immune deficiency syndrome or acquired immunodeficiency syndrome (AIDS or Aids) is symptoms and infections resulting from the damage to the human immune system caused by the human immunodeficiency virus (HIV) (1).

Anti-HIV (anti-retroviral) medications are used to control the reproduction of the virus and to slow the progression of HIV-related disease. The current standard of care for treatment recommended for HIV infection is highly active anti-retroviral therapy (HAART). The recommended treatment for HIV is a combination of three or more medications from different classes in a regimen including the use of two nucleoside reverse transcriptase inhibitors (NRTI) with either one or two protease inhibitors (PIs). Five drug classes have been developed: PIs, non-nucleoside reverse transcriptase inhibitors (NNRTIs), NRTIs, fusion inhibitors and integrase inhibitors. Plasma drug concentrations of the HIV PIs correlate with anti-viral efficacy [2–4]. Anti-retroviral drugs are presented in Table 1.

The purpose of this paper is to review the literature for high-performance liquid chromatography (HPLC), methods to simultaneous determination of anti-retroviral drugs in biological matrices covering the articles mainly from 2001 to 2009. The samples include various biological matrices such as human plasma, serum, breast milk, saliva, cerebrospinal fluid and seminal fluid. The review covers HPLC coupled with UV detection (HPLC-UV) and reversed-phase HPLC coupled with MS/MS (RP-HPLC–MS/MS). The separation methods for NRTIs were reviewed in 2001 by Pereira and Tidwell (5). Armagan Onal published a review on HPLC analysis of anti-retroviral drugs including NNRTIs, NRTIs and PIs in biological matrices for therapeutic drug monitoring in 2006 (6). Lai et al. reported a review NRTIs and their phosphorylated metabolites in human immunodeficiency virus-infected human matrices (7).

Chromatography is the general term for various separatory methods, all of which have in common the distribution of a component between a mobile phase (a liquid or gas) and a stationary phase (a solid or liquid). HPLC methods generally provide a good efficiency, precision and accuracy in biochemical analyses. HPLC methods in combination with UV detection and tandem mass spectrometry are the most frequently used technique for measuring plasma concentrations of anti-retroviral drugs.

UV detection is one of the most popular universal detection methods used in anti-retroviral drugs due to its simplicity, ruggedness, ease-of-use and low cost.

More recently, MS has been introduced as a highly sensitive and specific detector for HPLC analyses. Several approches have tried to interface LC to MS, including thermospray particle beam and electrospray (ES) interfaces. Recently, the most important detection used in biopharmaceutical analysis is MS with ESI and APCI techniques (8).

The author dedicates this article to his PhD supervisor, Dr. Zuhre Senturk, on the occasion of 30 years of her scientific and professional career.

Address correspondence to Cafer Saka, School of Health, Siirt University, Hospital Street, 56100 Siirt, Turkey. E-mail: sakaca1976@hotmail.com.

TABLE 1
Anti-retroviral drugs with brand names

Drug name	Brand name
Nucleoside reverse transcriptase inhibitors	
Abacavir	Ziagen
Zidovudine	Retrovir
Lamivudine	Epivir
Didanosine	Videx
Tenofovir	Viread
Stavudine	Zerit
Emtricitabine	Emtriva
Nonnucleoside reverse transcriptase inhibitors	
Delavirdine	Rescriptor
Efavirenz	Sustiva
Zalcitabine	Hivid
Nevirapine	Viramune
Protease inhibitors	
Amprenavir	Agenerase
Nelfinavir	Viracept
Saquinavir	Invirase
Fosamprenavir	Lexiva
Indinavir	Crixivan
Lopinavir	Kaletra
Tipranavir	Aptivus
Atazanavir	Reyataz
Darunavir	Prezista
Ritonavir	Norvir
Fusion inhibitor	
Enfuvirtide	Fuseon
Integrase inhibitor	
Raltegravir	Isentr

A wide variety of RP-HPLC columns are available. Most columns are silica-based. Silica offers good mechanical stability. A typical stationary phase is formed by chemically bonding a long-chain hydrocarbon group to porous silica. Typical ligands are n-octadecyl (C18), n-octayl (C8), n-butyl (C4), diphenyl, and cyano propyl (9).

Sample pre-treatment is a critical step in the analysis of drugs from biological fluids. There are three main methods used for extraction of anti-retroviral drugs. These include LLE, SPE and protein precipitation. Each of these methods has advantages as well as disadvantages.

HPLC-UV Methods

Dogan-Topal et al. (10) presented RP-HPLC with diode array detection procedure for the simultaneous determination of abacavir (ABC), EFV and valganciclovir in spiked human serum in 2007. Separation was performed using a 5-μm Waters Spherisorb column (250 $\times$ 4.6 mm ID) (Avondale, USA) with an isocratic mobile phase consisting of ACN:MeOH:KH_2PO_4 (at pH 5.00) (40:20:40 $v/v/v$) elution at a flow rate of 1.0 mL/min. Calibration curves were constructed in the range of 50–30,000 ng/mL for ABC and EFV, and 10–30,000 ng/mL for valganciclovir in serum samples. The LODs and LOQs were 3.80 and 12.68 ng/mL for ABC, 2.61 and 8.69 ng/mL for EFV and 1.30 and 4.32 ng/mL for valganciclovir.

Kappelhoff et al. (11) presented a RP-HPLC method for the simultaneous determination of EFV and NVP in human plasma with UV detection at 275 nm, using a pre-treatment consisting of protein precipitation with ACN. Carbamazepine was used as an internal standard. The calibration curve was linear over the concentration range of 0.05–15.0 mg/L and 0.25–15.0 mg/L for EFV and NVP, respectively. The chromatographic separation was performed at ambient temperatures on a Zorbax Extend C18 (150 $\times$ 2.1 mm ID, 5 μm) analytical column protected by a ChromGuard HPLC pre-column (10 $\times$ 3.0 mm I.D.) with a mobile phase consisting of 25 mM triethylamine in water–acetonitril (65:35, v/v), pH: 11.7. Flow-rate was 0.2 mL/min. Detection was measured at 275 nm. Retention times of NVP, carbamazepine and EFV were 2.8, 5.0 and 7.8 minutes, respectively. Total run time was 10 minutes.

Bouley et al. (12) developed a LC method for the simultaneous determination of four PIs (IDV, NFV, RTV, and SQV) in human plasma with UV detection (260 nm) using a liquid-liquid extraction with terbutyl methyl ether and a sequential washing of the reconstituted sample with hexane. The separation was performed using a Hypersil-phenyl column (250 $\times$ 4.6 mm, 5 μm, Merck) equipped with a pre-column Merck Lichrospher 100RP-18 (30 $\times$ 4.6 mm, 5 μm) using mobile phase consisting of a mixture of 0.04 mol/L ammonium acetate aqueous solution:ACN (48:52 v/v) adjusted to pH 7.5 with a 5N ammonium hydroxide solution at ambient temperature. The flow rate was 1 mL/min. The standard curves are linear in the range 0.025–1 μg/mL for SQV, 0.1–4 μg/mL for IDV and NFV, and 0.25–10 μg/mL for RTV. The LOQs were 0.025 μg/mL for SQV, 0.25 μg/mL for RTV and 0.1 μg/mL for IDV and NFV, respectively; 4 amino-quinaldine was used as an internal standard.

A LC method for the determination of the six PIs (APV, IDV, LPV, NFV, RTV, and SQV) with the M8 active metabolite of NFV and the non-nucleoside reverse transcription inhibitor EFV in human plasma was reported by Poirier et al. (13) using a solid-phase extraction, using a narrow-bore C18 RP column (Nantes, France). Separations were performed on a Novapak 4-μm C18 column (15 cm $\times$ 2.1 mm ID) (Nantes, France) equipped with a pre-column packed with the same material (waters) with gradient elution at 18°C. Mobile phase (A) was composed of pH 5 buffer (0.5% 5.8 mol orthophosphoric acid/0.02% triethylamine adjusted at pH 5.0 with 10 mol sodium hydroxide) ACN, and MeOH (42.5:28:29.5; v/v/v). Mobile phase (B) was composed of ACN and HPLC-grade water (75: 25; v/v). Double UV detection was monitored at 265 nm for APV and at 210 nm for all other drugs and the internal standard. Calibration curves were linear in the range 25 to 10,000 ng/mL. The LOQ was 25 ng/mL for all drugs.

Boffito et al. (14) reported a HPLC method for the simultaneous determination of rifampicin and EFV in human plasma

with UV detection (254 nm) using an ethyl acetate/n-hexane solution (3 mL; 80:20; v/v) extraction. Stock solutions (1 mg/mL) of rifampicin was used as the internal standard. The chromatographic separation was carried out on a reverse phase Luna 5-μm column (250 $\times$ 4.6 mm) (Macclesfield, UK) protected by a pre-column guard (Si 60, 5 μm, Merck, Germany) using gradient mobile phase consisting of sodium phosphate buffer 0.01 mol/L (pH adjusted to 5.2 with *o*-phosphoric acid), ACN, and MeOH [Buffer A (40:45:15; v/v/v) and Buffer B (20:65:15; v/v/v)] at a flow rate of 1.0 mL/min. Rifampicin, IS, and EFV retention times were 4.30, 5.90, and 13.05 minutes, respectively.

A simultaneous HPLC assay for Indinavir (IDV), SQV, RTV and NFV in human plasma with a diode array detector (210 nm) was reported by Walson et al. (15). The chromatographic separation on a Hypersil C18 (4.6 $\times$ 250 mm column, 5 μm, Phenomenex, Torrance, CA, USA) using gradient mobile phase consisting of ACN and 0.15% triethylamine adjusted to pH 5.0 with phosphoric acid. Flow rate was 1.5 mL/min. Detection range for all of drugs were 0.05–20 μg/mL.

Droste et al. (16) presented a RP-HPLC for the simultaneous quantitative determination of the HIV PIs (APV, IDV, LPV, NFV, RTV, SQV), the active NFV metabolite M8, and the NNRTI (NVP) in human plasma. After a liquid-liquid extraction from plasma, the chromatographic separation was achieved on an OmniSpher 5 C18 column (150 $\times$ 4.6 mm ID; particle size, 5 μm) with mobile phase consisting of ACN and 50 mmol/L potassium phosphate adjusted to pH 5.75 with 50 mmol/L sodium phosphate at a flow rate of 1.5 mL/min at ambient temperature. UV detection was monitored at a wavelength of 215 nm for the PIs and 280 nm for NVP. The runtime was 25 minutes. The LOQs were 0.07 mg/L for LPV and APV and 0.05 mg/L for M8 and NVP. The detection limits were 0.006 mg/L, 0.013 mg/L, 0.014 mg/L, and 0.014 mg/L for NVP, APV, M8, and LPV, respectively. The calibration curves were linear over the concentration range of 0.05 to 15 mg/Lfor M8 and NVP and 0.07 to 30 mg/L for APV and LPV.

Titier et al. (17) developed a HPLC method for the determination of the six PIs (APV, IDV, LPV, NFV, RTV, and SQV) and the NNRTIs (EFV and NVP) in human plasma with UV detection. After a liquid-liquid extraction with diethyl ether, the drugs are performed on a stability RP18 column (250 $\times$ 4.6 mm) using a mixture of ACN and phosphate buffer (50 mmol/L, pH 5.65) as a gradient mobile phase at a flow rate of 1.5 mL/min at 25°C. UV detection was monitored at 240 nm for NVP and 215 nm. UV detection for other anti-retroviral drugs was 260 nm for internal standard. Calibration curves were linear in the range 100 to 10,000 ng/mL. The limit of quantitation was 50 ng/mL for all drugs except NVP (100 ng/mL). The total run time of the analysis was 45 minutes.

Tribut et al. (18) performed a LC method for simultaneous assay of nine anti-retroviral drugs; six approved PIs (APV, IDV, LPV, NFV, RTV, and SQV) and two approved NNRTIs (EFV and NVP) in human plasma. UV detection was monitored at 222 nm for LPV, 240 nm for RTV and SQV and 260 nm for the others. After a liquid-liquid extraction with diethyl ether from 250-microL plasma samples, the separations were carried out on an X-TERRA column (150 $\times$ 4.6 mm, particle size 5 μm) (Saint Quentin, France) with the mobile phase water (with 3 mmol/L pyrrolidine) and ACN (58:42, v/v) at a flow rate of 1.0 mL/min at 22°C. The method was linear within a concentration range of 25–9000 ng/mL. The lower LOD for the different compounds was 20 ng/mL. The smallest quantification was 25 ng/mL except for LPV (50 ng/mL).

An isocratic RP-LC method for simultaneous quantitative assay of ATV and six other HIV protease inhibitors (APV, IDV, LPV, NFV, RTV, and SQV) in human plasma with UV detection was developed by Tribut et al. (19). After a liquid-liquid extraction with diethyl, chromatographic separation was achieved on an X-TERRA column (150 $\times$ 4.6 mm, particle size 5 μm, Waters, Saint Quentin, France) using a mixture of water (with 3 mM pyrrolidine) and ACN (58:42, v/v) as at a flow rate of 1.0 mL/min at 22°C. Absorbance was monitored at 240 nm for Atazanavir (ATV), RTV, and SQV; at 260 nm for NVP, EFV, IDV, APV, and NFV; and at 222 nm for LPV. The retention time was 15.43 minutes, and the quantification threshold was 100 ng/mL.

Takahashi et al. (20) developed a simple HPLC method for the simultaneous quantitative determination of seven HIV PIs (APV, ATV, IDV, LPV, NFV, RTV, SQV) and a non-nucleoside reverse transcription inhibitor (EFV) in human plasma with UV detection (205 nm) using a liquid–liquid drug extraction from plasma. The separation was achieved on a Radial-Pak Nova-Pak RP C18 column (8 $\times$ 100 mm, 4 μm, Waters, Milford, MA, USA) using mobile phase consisting of 50 mM phosphate buffer (pH 5.9), MeOH and ACN (39:22:39, v/v/v) with a flow rate of 1.8 mL/min at 30°C. The run time was 30 minutes.

Schuman et al. (21) developed and validated a RP HPLC method using photo diode array detection for the simultaneous quantification of 3TC, d4T, NVP, AZT, methyl paraben and propyl paraben in solid and liquid drug formulations. The separation was performed on a Waters Symmetry C8 column with a mobile phase gradient consisting of 50 mM NaH2PO4 (pH 3.8) and ACN (95:5 to 45:55, v/v) and a flow gradient (0.5 to 1.0 mL/min). The LODs and LOQs were below 19 and 55 ng/mL respectively.

Alnouti et al. (22) developed and validated a sensitive HPLC method for the simultaneous quantification of AZT and 3TC in rat plasma, amniotic fluid and placental and fetal tissues with UV detection (254 nm) using SPE using C2 cartridges. Chromatography was carried out on a phenyl column (150 $\times$ 2 mm ID, 5 μm) using a mixture of 8% ACN in 5 mm 1-heptane sulfonic acid dissolved in 30 mm ammonium formate buffer (pH 3.3) as the mobile phase. Flow rate was 0.2 mL/min. The method was validated in the range 0.25–50 μg/mL for both AZT and 3TC in the four biological matrices.

Donnerer et al. (23) reported an analytical procedure for monitoring the plasma concentrations of seven drugs (ABC, AZT, EFV, NVP, IDV, LPV, and NFV) in human plasma with UV detection using a liquid/liquid extraction. The compounds were monitored by UV detection: IDV, LPV, and NFV at 215 nm;

EFV at 254 nm; and ABC, AZT, and NVP at 266 nm. Two different extraction procedures and two different HPLC eluents on a C8 RP HPLC column were used to monitor all seven compounds. Separation for IDV, LPV, NFV, and EFV was achieved on a 15-cm C8 column (LiChroCART 5 Ìm, Merck, Darmstadt, Germany) with mobile phase consisting of 450 mL/L ACN and 50 mL/L MeOH in 15 mmol/L phosphate buffer at pH 7.5, with a flow of the mobile phase of 1 mL/min throughout the run. Separation for AZT, ABC, and NVP was achieved on a 15-cm C8 column (LiChroCART 5 Ìm) with mobile phase consisting of 170 mL/L ACN in 15 mmol/L phosphate buffer at pH 7.5, with a flow of the mobile phase of 1.5 mL/min.

Bezy et al. (24) presented a RP HPLC method for analysis of simultaneous analysis of several anti-retroviral nucleosides (DDC, 3TC, DDI, d4T, carbovir, AZT, TNF and its administrated form (TNF diisoproxyl fumarate) in rat plasma with UV detection (260 nm) using acetic acid/hydroxylamine buffer. After a SPE on Oasis HLB Waters cartridges, HPLC separation was achieved on an Atlantis^{TM} dC18 column (Manchester, UK) with acetic acid–hydroxylamine buffer (ionic strength 5 mM, pH 7)-ACN elution gradient. The flow rate was 0.2 mL/min. Linear calibration curves were obtained within a 30–10,000 ng/mL plasma concentration range. Also, buffer obtained with acetic acid and hydroxylamine was tested in HPLC-ESI-MS/MS.

Rezk et al. (25) presented a RP HPLC assay for the simultaneous quantitative determination of three HIV NNRTIs (NVP, DEL, and EFV) in human blood plasma with UV absorbance detection using hexobarbital as an internal standard. The assay was performed on an Eclipse XDB C8 (150 $\times$ 4.6 mm, 5.0 μm particle size) analytical column (Wilmington, DE, USA), with an SB C18 guard column (5.0 μm particle size), with two mobile phases (Mobile Phase A: 50 mM sodium phosphate buffer, pH 4.8 and Mobile Phase B: 200 mL of Mobile Phase A was mixed with 800 mL of ACN.) with a mobile phase flow rate of 1.5 mL/min at 35°C. The method was validated over the range of 10 ng/mL–50 μg/mL for NVP, 25 ng/mL – 25 μg/mL for DEL, and 10 ng/mL–10 μg/mL for EFV. The lowest LOQ was 10.0 ng/mL for NVP, 25 ng/mL for DEL and 10 ng/mL for EFV. Absorbance wavelength was monitored at 220 nm for NVP, 224 nm for DEL and 248 nm for EFV.

In 2003, Rezk et al. (26) developed a RP-HPLC method for the simultaneous quantitative determination of the NRTIs DDC, 3TC, DDI, d4T, AZT, and ABC with the NNRTI NVP in human blood plasma using SPE and hexobarbital as an internal standard. The chromatographic separation was performed on a Polarity dC C silica (150 $\times$ 3.9 mm, 5.0-μm particle size, Waters) column using gradient mobile phase at flow rate of 1.1 mL/min at 40°C. Mobile Phase A, 10 mM ammonium acetate buffer (pH 6.5), pH adjusted with diluted acetic acid. Mobile Phase B, 200 mL of Mobile Phase A (pH 6.5) were mixed with 500 mL of ACN and 300 mL of MeOH. The method was validated over the range of 10–10, 000 ng/mL for all analytes except DDC (10–5000 ng/mL). The lowest LOQ was 10.0 ng/mL for all analytes. UV detection was monitored at 271 nm for DDC and 3TC, 250 nm for DDI, 269 nm for d4T and ZDV and 230 nm for ABC, NVP and hexobarbital.

A RP-HPLC method for the simultaneous quantitative determination of HIV-PIs (IDV, APV, SQV, NFV, RTV, and LPV) and NNRTIs (NVP, DEL, and EFV) in human blood plasma using SPE was presented by Rezk et al. (27). Midazolam was used as an internal standard. The chromatographic separation was performed on a Zorbax C18 (150 mm $\times$ 4.6 mm, 3.5 μm particle size, Agilent (Wilmington, DE, USA) analytical column with a Zorbax C18 (12.5 mm $\times$ 4.6 mm, 3.5 μm, Agilent) guard column with a gradient elution. Mobile Phase A was composed of 50 mM phosphate monobasic (pH 4.5 adjusted with diluted phosphoric acid) and 150 mL MeOH. Mobile Phase B : 250 mL of Mobile Phase A (pH 4.5) was mixed with 600 mL of ACN, 150 mL of MeOH and 0.75 mL TFA.The low LOQ for NVP, IDV, EFV, and SQV was 10 ng/mL and for all other analytes was 0.025 μg/mL. The upper LOQ was 10 μg/mL for all analytes except DLV, NFV, RTV, and LPV (5 μg/mL).

Rezk et al. (28) reported a gradient RP-HPLC assay for the simultaneous quantitative determination of emtricitabine and TNF in human blood plasma with UV detection using 2,3 didoxyuridine as an internal standard. After SPE, chromatographic separation was achieved on an Atlantis dC18 analytical column (150 $\times$ 3.9 mm, 5.0 μm particle size, Waters Corp., Milford, MA, USA) with an Atlantis dC18 guard column (20 $\times$ 3.9 mm, 5.0 μm particle size, Waters Corp.) using Mobile Phase A consisting of of 20 mM phosphate monobasic and 4 mM of tetrabutylammonium hydrogen sulfate (pH 5.7) and Mobile Phase B consisting of 100% MeOH. The flow rate was 1.0 mL/min at 25°C. Detection was monitored for emtricitabine at 280 nm, TNF at 259 nm, and the internal standard at 262 nm. The method was validated over the range of 10–10,000 ng/mL for both analytes. The low LOQ for both analytes was 10 ng/mL. The upper LOQ was 10,000 ng/mL.

Full validation of an analytical method that combines ATV with 6 HIV-PIs (IDV, APV, SQV, NFV, RTV, and LPV) and 2 NNRTIs (NVP and EFV) in plasma using a simple liquid-liquid extraction method with midazolam as an internal standard reported by Rezk et al. (29). The assay was carried out on a Zorbax C18 (150 $\times$ 4.6 mm, 3.5 μm) analytical column with a gradient elution of the mobile phase at 30°C. Mobile Phase A consisted of 50 mM phosphate monobasic solution (adjusted to a pH of 4.5 with diluted phosphoric acid) with 150 mL of MeOH. Mobile Phase B consisted of a mixture of 250 mL of the pH 4.5 phosphate buffer of Mobile Phase A with 600 mL of ACN, 150 mL of MeOH, and 0.75 mL of trifluoroacetic acid with a flow gradient (0.9 to 1.2 mL/min). UV detection was monitored at 210 nm. The assay was validated over a range of 25 to 10,000 ng/mL. There was a low LOQ quantitation of 0.025 μg/mL for all analytes.

Rezk et al. (30) presented a HPLC assay for the simultaneous quantitation of four anti-retroviral drugs (3TC, d4T, AZT, and NVP) in human breast milk with UV detection using a SPE and hexobarbital as an internal standard. The chromatographic

separation of analytes was performed on an Atlantis dC_{18} (150 × 3.0 mm, 3.0 μM, Waters) analytical column with an Atlantis dC18 (20 × 3.9 mm, 5.0 μM particle size, Waters) guard column using a gradient elution in both mobile phase composition (Mobile Phase A was 10 mmol/L ammonium acetate solution buffer, at pH 6.3, adjusted using diluted acetic acid. Mobile Phase B was composed from 60% ACN and 40% MeOH) at 30°C. The run time was 23 minutes. All drugs were linear over a wide range of concentrations, from 0.02 to 20 μg/mL.

Rebiere et al. (31) developed two methods of RP-LC for the analysis of 19 anti-retroviral molecules (DDC, 3TC, amdoxovir, emtricitabine, DDI, d4T, AZT and ABC, fosamprenavir, NVP, IDV, APV, SQV, ATV, RTV, LPV, EFV, NFV and tipranavir). The first HPLC method was used for nucleoside/nucleotide reverse transcriptase inhibitors molecules identification and assay. The separation was carried out using a YMC-pack ODS-AM, (250 × 4.6 mm, 5 μm) analytical column using a gradient mobile phase (Mobile Phase A, an ammonium acetate buffer, 25 Mm adjusted to pH 4.0 with acetic acid 100% and Mobile Phase B, MeOH) at a flow rate of 1 mL/min. UV detection was monitored at 270 nm. The run time was 60 minutes. The second HPLC method was used for NNRTIs and PIs molecules identification and assay. The separation was carried out using a Symmetry C18 (250 × 4.6 mm, 5 μm column, Waters, France) with gradient mobile phase (Mobile Phase A, potassium phosphate buffer, 50 mM adjusted to pH 5.65 with potassium hydroxide 100% and Mobile Phase B, ACN) at a flow rate of 1.5 mL/min at 30°C. UV detection was monitored at 260 nm. The run time was 50 minutes.

A RP gradient HPLC assay for the simultaneous quantitative determination of tipranavir with nine other anti-retroviral drugs (NVP, IDV, EFV, SQV, APV, ATV, RTV, LPV and NFV) in plasma using a liquid-liquid extraction of the drugs in tert-butylmethylether with UV detection at 210 nm presented by Choi et al. (32). Clozapine was used as an internal standard. The chromatographic separation of analytes was performed on a Zorbax C18 (150 × 4.6 mm, 3.5 μm particle size, Agilent) analytical column and a Zorbax C18 (12.5 × 4.6 mm, 5 μm, Agilent) guard column using a gradient elution. Mobile Phase A consisted of 50 mM phosphate monobasic solution (adjusted to a pH of 4.5 with diluted phosphoric acid) with 150 mL of MeOH. Mobile Phase B consisted of a mixture of 250 mL of the pH 4.5 phosphate buffer of Mobile Phase A with 600 mL of ACN, 150 mL of MeOH, and 0.75 mL of trifluoroacetic acid with a flow gradient (0.9–1.2 mL/min). The standard curve for the drug was linear in the range of 80–80,000 ng/mL for tipranavir; 10–10,000 ng/mL for NVP, IDV, EFV, and SQV; and 25–10,000 ng/mL for APV, ATV, RTV, LPV, and NFV. The low LOQ for tipranavir was 80 ng/mL. The lower LOQ for NVP, IDV, EFV, and SQV was 10 ng/mL; was 25 ng/mL for all other analytes.

Notari et al. (33) presented a HPLC method for simultaneously determining seven HIV PIs (APV, ATV, IDV, LPV, NFV, RTV, and SQV), seven NRTIs (ABC, DDI, emtricitabine, 3TC, d4T, DDC, and AZT), and two NNRTIs (EFV and NVP) in human plasma with UV detection. After a SPE with Oasis HLB Cartridge, separation was performed on an analytical C_{18} Symmetry column (250 × 4.6 mm ID) with a particle size of 5 μm with mobile phase composed of 0.01 M KH_2PO_4 and ACN. The flow rate was 1.0 mL/min and UV detection were monitored at 240 and 260 nm. The total run time was 35 minutes. The calibration curves were linear up to 10 μg/mL. The LOQs for APV, ATV, 3TC, LPV, NVP, SQV, and RTV were 0.005 μg/mL, 0.025 μg/mL for ABC, DDI, IDV, and AZT, and 0.10 μg/mL for EFV, emtricitabine, NFV, d4T, and DDC.

Justesen et al. (34) reported a HPLC method for determination of six PIs (IDV, APV, RTV, LPV, SQV, NFV) and the NFV active metabolite M8 in human plasma with UV detection (210 and 239 nm) using liquid–liquid extraction with a mixture of heptane and ethyl acetate. Chromatographic separation was performed isocratic on an analytical C18 column (3.9 × 150 mm, Waters) using a mobile phase consisting of 140 mL of ACN, 75 mL triethylamine and 160 mL of 5 mM sodium dihydrogenphosphate (pH 6) at a flow rate of 1.0 mL/min. The run time was 30 minutes. Detection limits were 10.8 ng/mL for IDV, 23.1 ng/mLfor APV, 12.9 ng/mL for RTV, 18.0 ng/mL for LPV, 12.6 ng/mL for SQV, 15.9 ng/mL for NFV and 6.9 ng/mL for M8. The LOQ was 25 ng/mL.

Verbesselt et al. (35) reported a method for determination of the HIV PIs APV, IDV, ATV, RTV, LPV, NFV, M8-NFV metabolite and SQV in human plasma using liquid-liquid extraction with hexane/ethylacetate from buffered plasma samples with a borate buffer of pH 9.0 with UV and fluorescence detection. Isocratic chromatographic separation was performed on an Allsphere hexyl HPLC 5 μ column (150 × 4.6 mm ID) (Milford, MA, USA) using a mobile phase consisting of a mixture of ACN, MeOH and 15 mM sodiumdihydrogenphosphate buffer at pH 4.5 (35:20:45, v/v/v) at a flow rate of 1.0 mL/min. Calibration curves were constructed in the range of 0.025–10 mg/L. The UV detector was set at 215 nm and the fluorescence detector at 280 and 340 nm as excitation and emission wavelengths, respectively. The LLOQ was defined as 25 ng/mL for all PIs. The LOQ was set at 10,000 ng/mL.

Marchei et al. (36) presented a RP-LC for the simultaneous determination of AZT and NVP in human plasma with UV detection (265 nm) using a solid-liquid extraction. The analysis was achieved a Zorbax SB-C18 column (250 × 4.6 mm ID) (Hewlett Packard, USA) with a mobile phase consisting of potassium dihydrogen phosphate (10 mM; pH 6.5)-ACN (83:17, v/v). Flow rate was 1.0 mL/min. The LODs for AZT and NVP were 0.025 and 0.05 μg/mL, and the LOQs were 0.05 and 0.150 μg/mL, respectively.

Leibenguth et al. (37) reported a HPLC assay for simultaneous determination of six PIs (RTV, SQV, IDV, NFV, APV, and LPV) with UV spectrophotometer by a photodiode array detector, using carbamazepine as internal standard. The assay was on a Symmetry C18 (250 × 4.6 mm) (Quentin-en-Yvelines, France) equipped with a Sentry guard Symmetry C18 column (20 × 3.9 mm) (Quentin-en-Yvelines, France) using gradient

mobile phase consisting of phosphate buffer and ACN. The flow rate was 1 mL/min. Detection was carried out at 211 nm. LOQs were: 0.1, 0.025, 0.05 and 0.1 mg/L for RTV, SQV, IDV and NFV, respectively. Through concentrations were 0.15–13.6 mg/L for RTV, 0.06–9.7 mg/L for IDV, 0.03–5.5 mg/L for SQV, and 0.15 to 4.15 mg/L for NFV.

Faux et al. (38) developed a HPLC method for the determination of six PIs (APV, IDV, LPV, NFV, RTV, and SQV) and two NNRTIs (EFV and NVP) in a single run using liquid-solid extraction on OASIS HLB column using prazepam as internal standard. Chromatographic separation was performed on an XTERRA, C_{18} (150 × 3.9 mm ID) column (Quentin-en-Yvelines, France) using a mixture of ACN and 3-(cyclohexylamino)-1-propanesulfonic acid buffer, pH 10.5 (37:63, v/v) as mobile phase at a flow rate of 1.5 mL/min. UV detection was monitored at 320 nm for NVP and at 210 nm for other drugs and internal standard. The LODs were 0.06 μg/mL for IDV and LPV, 0.05 μg/mL for NVP, APV, EFV, RTV and NFV, 0.04 μg mL for SQV and 0.025 μg/mL for NFV M8.

Yamada et al. (39) presented a RP-HPLC method for the simultaneous quantitative determination of five HIV PIs (IDV, APV, SQV, RTV and NFV) in human plasma with UV detection (215 nm), using extraction with 0.5 mL of 0.1 M NH_4OH and 5 mL of methyl tert.-butyl ether. The separation was achieved on a C18 RP column with a mixture of ACN and 50 mM KH_2PO_4 adjusted to pH 5.6 with 50 mM Na_2HPO_4 (43:57, v/v) as mobile phase at ambient temperature and a flow rate of 1.5 mL/min. Linearity of the method was obtained in the concentration range of 0.05–20 μg/mL for all five PIs. LLOQ for all PIs were 0.05 mg/mL.

A HPLC using UV detection for the determination of nine anti-retroviral (IDV, SQV, RTV, APV, LPV, DEL, EFV, NFV and its M8 metabolite) in human plasma with UV detection (210 nm) reported by Turner et al. (40). After an extraction of tert.-butyl methyl ether, separation was performed on a C_8 column (25 cm × 4.6 mm ID, 5 μm, Waters) with a gradient mobile phase consisting of (A) 25 mM potassium phosphate buffer, pH 3.1; (B) ACN and (C) MeOH at 27°C and a flow rate of 1.5 mL/min in 40-minute run time. LOQ is 50 ng/mL.

Sarasa-Nacenta et al. (41) reported a HPLC assay for the simultaneous quantification of the HIV-PIs IDV, APV, RTV, SQV and NFV in human plasma with UV detection at a variable wavelength, using SPE and the internal standard (verapamil). The HPLC analysis was performed on a RP Nova Pak C18 column (150 × 3.9 mm, 5 μm, Waters) with a Nova pak C18 guard column (Waters) using a mobile phase consisting of a gradient with 15 mM phosphate buffer (pH 5.75)-ACN at flow rate of 1.5 mL/min over 23 minutes. The LOQs were 0.040 mg/mL for IDV, 0.050 mg/mL for APV, 0.10 mg/mL for RTV, 0.044 mg/mL for SQV and 0.085 mg/mL for NFV. UV detection was set at 210 nm (first 10 minutes).Then detection changed to 240 nm, and in minute 19 the wavelength changed again to 220 nm.

Tarinas et al. (42) reported methods for the determination of AZT, d4T, 3TC and IDV in human plasma by RP-LC with UV detection, using solid-liquid extraction procedures. Chromatography was performed on a C18 analytical column (250 × 4 mm, 5 μm particle size, Merck) with mobile phase in the isocratic mode at room temperature. The mobile phase consisted of a mixture of ACN and water (9:91, v/v) for d4T, 3TC; MeOH and water (20:80, v/v) for AZT and buffer phosphate (pH 5.7, adjusted with Na2HPO4) and ACN (60:40, v/v) with 0.2% triethylamine for IDV. The UV detector was monitored at 271 nm for d4T, 3TC, 267 nm for AZT and 210 nm for IDV. The flow rate was 0.7 mL min for d4T, 3TC, 1.2 mL min for AZT and 1.3 mL min for IDV. LOQs were 10 ng mL for d4T, 50 ng mL for 3TC, 0.05 μg mL for AZT 0.1 μg mL for IDV. LODs were 5 ng mL for d4T, 11.5 ng mL for 3TC, 0.01 μg mL AZT and 0.05 μg mL for IDV.

Albert et al. (43) presented two RP-HPLC methods for the quantitation of SQV and RTV in human plasma with UV detection, using single liquid–liquid extraction procedure with ethyl acetate–hexane (50:50, v/v). The analyses were achieved on a Luna C18 column (150 × 4.6 mm ID) with a C18 guard column using a mixture of ACN and 70 mM KH_2PO_4 adjusted to pH 5 with 80 mM Na_2HPO_4 (46:54, v/v) as mobile phase at a flow rate of 1 mL/min at room temperature. The UV detection was monitored at 240 nm for SQV and at 210 nm for RTV. The retention times were 6.4 minutes for SQV and 8.3 minutes for RTV. The methods were linear over the range of 100–2500 ng/mL for SQV and 200–2500 ng mL for RTV. The LOQs were 100 and 200 ng/mL for SQV and RTV, respectively.

Hsieh et al. (44) developed a method for simultaneous determination of plasma concentrations of IDV, RTV and SQV by HPLC in a single run UV detection (215 nm) using propylparaben as an internal standard. Chromatographic separation was performed a on C18 column using a mixture of phosphate buffer (50 mM, pH 5.6) and ACN (55:45, v/v) as mobile phase after an extraction with methyl tert.-butyl ether. The flow rate was 1.5 mL min. The calibration curves were linear for all drugs in the concentration range from 0.1–5 μg mL.

Kuschak et al. (45) reported a HPLC method for the simultaneous determination of LPV, IDV, APV, SQV, RTV and NFV in human plasma UV detection at 215 nm. After an extraction with 500 microL 0.1 M ammonium hydroxide solution and 5 mL tert-butyl ether, chromatographic separation was carried out on a C18 column with a non-linear gradient mobile phase consisting of 50 mM phosphate buffer (pH 5.40) and ACN (50:50, v/v).

A HPLC for the simultaneous determination of six HIV PIs (APV, IDV, LPV, NFV, RTV and SQV), the active metabolite of NFV (M8) and the NNRTI (EFV) in human plasma with UV detection was reported by Hirabayashi et al. (46). After extraction from plasma with an ethyl acetate-ACN mixture, the analytes were separated on a Develosil Ph-UG-3 (150 × 2.0 mm, 3 μm particle size) column (Seto City, Japan) with a gradient of ACN and phosphate solutions at 40°C. The Mobile Phase A consisted of 34% (v/v) ACN and 66% (v/v) 25 mM NaH_2PO4 containing 6 mM sodium 1- hexanesulfonate and exactly adjusted to

pH 5.1 with 25 mM Na2HPO4. The Mobile Phase B consisted of 64% (v/v) ACN and 36% (v/v) 25 mM NaH_2PO4 containing 6 mM sodium hexanesulfonate and adjusted to pH 5.3. The flow rate was 200 μL/min. Calibration curves were linear over the range 0.025–15 μg/mL for SQV and 0.05–15 μg/mL for the other analytes. Absorbance was monitored at a dual wavelength. Channel 1, 212 nm and channel 2 for APV, SQV and the other six analytes were 266, 239 and 212 nm, respectively.

Xu et al. (47) presented a RP-HPLC method using an underivatized silica column for the separation and analysis of DDC and d4T with UV detection from rat plasma, amniotic fluid, placental, and fetal homogenate using 3TC as internal standard. After a liquid-liquid extraction of DDC, d4T and internal standard using a saturated solution of ammonium sulfate from matrices, chromatographic separation was achieved on a Waters Spherisorb S3W silica column (4.6 × 100 mm) equipped with a Phenomenex guard column using a mixture of 3% MeOH in 22 mM formic acid as mobile phase. The flow rate was 0.5 mL/min, and the detection was at 265 nm.

Bezy et al. (48) developed an HPLC method for the simultaneous analysis of several nucleosides anti-HIV (DDI-dideoxynucleosides, 3TC, d4T, AZT, carbovir and TNF) in rat and human plasma with UV detection. The separation was achieved on an Atlantis dC18 (3 μm) reversed stationary phase with mobile phase consisting of ACN-water elution gradient.

Verweij-Van Wissen et al. (49) reported a RP-HPLC method for the simultaneous quantitative determination of 3TC, DDI, d4T, AZT and ABC with UV detection (260 nm) in plasma using SPE with Oasis MAX cartridges. Chromatographic separation was performed on a SymmetryShield RP 18 column (150 × 4.6 mm ID, 3.5 μm particle size, Waters Etten-Leur, The Netherlands) with a SymmetryShieldRP18 guard column (20 × 3.9 mm ID, 3.5 μm particle size, Waters) using Mobile Phase A consisting of acetate buffer:ACN (95:5, v/v) and Mobile Phase B consisting of acetate buffer:ACN (76:24, v/v) at 30°C. The flow rate was set at 1.0 mL/min. The assay was validated over the concentration range of 0.015–5 mg/L for all five drugs. The LLOQ was 0.015 mg/L for all five drugs.

Djurdjevic et al. (50) presented a HPLC for the separation of ABC, 3TC, and AZT in tablets on a C18 column with UV detection at 270 nm. The mobile phase consisted of water:MeOH (60:40 v/v) with 0.2% TEA and pH 3.20 (pH adjusted with H_3PO_4). Also, a HPLC method for the analysis of Trizivir tablets with an extraction with MeOH:water mixture (50:50 v/v) was achieved using d4T as an internal standard.

Savaser et al. (51) reported a HPLC method for the simultaneous determination of ABC, 3TC and AZT in pharmaceutical tablets, human serum samples and drug dissolution studies using Granisetron as an internal standard with UV detection at 275 nm in less than 7 minutes. Chromatographic separation was achieved on a 5 μm Zorbax C18 column (150 × 4.6 mm ID) using a mixture of MeOH:water:phosphate buffer at pH 5.65 (80:10:10; v/v/v) as mobile phase at ambient temperature. Flow rate was 0.6 mL/min. LODs were 21.87 ng/mL for ABC, 63.03 ng/mL for 3TC and 110.32 ng/mL for AZT in mobile phase and 11.01 ng/mL for ABC 95.65 ng/mL for 3TC and 41.18 ng/mL for AZT in serum. LOQs were 72.90 ng/mL for ABC 210.09 ng/mL for 3TC and 367.74 ng/mL for AZT in mobile phase and 33.36 ng/mL for ABC 290.47 ng/mL for 3TC and 124.79 ng/mL for AZT in serum.

A simple HPLC method for the simultaneous determination of ABC and AZT with UV detection (270 nm) in rat plasma, amniotic fluid, fetal, and placental tissues using an internal standard (azidouridine) was developed by Summer et al. (52). Extraction of ABC, AZT, and internal standard in amniotic fluid was achieved with protein precipitation. Extraction from plasma, fetal and placental homogenates was achieved by using a salting out technique. Chromatographic separation was carried out on a Agilent Eclipse XDB C8 column (150 × 4.6 mm, 5 μm) (Palo Alto, CA, USA) with a Phenomenex Security Guard C18 guard column (Torrance, CA, USA) using mobile phase consisting of 12% ACN in 25 mM sodium phosphate buffer (adjusted to pH 7 with sodium hydroxide) for the fetus, placenta, plasma and amniotic fluid samples. The flow rate was 0.8 mL/min. The LOQ was 0.05 μg/mL. Retention times of ABC, AZT and azidouridine eluted at 8.3, 13.5, and 5.7 minutes respectively.

Anbazhagan et al. (53) reported RP-HPLC and HPTLC methods for simultaneous determination of d4T, 3TC and NVP in tablets with UV spectroscopy (265 nm). The RP-HPLC method was performed on a C18-ODS-Hypersil (250 × 4.6 mm, 5 μm) column using a mixture of 20 mM sodium phosphate buffer (containing 8 mM 1-octanesulphonicacid sodium salt): ACN adjusted to pH 3.5 using phosphoric acid (4:1, v/v) as mobile phase. The flow rate was 1.5 mL/min. The retention time of d4T, 3TC and NVP was 2.85, 4.33 and 8.39 minutes respectively. According to the authors, the results have shown that HPLC method is best for a simultaneous quantification of d4T, 3TC and NVP in tablets.

Sarkar et al. (54) reported a HPLC and an UV spectrophotometric method for the quantitative determination of three antiretroviral drugs (3TC, D4T and NVP). Chromatography was performed on a RP C18 Symmetry column (4.6 mm × 250 mm, 5 μm, Waters) using isocratic mobile phase. Mobile phase for 3TC was MeOH:water (70:30, v/v) at a flow rate of 0.75 mL/min. Mobile phase for d4T was MeOH:water (20:80, v/v) at a flow rate of 0.80 mL/min. Mobile phase for NVP was Solvent A MeOH:water (20:80, v/v) at a flow rate of 0.18 mL/min and Solvent B ACN:isopropyl alcohol (50:50, v/v) at a flow rate of 0.42 mL min^{-1}. The LOQs of d4T, 3TC and NVP were 0.52, 0.09 and 0.37 μg/mL, respectively. The LODs of d4T, 3TC and NVP were 0.17, 0.03 and 0.12 μg/mL, respectively. UV detection with HPLC method was 270 nm for all drugs. The UV spectrophotometric determinations were monitored at 270, 265 and 313 nm for 3TC, d4T and NVP, respectively.

Marzolini et al. (55) reported an HPLC method after SPE with phosphate buffer pH 7 on a C 18 cartridge for the separate analysis of LPV and NVP using as internal standard (clozapine) with UV detection. The separation was achieved on a

ChromCart cartridge column (125 × 4 mm I.D.) filled with Nucleosil 100–5 μm C18 AB (Macherey-Nagel) equipped with a guard column (8 × 4 mm ID) filled with the same packing material using a gradient mobile phase consisting of ACN and phosphate buffer adjusted to pH 5.07 and containing 0.02% sodium heptanesulfonate at a flow rate of 1.0 mL/min at room temperature. LPV and NVP were monitored at 201 and 282 nm, respectively. The LODs for LPV and NVP were 0.05 and 0.015 mg/mL, respectively. The LOQs for LPV and NVP were 0.10 and 0.06 0.015 mg/mL, respectively.

A RP-HPLC method for the simultaneous quantitative determination of the NRTIs (3TC, d4T) and the NNRTI (NVP) in pharmaceutical fixed dose combinations with UV detection (270 nm) was presented by Kapoor et al. (56). Chromatographic separation was performed on C18 (250 × 4.6 mm ID, 5 μm) column with guard column (Nova-Pak, 4 μm, 60 A°, C18 Guard-Pak, Waters) using two mobile phases consisting of 10 mM acetate buffer (adjusted to pH 3.5 with glacial acetic acid) and MeOH (80:20, v/v) and other mobile phases 50% ACN and isopropyl alcohol (50:50, v/v). Flow rate was 0.6 mL/min. The average retention times for 3TC, d4T and NVP were 5.9, 8.8 and 14.2 minutes, respectively. LOQs for all drugs was 0.5 μg/mL.

Pai and Desai (57) reported a HPLC method for the simultaneous estimation of 3TC, AZT and NVP from tablets by external standard method. The separation was achieved on an Inertsil ODS 3V (250 × 4.6 mm, 5 μ) column with a mobile phase consisting of MeOH:buffer (0.1 M ammonium acetate in 0.5% glacial acetic acid) (50:50, v/v). UV detection was monitored at 270 nm. The flow rate was 1.0 mL/min at ambient temperature. The calibration curve was linear in the concentration range of 75–225 μg/mL of 3TC, 100–300 μg/mL for NVP and 150–450 μg/mL for AZT.

D'Avolio et al. (58) developed a HPLC method with photo diode array detection for the quantification of integrase inhibitor (raltegravir), the NNRTI (etravirine), and 11 other anti-retroviral agents (RTV, ATV, LPV, NVP, EFV, SQV, IDV, NFV, and its metabolite M8, APV, and darunavir) in human plasma using an internal standard (quinoxaline). After a SPE procedure, the chromatographic separation of the drugs was carried out on a Luna 5 μ C18 column (150 × 4.6 mm ID) (Milan, Italy) with a SecurityGuard with C18 (4.0 × 3.0 mm ID, Phenomenex) at 45° using a gradient mobile phase consisting of solvent A (KH_2PO_4 50 mM with orthophosphoric acid, pH 3.23) and Solvent B (ACN) with a 28-minute analytical run time. UV detection was monitored in the range of 210–320 nm. The flow rate was 1.0 mL/min.

In 2003, Usami et al. (59) reported a HPLC method for the simultaneous determination of LPV, RTV and EFV in human plasma with UV detection (205 nm). The separation was achieved with a Radial-Pak Nova- Pak C18 column (8 × 100 mm, 4 μm, Waters) with Guard-Pak Inserts in a Nova-Pak C18 pre-column using a mixture of ACN, MeOH and 0.02 M tetramethylammonium perchlorate (45:5:50, v/v/v) as mobile phase at a flow rate of 1.5 mL/min at 30°C. Standard curves were linear in the range 0.060–24.06 μg/mL for LPV, 0.010–4.16 μg/mL for RTV, and 0.047–37.44 μg/mL for EFV.

Dogan-Topal et al. (60) presented a RP-HPLC with diode array detection procedure for the simultaneous determination of ABC, EFV and valganciclovir in spiked human serum in 2007. Separation was performed using a 5 μm Waters Spherisorb column (250 × 4.6 mm ID) with a isocratic mobile phase consisting of ACN:MeOH:KH_2PO_4 (at pH 5.00) (40:20:40 $v/v/v$) elution at a flow rate of 1.0 mL/min. Calibration curves were constructed in the range of 50–30,000 ng/mL for ABC and EFV, and 10–30,000 ng/mL for valganciclovir in serum samples. The LODs and LOQs were 3.80 and 12.68 ng/mL for ABC, 2.61 and 8.69 ng/mL for EFV, 1.30 and 4.32 ng/mL for valganciclovir.

Checa et al. (61) reported a RP-HPLC method for the determination of anti-retroviral drugs; NRTI drugs [AZT, DDI, d4T, DDC, 3TC, emtricitabine (FTC)] NNRTI drugs (NVP and DEL); PI (IDV, SQV, RTV and LPV) and NFV in human plasma samples using SPE for sample pre-treatment. The separation was carried out on a Synergy Hydro-RP C18 column (150 × 4.6 mm ID, 4 μm) equipped with a guard column (4 × 3 mm ID) using an gradient mobile phase consisting of 1% acetic acid/ammonium acetate aqueous solution (pH 4.5) and ACN. LODs are around 5 ng/mL for reverse transcriptase inhibitors (nucleoside and non-nucleoside) and 20 ng/mL for PIs. Spectrophotometric detection was at 240 nm for SQV and RTV, 250 nm for DDI, 260 nm for d4T, IDV, LPV, NFV and 280 nm for ddc, 3TC, FTC, AZT, NVP, DLV.

A RP-HPLC method for the analysis of APV, EFV, IDV, LPV, NFV and its active metabolite (M8), RTV, and SQV in heparinized human plasma using a photodiode-array detector reported by Keil et al. (62). Chromatographic separation was performed on a Symmetry shield RP8 (3.0 × 150 mm, 5 μm, Waters) column protected with a Waters Symmetry C8 5-μm guard column using isocratic mobile phase consisting of an ACN and 50 mmol/L formic acid buffer, pH 4.26 to 4.27 (40:60, v/v) at 33°C. The flow rate was started at 0.75 mL/min for 12 minutes and 1.6 mL/min for 12.5 minutes. Detection was monitored at 215 nm for IDV, RTV, LPV, NFV and its metabolite M8, 235 nm for SQV, 248 nm for EFV, and 265 nm for APV. The retention times for IDV, NFV's metabolite M8, SQV, APV, NFV, RTV, LPV, and EFV are 3.4, 6.3, 7.2, 8.8, 13.1, 16.7, 19.1, and 20.4 minutes, respectively. The LLOQ were ranged from 0.1–0.2 μg/mL.

Uslu and Özkan (63) developed first derivative spectrophotometric, first derivative of the ratio-spectra and HPLC–UV (265.0 nm for both drugs) methods for the simultaneous determination of 3TC and AZT in binary mixtures using finasteride as an internal standard. Chromatographic separation was performed on a Spherisorb (4.6 × 150 mm ID, 5 μm, Waters) column with a mobile phase of MeOH:water:ACN (70:20:10, v/v/v)) at 0.9 mL/min flow rate at ambient temperature. Linearity was obtained in the concentration range of 0.025–50 μg/mL for 3TC and 0.15–50 μg/mL for AZT. The retention times were 2.06 minutes for 3TC, 3.36 minutes for AZT and 4.32 minutes

for finasteride. The LODs for 3TC and AZT were 0.00098 and 0.0049 μg/mL, respectively. The LOQs for 3TC and AZT were 0.0033 and 0.016 μg/mL, respectively.

Ramachandran et al. (64) developed a RP-HPLC method for simultaneous determination of plasma AZT and NVP with UV detection (260 nm) using liquid–liquid extraction with ethyl acetate, and 3-isobutyl 1-methyl xanthine as an internal standard. Chromatographic separation was performed on a C18 column (150 × 4.6 mm ID) using a mixture of potassium dihydrogen phosphate (15 mM; pH 7.5) and ACN (80:20,v/v) as mobile phase at a flow rate of 1.5 mL/min at ambient temperature. The assay was linear from 0.025–10.0 μg/mL for AZT and 0.05–10.0 μg/mL for NVP. The run time was 10 minutes, LOQs for AZT and NVP were 0.025 and 0.05 μg/mL, respectively.

Two HPLC methods using UV detection for the determination of two different groups (A and B) of drugs used in the suppression of HIV with SPE were reported by Simon et al. (65). Group A consisted of six nucleosidic reverse transcriptase inhibitors (DDC, 3TC, d4T, DDI, AZT and ziagen). Group B consisted of PIs (IDV, NFV, SQV and RTV (Pis) and non-nucleosidic reverse transcriptase inhibitors (NVP, DEL and EFV.) Chromatography for Method A was performed on two 150 × 4.6 mm, 3-mL-Luna C18 columns using gradient mobile phase consisting of HPLC-grade water and ACN at 60°C at flow rate of 0.85 mL/min. The UV detection was monitored at 250 nm. Chromatography for Method A was performed using gradient mobile phase consisting of 0.004 M sulfuric acid and ACN (8–63% ACN in 45 minutes with a 5-minute hold at 63%) with SPE column. UV detection was monitord at 265 nm during the first 31 minutes and at 240 nm. Method detection limits in serum for six HIV drugs in Group A were 0.44 for DdC, 0.26 for 3TC, 0.12 for DdI, 0.040 for D4T, 0.030 for AZT and 0.075 μg/mL for Ziagen. Method detection limits in serum for seven HIV drugs in Group B were 0.084 for NVP, 0.21 for IDV, 0.11 for DEL, 0.40 for NFV, 0.10 for SQV, 0.51 for RTV and 0.062 μg/mL for EFV.

Weller et al. (66) developed an isocratic HPLC method for determining EFV, NVP, APV, ATV, IDV, LPV, NFV, RTV, and SQV drugs in plasma UV detection (212 nm) using DEL as internal standard. After a liquid–liquid extraction, chromatographic separation was on an S-3, 3.0 × 150 mm YMC-Pack Octyl C8 column (Milford, MA, USA) with mobile phase consisting of 25 mM monobasic potassium phosphate (pH 4.90), ACN(52:48,v:v) at 35°C at flow rate of 0.4 mL/min. Retention times for NVP, IDV, DEL, APV, SQV, ATV, RTV, LPV, EFV, and NFV were 2.9, 4.5, 5.7, 6.2, 7.7, 10.1, 12.3, 14.0, 15.0, and 17.1 minutes, respectively.

Caufield and Stewart (67) reported a HPLC assay for the simultaneous determination of AZT and levofloxacin in human plasma with UV detection at 266 nm using SPE procedure. The chromatographic separation was on an octadecylsilane column (150 × 4.6 mm ID, 5 μm particle size, Hewlett Packard) using a mobile phase of 25 mM sodium phosphate monobasic monohydrate and 0.1% trifluoroacetic acid (pH 2.4) – ACN (86:14, v:v) at flow rate of 1.5mL/min at ambient temperature. Ciprofloxacin was used as the internal standard. Retention times were 4.9 minutes for AZT and 8.0 minutes for levofloxacin. The LODs were 8.8 ng/mL for AZT and 25.0 ng/mL for levofloxacin. The LOQs were 26.3 ng/mL for AZT and 51.2 ng/mL for levofloxacin.

Brown et al. (68) reported a gradient HPLC assay for the separation and analysis of acyclovir and AZT from plasma, amniotic fluid, placental homogenate, and fetal homogenate with UV detection (254 nm). The plasma and amniotic fluid samples were prepared using a combination of protein precipitation and filtration. The more complex tissues were prepared with SPE. Chromatographic separations were achieved using an Agilent Eclipse XDB C-8 column (150 × 2.1 mm, 5 mm) with a Phenomenex Security Guard C18 guard column with mobile phase consisting of 30mM acetate/citrate buffer (pH 3) and MeOH.

Ray et al. (69) presented an isocratic RP-HPLC for the determination of IDV, RTV and LPV with UV detection (205 nm) in human plasma using RTV analogue A-86093.0 as an internal standard. Chromatographic separation was achieved on a Phenomenex Luna phenyl hexyl column (250 × 4.6 mm ID, particle size 5 μm; Phenomenex) using a mobile phase consisting of ACN and water containing 10 mM potassium phosphate buffer adjusted to pH 7.0–85% orthophosphoric acid (50:50, v/v) with a flow of the mobile phase of 1.0 mL/min. The LLOQs for IDV, RTV and LPV were 50, 100 and 100 μg/L, respectively.

A stability-indicating HPLC method for determination RTV and LPV in soft gelatin capsules with UV detection (210 nm) was reported by Donato et al. (70) using a photodiode array detector. The method was achieved a LiChrospher 100 RP-18 (250 × 4.6 mm, 5 μm, Merck) column with mobile phase consisting of a mixture of ACN-water-MeOH (53:37:10, v/v/v).The flow rate was 1.0 mL/min. The authors showed that LPV is stable in thermal, alkaline and oxidative conditions, while RTV degraded under these conditions.

HPLC-MS Methods

Colombo et al. (71) reported a ESI-LC–MS/MS method for the intra-cellular determination of nine anti-retroviral drugs (IDV, APV, SQV, RTV, NFV, LPV, ATV, EFV and NVP) in human peripheral blood mononuclear cells using single-step extraction with clozapine as an internal standard. The separation was achieved a on 2.1 × 30 mm Symmetry Shield 3.5 μm-RP18 column (Waters, Milford, MA, USA) equipped with a 2.1 × 10 mm guard column using a gradient program with a mixture of 2 mM ammonium acetate containing 0.1% formic acid (pH 2.85) and ACN with 0.1% formic acid at 30°C. The calibration curves were at concentrations ranging from 0.5–100 ng/mL of cell extracts. The LLOQ is less than 0.5 ng/mL. The LODs were 6 pg/mL for IDV, 25 pg/mL for NVP, 1 pg/mL for SQV and NFV, 6 pg/mL for ATV and APV, 3 pg/mL for RTV, 13 pg/mL for LPV and 100 pg/mL for EFV. LOQs were 0.5 ng/mL for NFV, 0.2 ng/mL for ATV and APV, 0.4 ng/mL for other drugs.

Mesplet et al. (72) reported HPLC and mass spectrometry analysis of the enzymatic hydrolysis of anti-HIV pronucleotide

diastereomers (d4T, AZT, 3TC and lodenosine) in pig liver esterase. After a liquid–liquid extraction, separation was performed on a Chiralcel OD-RH analytical column (150 × 4.6 mm ID, 5 μm) (Les Ulis, France) with mobile phase MeCN/water (30:70, v/v for d4T; 40:60, v/v for AZT; 30:70, v/v for 3TC and ddA). UV detection was performed at 254 nm. The flow rate was 0.5 mL/min. Mass spectrometry experiments were performed using a Perkin–Elmer API 300 triple quadrupole ESI mass spectrometer (Foster City, CA, USA).

Alnouti et al. (73) reported an ESI-LC/MS/MS method for the simultaneous determination of AZT and 3TC in rat plasma, amniotic fluid, placental, and fetal tissues with ACN precipitation. Separation was achieved on a Symmetry C18 column (150 × 3.9 mm ID, 5 μm, Waters) equipped with a Phenomenex C18 guard column using a mixture of 30% MeOH and 7.5 mM ammonium acetate (pH 6.5) as gradient mobile phase at a flow rate of 0.65 mL/min. The assay was validated in the range of 0.05–25 μg/mL for both 3TC and AZT in the four biological matrices.

A LC coupled with ESI-MS method for the simultaneous determination of five PIs (SQV, IDV, RTV, NFV, and APV) and three NNRTIs (NVP, DEL, and EFV) in human plasma was developed by Villani et al. (74). After a liquid-liquid extraction, chromatographic separation was performed on a Supelcosil LC18-DB (7.5 cm × 4.6 mm, 3 μm) (BAKKKK) analytical column using a linear gradient with water and ACN. The column flow rate was 0.8 mL/min, and analyses were performed at room temperature. The calibration curves were linear in a range of concentrations between 20–10,000 ng/mL (40–10,000 ng/mL for EFV). The LOD was approximately 10 ng/mL for seven drugs (25 ng/mL for EFV). The authors suggested that the ESI-LC-MS/MS assay provides an excellent method for simultaneous determination of different components of the highly active anti-retroviral treatments in patients treated simultaneously with PIs and NNRTIs.

Fan et al. (75) reported a HPLC method utilizing triple quadrupole MS detection with ESI and Polarity Switching in human serum for the determination of a 3TC, d4T and EFV using a SPE and internal standard (aprobarbital). The assay was carried out on a Sphereclone hexylsilane column (150 × 2.0 mm ID 3 μm) (Torrance, CA, USA) with a gradient mobile phase consisting of ACN and 20 mM ammonium acetate buffer (pH adjusted to 4.5 using glacial acetic acid) at ambient temperature. The total run time between injections was 18 minutes. The method was validated over the range of 1.1–540 ng/mL for 3TC, 12.5–6228 ng/mL for d4T and 1.0–519 ng/mL for EFV. The LODs for 3TC, d4T and EFV were 0.5, 6.2 and 0.5 ng/mL, respectively. The LOQs for 3TC, d4T and EFV were 1.1, 12.5 and 1.0 ng/mL, respectively.

Huang et al. (76) reported a LC-MS-MS spectrometric method using ESI positive mode for the simultaneous determination of DDI and d4T in human plasma, bronchoalveolar lavage fluid, alveolar cells, peripheral blood mononuclear cells, seminal plasma, cerebrospinal fluid, and tonsil tissue using a SPE. Plasma, alveolar cells, peripheral blood mononuclear cells and cerebrospinal fluid were achieved on a BDS C18 column (4.6 × 150 mm, 5 μm) (Milford, MA, USA) with isocratic HPLC method [mobile phase consisting of MeOH–water (16:84, v/v), 0.05% trifluoracetic acid, and 1mM ammonium formate] at a flow rate of 0.8 mL/min, while bronchoalveolar lavage fluid supernatant, semen, and tonsil tissue utilized a gradient elution (Phase A: MeOH–water (16:84, v/v) containing 0.05% TFA, 1mM ammonium formate and Phase B: MeOH–water (80:20, v/v) containing 0.05% TFA, 1 mM ammonium formate).The flow rate was 0.8 mL/min. The LOQs for DDI and d4T were 2.0 ng/mL in plasma, 0.5 ng/mL in cerebrospinal fluid, 0.4 ng/mL in alveolar cells, peripheral blood mononuclear cells, and bronchoalveolar lavage fluid, 1.0 ng/mL in seminal plasma and 0.01 ng/mg in tonsil tissue. Retention times for DDI, d4T and IS with isocratic HPLC method were 5.0, 5.6 and 7.0 minutes, respectively. Retention times for DDI, d4T and IS with gradient elution were 1.7, 2.1 and 2.6 minutes, respectively

A SPE method on a LC-MS by ESI-MS in the positive ion mode for the determination of concentrations of TNF and emtricitabine in human plasma was reported by D'Avolio et al. (77). Chromatographic separation was performed on an Atlantis (4.6 × 150 mm) RP analytical column using mobile phase consisting of ACN and water with formic acid 0.05%. Calibration curve ranged from 15.6–4000 ng/mL for TNF and 11.7–3000 ng/mL for emtricitabine. The LOQs were 15.6 ng/mL for TNF and 11.7 ng/mL for emtricitabine; LODs were 2 ng/mL for TNF and 1.5 ng/mL for emtricitabine.

Estrela et al. (78) developed a HPLC method with ESI-MS/MS detection for the simultaneous determination of LPV and RTV in human blood, semen and saliva samples using a liquid-liquid extraction. Chromatographic separation was achieved on a LiChrocart1 (125 × 4 mm ID LiChrospher1 100 RP-18 endcapped, 5 mm) analytical column, coupled to a LiChrospher1 100 RP-18, 4 × 4 mm ID, 5mm guard column using isocratic mobile phase consisting of a mixture of 5 mM ammonium acetate-MeOH (20:80 v/v), pH 3.2, containing formic acid. The flow rate was 1 mL/min.The total run time was 4.5 minutes. The LOQ was 1 ng/mL for both analytes in all matrices. The method was linear in the range 1–2000 ng/mL for LPV and 1–200 ng/mL for RTV.

For the determination of LPV and RTV in human plasma, plasma ultrafiltrate, and peripheral blood mononuclear cells Ehrhardt et al. (79) developed a LC/MS/MS method with ESI detection using a liquid/liquid-extraction. Isocratic chromatographic separation was achieved on a Jupiter Proteo C12 column (100 × 2 mm ID, 4 μm, Phenomenex) with integrated guard column with mobile phase consisting of 0.1% aqueous acetic acid including 20 mM ammonium acetate and ACN (45:55, v/v) using $^2H_5$-SQV as an internal standard at 40°C. The flow rate was 0.35 mL/min. LOQs for both analytes were 4.0 ng/mL in plasma, 0.2 ng/mL in ultrafiltrate, and 0.1 ng/cell pellet ($\sim$3 × 10^6 cells) in peripheral blood mononuclear cells.

Rentsch (80) reported a LC–MS with detection atmospheric pressure chemical ionization (APCÝ) method for determination

of the different proteinase inhibitors (APV, IDV, LPV, NFV, RTV, SQV) and NNRTIs (EFV, NFV) after SPE within 21 minutes. The separation was performed on a Nucleosil C18 HD (12.5 × 2 mm, 5-μm) protected with a guard column (8 × 2 mm) using a mixture of Mobile Phase A consisting of ACN containing 30% MeOH and ammonium carbonate buffer pH 9.3 (5:95, v/v) and Mobile Phase B consisting of ACN containing 30% MeOH and ammonium carbonate buffer, pH 9.3 (95:5, v/v). The flow rate of the Mobile Phase was set at 200 mL/min. The quantification limits were 1 μg/L for IDV, 10 μg/L for APV and EFV, 50 μg/L for SQV, 90 μg/L for NFV, 200 μg/L for NVP and RTV, and 250 μg/L for LPV.

An ESİ-HPLC-MS-MS method for the simultaneous quantification of EFV, emtricitabine and TNF in human plasma developed by Nirogi et al. (81) using SPE. Mass spectrometric detection was performed on an API 4000 triple quadrupole instrument. Separation was performed on a Chromolith Performance RP C18e (100 × 4.6 mm) (Kyoto, Japan) column using a gradient Mobile Phase consisting of Mobile Phase A (0.1% formic acid) and Mobile Phase B (ACN) at a flow rate of 1.5 mL/min at 25°C temperature. The lower LOQs were 2 ng/mL for emtricitabine and 20 ng/mL forEFV and TNF. The calibration curve was linear over the concentration range 2–200 ng/mL for emtricitabine and 20–2000 ng/mL for EFV and TNF. The LLOQs was 2 ng/mL for emtricitabine and 20 ng/mL for both EFV and TNF.

Volosov et al. (82) presented a LC-MS-MS method for quantification of anti-HIV drugs (APV ABC DEL, IDV, DdI, EFV, NFV, 3TC, NVP, RTV, D4T, SQV, DdC, LPV, AZT) in human plasma, with an internal standard (cimetidine) using an online extraction with ammonium acetate. Separation was performed on a Supelco LC18-DB (3.3 × 3.0 mm, 3.0 μm ID) (St. Louis, MO, USA) chromatographic column equipped with Supelco Discovery C18 (3.0 mm) guard column with an aqueous solution of ammonium acetate (15 mM) and MeOH. Flow rate was 1 mL/min. The run time was 4.5 minutes. The assay was linear over the range of 2–2000 ng/mL for d4T, DDI, DDC and AZT, and 10–10000 ng/mL for all other drugs. The LLOQs were 3 ng/mL for AZT and d4T, 1 ng/mL for EFV, ABC, NVP, 3TC, DDC and DDI, and <1 ng/mL for other drugs. Also, Ghoshal and Soldin (83) improved the LC-MS-MS system presented by Volosov et al. The procedure was modified by adding TNF to 15 other anti-HIV drugs, extending the upper limit of the calibration curves to10,000 ng/mL, and changing the MeOH standard matrix to serum.

A LC-MS method for the simultaneous determination of four HIV PIs, IDV, SQV, NFV and APV, in rat plasma and liver dialysate by a microdialysis method was presented by Gao et al. (84). MS was performed utilizing APCI at a negative mode. After extraction with ether, separation of analytes was achieved on a RP semi-micro QUICKSORB ODS column (2.1 × 150 mm ID, 5 μm size, Chemco, Osaka, Japan) at 60°C using 50% of ACN containing 1% acetic acid as mobile phase at a flow rate of 0.2 mL/min. The separation run time was 5 minutes. Retention times for IDV, SQV, NFV and APV were 1.95, 2.28, 2.29 and 3.67 minutes, respectively.

Vela et al. (85) developed an ion-pairing LC-MS-MS with API method for determination of adefovir and its phosphorylated metabolites in cellular samples. Chromatographic separation was achieved on microbore RP columns [XTERRA MS, C18, 3.5 μM, 1.0 × 100 mm, Waters; YMC J'sphere, C18, 4 μm, 1.0 × 150 mm, Waters; or a phenomenex Luna, C18(2), 3 μM, 1.0 × 100 mm column] using gradient elution at flow rate of 50 μl/min (Mobile Phase A containing 0.25 mM TBAH, 4 mM ammoniumphosphate (pH 6.0) and 6% ACN (isocratic for 5 minutes) and Mobile Phase B containing 0.25 mM TBAH, 4 mM ammonium phosphate (pH 6.0) and 20%) with a step gradient from. LLOQs for adefovir, adefovir-MP and adefovir-DP were 25, 50 and 25 fmols, respectively.

Crommentuyn et al. (86) reported an ESİ-LC/MS/MS method for the quantification of the six PIs (APV, IDV, LPV, NFV, RTV and SQV) and NFV metabolite M8 in plasma consisting of protein precipitation with a mixture of MeOH and ACN using only 100 microL of plasma. Chromatographic separation was performed on an Inertsil ODS3 column (50 × 2.0 mm ID, 5 μm) (Middleton, The Netherlands) using a gradient mobile phase consisting of MeOH and an acetate buffer (pH 5) (35:65, v/v)) with a flow rate of 0.5 mL/min. The analytical run time was 5.5 minutes. SQV-d_5 and IDV-d_6 were used as internal standards. LLQs for APV, IDV, LPV, M8, NFV, RTV, SQV were 0.108, 0.0100, 0.0984, 0.00999, 0.0493, 0.0511, and 0.0108 μg/mL, respectively.

Crommentuyn et al. (87) developed and validated an ESİ-LC–MS/MS assay for the quantification of ATV and tipranavir in human plasma with sample pre-treatment consisting of protein precipitation using a mixture of MeOH and acetronitrile. Chromatographic separation was achieved on an Inertsil ODS3 column (50 × 2.0 mm ID, particle size 5 μm) using a gradient mobile phase consisting of acetate buffer (pH 5) and MeOH (35:65, v/v) at a flow rate of 0.5 mL/min. The analytical run time was 5.5 minutes. The triple quadrupole MS operated in the positive ion-mode and multiple reaction monitoring (MRM) was used for drug quantification. The assay was linear over a concentration range of 0.05–10 μg/mL for ATV and 0.1–75 μg/mL for tipranavir. SQV-d5 was used as an internal standard. Retention times were approximately 3.3, 3.5, and 3.4 minutes for ATV, tipranavir, and the internal standard SQV-d5, respectively.

An ESI-LC/MS/MS method for the detection and qualitative identification of human microsomal metabolites of the PIs (RTV and IDV) with separation on either an HP series 1090 or 1100 HPLC equipped with an auto-sampler and diode array detector was reported by Gangl et al. (88). The assay was performed a Waters Symmetry C18 RP LC column (2.1 × 150 mm, 3.5 mm) (Milford, MA, USA) using a gradient elution mode with Mobile Phase A consisting of 10 mM ammonium acetate (adjusted to pH 4.7 with acetic acid) and Mobile Phase B consisting of ACN. Flow rate was 210 μL/min.

Chi et al. (89) presented a LC-MS-MS with an API Turbo ion spray source method for determination of the levels of five HIV PIs, NFV, IDV, RTV, SQV and APV, in human plasma. The assay was performed on a Zorbax column XDB-C8 RP column (2.1 Ã–50 mm with 5 μm particle size packing) and a Zorbax XDB-C8 (2.1 $\times$ 12.5 mm, 5 μm) guard column using mobile phase with a linear gradient of 10 mM ammonium formate buffer (pH 4.10) and ACN. The flow rate was at 400 μL/min. The calibration curve ranged from 5.0–10,000 ng/mL for each analyte. The LLOQs for NFV, IDV, RTV, SQV and APV were all 5.0 ng/mL.

Divi et al. (90) reported an assay for determination using LC-MS-MS equipped with an ESI analysis related with metabolism and pharmacokinetics of the combination AZT and 3TC in the adult Erythrocebus patas monkey. Chromatographic separation was performed on a Polaris C18 analytical column (150 $\times$ 2.0 mm, 3 μm) (Milford, MA, USA) equipped with a C18 security guard (4 mm $\times$ 2.0 μm,) with gradient mobile phase consisting of 0.1% formic acid and ACN at ambient temperature. The flow rate was 200 μL/min. Total run time was 15 minutes. LOQs for 3TC, 3MT, AZT and AZT-G were 0.005, 0.002, 0.01, and 0.003 μg/mL, LODs for 3TC, 3MT, AZT and AZT-G were 0.01, 0.003, 0.04, and 0.01 μg/mL respectively.

A chromatographic method to detect and quantify NVP, DEL, APV, IDV, RTV, LPV, EFV, SQV, NFV and M8 (NFV metabolite) in human plasma and in peripheral blood mononuclear cells using either LC–UV or LC–MS/MS was reported by Pelerin et al. (91). A solid–liquid extraction was carried out on 500 μL of plasma as pre-treatment. Chromatographic LC/UV (215 nm) system and LC–MS/MS system were performed on a column was a Nova Pak C1860 A$^\circ$ Waters (4 μm particle size, 150 $\times$ 3.9 mm) with a C18 guard column, cartridge modulocart 10 $\times$ 2 mm with mobile phase consisting of (A) ACN-25 mM ammonium acetate buffer (0.1% acetic acid) (10/90, v/v), (B) ACN-25 mM ammonium acetate buffer (0.1% acetic acid) (90/10, v/v), and (C) MeOH. The LC–MS/MS system consisted of an HPLC system 1100 connected to an API 3000 tandem mass-spectrometer equipped with ESI. The flow rate was 1 mL/min. The run time was 20 minutes. The LLOQ was 50 ng/mL for all compounds except IDV (100 ng/mL).

Frerichs et al. (92) presented a method for the analysis of six PIs and one metabolite (APV, RTV, SQV, LPV, IDV, NFV, and an active metabolite of NFV, M8) using ESI-LC-MS-MS. Analytes were separated on a Waters Symmetry C18 column (30 $\times$ 2.1 mm ID, 3.5-μm) with a Waters Symmetry Shield Guard column (2.1 $\times$ 10 mm). The mobile phase consisted of ACN and 5 mM acetate buffer, pH 3.25. The flow rate was 350 mL/min. The lowest LODs for APV NFV, M8, RTV, SQV, LPV were 380, 330, 550, 650, 780, 750 pg/mL, respectively. The lowest LOD for IDV was 1.50 ng/mL. The lowest LOQ was 8.19 ng/mL for M8, 51.2 ng/mL for RTV, 16.3 ng/mL for other drugs.

Van Den Broek (93) presented a method for the quantification of two peptide HIV-1 fusion inhibitors (enfuvirtide and tifuvirtide) and one metabolite of enfuvirtide (M-20) in human plasma with ESI-LC-MS/MS using SPE on vinyl-copolymer cartridges. Chromatographic separation was performed on a Waters Symmetry 300 C_{18} column (50 $\times$ 2.1 mm ID, particle size 3.5 μm) using a water-ACN gradient containing 0.25% (v/v) formic acid. Deuterated enfuvirtide and tifuvirtide were used as internal standards. The triple quadrupole mass spectrometer was operated in the positive ion-mode and multiple reaction monitoring (MRM) was used for peak detection. Total run time was 11.0 minutes. The flow rate was 0.2 mL/min (0.4 mL/min. between 5 and 9 minutes) The assay was linear over a concentration range of 20–10,000 ng/mL for enfuvirtide and tifuvirtide and of 20–2000 ng/mL for M-20.

Wıllıams et al. (94) presented a gradient LC-ESI/MS method to measure serum concentrations in maternal and fetal mice of the nucleoside analogs AZT, 3TC and several metabolites using a SPE. Chromatographic separation was performed on a Polaris C18 analytical column (150 $\times$ 2.0 mm $\times$ 3 μm particles, Waters), equipped with a C18 security guard (4 mm $\times$ 2.0 μm, Phenomenex) with mobile phase a mixture of 0.1% formic acid and ACN at ambient temperature. Flow rate was 200 μL/min.

Wang et al. (95) reported a APCI-LC/MS/MS method using for the quantification of LPV and RTV in human plasma using a liquid-liquid extraction with a mixture of hexane: ethyl acetate (1:1, v/v). Chromatographic separation was achieved on a Waters Symmetry C_{18} column (150 $\times$ 3.9 mm, particle size 5 μm) with RP isocratic using mobile phase consisting of ACN and 2 mM ammonium acetate aqueous solution containing 0.01% formic acid (70:30, v/v) at room temperature. Flow rate was 1.0 mL/min. The run time was 4 minutes. The mass spectrometer was operated in a positive ion MRM mode. The method was validated over the concentration ranges of 19–5300 ng/mLfor LPV and 11–3100 ng/mLfor RTV. The LLOQs were 19 ng/mL for LPV, and 11 ng/mL for RTV, using 0.10 mL normal human plasma.

Koal et al. (96) reported a direct and fast LC-MS method for determination of anti-retroviral drugs (APV, NFV, IDV, LPV, SQV, RTV, ATV, NVP and EFV) in human plasma using automated online SPE by the Symbiosis Pharma system (Spark Holland) for XLC coupled to an API 2000 for MS/MS analysis. Reserpine was used as an internal standard. The LOD was 2–70 ng/mL) and LLOQ was 78–156 ng/mL.

Jung et al. (97) presented a sensitive ESI-LC-MS/MS method for the simultaneous determination of 17 anti-retroviral drugs (EFV, NVP, AZT, d4T, ABC, 3TC, DDC, DDI, IDV, NFV, RTV, ATV, TNF, SQV, LPV, APV and DEL) in human plasma. After a liquid-liquid extraction and protein precipitation, separation was performed on an Aquasil C18 (50 $\times$ 2.1 mm column , 5 μm particle size) (San Jose, CA, USA) with a gradient mobile phase consisting of 0.05% formic acid in either water (v/v, Mobile Phase A) or MeOH (v/v, Mobile Phase B) at a flow rate of 0.5 mL/min. Cimetidine was used as an internal standard. The LLOQ were 1 ng/mL for NFV, IDV and ABC, 10 ng/mL for DDI and D4T, and 5 ng/mL for the rest of the analytes.

D'Avolio et al.(98) developed a ESI-HPLC/MS for the quantification of plasma concentration of the darunavir and other 11

anti-retroviral agents (RTV, APV, ATV, LPV, SQV, IDV, NFV and its metabolite M-8, NVP, EFV and tipranavir), using an internal standard (quinoxaline). After a protein precipitation extraction procedure, the chromatographic separation of drugs was carried out on Atlantis dC18 column (Waters, 150 × 2.1 mm ID, 3μ) protected by a Security Guard with C18 (4.0 × 3.0 mm ID) pre-column using gradient mobile phase consisting of Buffer A (HPLC grade water + 0.05% formic acid) and Buffer B (HPLC grade ACN + 0.05% formic acid) at 35°C. The run time was 25 minutes.

Reynolds et al. (99) developed and validated a API-HPLC-MS/MS method to determine the concentrations of four HIV PIs (IDV, RTV, SQV and NFV) in human plasma with internal standard (IS: Ro 31–9564). After extraction with acetronitrile by protein precipitation, separation of compounds was achieved on a Hypurity Elite 5C18 Column (250 × 4.6 mm, 5μm, Hypersil) using a mixture of 20 mmol/L ammonium formate buffer-acetronitrile (30:70; v/v) as mobile phase at 1.2 mL/min. Run time was 10 minutes. The LLODs were between 100–200 pg/mL.

Rezk et al. (100) developed a HPLC-MS/MS with a TurboSpray ion source for the simultaneous quantification of 3TC, d4T, ZDV, NVP NVP, NFV, RTV, and LPV in breast milk using cimetidine as an internal standard. After a SPE, separation was performed on an Aquasil C18 (50 × 2.1 mm column, 5-μm particle size) using Mobile Phase A 100% 10 mM ammonium acetate and Mobile Phase B 95% MeOH as mobile phase for negative ion mode at flow rate of 0.75 mL/min and Mobile Phase A 100% of 0.1% formic acid in water and Mobile Phase B 7% of 0.1% formic acid in MeOH as mobile phase for positive ion mode at flow rate of 0.5 mL/min. All drugs were linear over 10–10,000 ng/mL.

Gomes et al. (101) reported a ESİ-LC-MS/MS using an API 5000 instrument method for simultaneous quantification of TNF (TEN) and emtricitabine (EMT) in human plasma using SPE. 3TC was used as the internal standard. The linearity was validated with a linear range of 10–600 ng/mL for TEN and 25–2500 ng/mL for EMT. Total run time was 2 minutes. Chromatographic separation was performed on a Shimadzu HPLC with a Chromolith Speed Rod RP18 column (50 × 4.6 mm, Merck Mumbai, India) using a mobile phase consisting of ACN and ammonium acetate (pH 3.0, 40 mM) (20:80, v/v) at flow rate of 0.7 mL/min. The LOQs for TEN and EMT were 10 ng/mL and 25 ng/mL.

Heine et al. (102) reported a ESİ-LC-MS/MS method for the quantification of APV, ATV, EFV, IDV, LPV, NFV, the active NFV metabolite M8, NVP and RTV, SQV, darunavir, and tipranavir using sample pre-treatment consisting of a protein precipitation with a mixture of MeOH and ACN. Chromatographic separation was performed on a RP C18 column (150 × 2.0 mm, particle size 5 μm) with a gradient mobile phase using an acetate buffer (pH 5) and MeOH, at a flow rate of 0.25 mL/minutes. The analytical run time was only 10 minutes. The triple quadrupole mass spectrometer was operated in the positive ion mode and MRM was used for drug quantification. The method was validated over a range of 0.1–20 μg/mL for APV, ATV, EFV, IDV, LPV, NFV, the active NFV metabolite M8, NVP and RTV, a range of 0.05–10 μg/mL for SQV and darunavir and a range of 0.5–100 μg/mL for tipranavir, based on observed concentration ranges in patients treated with these drugs. D5-squinavir, D6-IDV, 13C6-EFV and dibenzepine were used as internal standards.

Heine et al. (103) reported a ESİ-LC-MS/MS method for the determination of PIs (ATV, darunavir, LPV and RTV) and NNRTIs (EFV and NVP). The analytes were extracted using a mixture of ACN, MeOH and 0.2 M zinc sulphate in water (1:1:2, v/v/v) containing the internal standards dibenzepine, 13C6-EFV and D5-SQV. Chromatographic separation was performed on a Phenomenex Gemini RP C18 column (150 × 2.0 mm, 5 μm) with a Phenomenex Security guard Gemini C18 pre-column (4.0 × 2.0 mm, 5 μm) using gradient mobile phase consisting of acetate buffer (pH 5) and MeOH. The flow rate was 0.25 mL/min. The analytical run time was only 10 minutes. The assay was linear over the concentration ranges of 0.1–20 mg/L for ATV, LPV, NVP and EFV and 0.05–10 mg/L for darunavir and RTV.

Le Saux (104) presented a ESİ-HPLC/MS/MS method in the positive mode using selected reaction monitoring in human plasma for the simultaneous seven nucleoside/nucleotide reverse transcriptase inhibitors (ABC, DDI, emtricitabine, 3TC, d4T, TNF, and AZT) with protein precipitation using 6-beta-hydroxy-theophyline as an internal standard. The chromatographic separation was performed with an Atlantis T3 column (100 × 2.1 mm, 3 μm particle diameter, Waters) C18 column using a gradient mobile phase consisting of a mixture of water and ACN, both containing 0.05% formic acid at 10°C.

Dickinson et al. (105) reported a ESİ-HPLC-MS/MS method for simultaneous quantification of PIs, APV, ATV, IDV, LPV, NFV, RTV and SQV, in plasma using protein precipitation with ACN followed by addition of ammonium formate buffer. APV, ATV, IDV, LPV, NFV, RTV and SQV were eluted using a gradient mobile phase consisting of ACN:ammonium formate buffer [(20 mM); 50/50 and 70/30, v/v] at a flow rate of 0.4 mL/min at a temperature of 26°C. The chromatographic separation was a HyPURITY C18 column (100 × 2.1 mm, 5 μm). LLQ, upper limit of quantitation (ULQ) and LOD were 56, 5136 and 29 ng/mL (APV); 47, 6239 and 4.6 ng/mL (ATV); 102, 9481 and 2.4 ng/mL (IDV); 95, 15,584 and 3.9 ng/mL (LPV); 62, 4670 and 2.4 ng/mL (NFV); 25, 4941 and 1.2 ng/mL (RTV); and 71, 4958 and 3.5 ng/mL (SQV), respectively.

CONCLUSIONS

The review has shown that a variety of HPLC methods for determination of antiretroviral drugs have been developed and described with HPLC-UV and HPLC-MS or HPLC-MS-MS detection systems. HPLC methods are the most common group for determination of anti-retroviral drugs. HPLC establish high separation potential, selectivity and sensitivity. Coupling HPLC with single MS or MS-MS which is very sensitive, selective and specific gives the most reliable results for determination

of anti-retroviral drugs. Despite being a versatile, sensitive and reproducible technique, HPLC is tedious and time consuming because it requires previous purification of the samples and the use of several columns.

In this review, I focused on HPLC-UV and HPLC–MS methods for the simultaneous quantitation of anti-retroviral drugs in biological samples. Most of the methods have used the RP chromatography and in sample preparations LLE, SPE, protein precipitation are preferred. RP (e.g.,C18 and C8) analytical columns are most commonly used in the separation of anti-retroviral drugs. In most of the reported methods MS was conducted in ESI mode. Typical quantification limits are in ng/mL and μg/mL range.

ABBREVIATIONS

3TC	Lamivudine
ACN	Acetonitrile
AIDS	Acquired immunodeficiency syndrome
APCI	Atmospheric pressure chemical ionization
APV	amprenavir
API	Atmospheric pressure ionization
ATZ	Atazanavir
d4T	Stavudine
DDC	Zalcitadine
DDI	Didanosine
DEL	Delavirdine
EFV	Efavirenz
ESI	Electrospray ionization
HAART	Highly active anti-retroviral therapy
HIV-1	Human immunodeficiency virus type 1
HPLC	High-performance liquid chromatography
I.S.	Internal standard
LC	Liquid chromatography
LLE	Liquid–liquid extraction
LOD	Limit of detection
LLOQ	Lower limit of quantitation;
MeOH	methanol
IDV	Indinavir
MRM	Multiple reaction monitoring
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
LPV	Lopinavir
NFV	Nelfinavir
NRTIs	Nucleoside reverse transcriptase inhibitors
NNRTIs	Non-nucleoside analog reverse transcriptase inhibitors
PIs	Protease inhibitors
NVP	Nevirapine
RTV	Ritonavir
SPE	Solid-phase extraction
SQV	Saquinavir
TDM	Therapeutic drug monitoring
TNF	Tenofovir
AZT	Zidovudine
UV	Ultraviolet

REFERENCES

1. R. A. Weiss, How does HIV cause AIDS? *Science* 260 (1993):1273–1279.
2. T. N. Kakuda, L. M. Page, P. L. Anderson, K. Henry, T. W. Schacker, and F. S. Rhame, Pharmacological basis for concentration-controlled therapy with zidovudine, lamivudine and indinavir. *Antimicrobial Agents and Chemotherapy* 45 (2001):236–42.
3. http://www.aidsinfo.nih.gov/guidelines/About the HIV Treatment Guidelines/pdf, accessed: October 26, 2008.
4. D. J. Back, S. H. Khoo, S. E. Gibbons, and C. Merry, The role of therapeutic drug monitoring in treatment of HIV infection. *British Journal of Clinical Pharmacology* 51 (2001):301–308.
5. A. S. Pereira and R. R. Tidwell, Separation methods for nucleoside analogues used for HIV-1 infection. *Journal of Chromatography B* 764 (2001):327–347.
6. A. Onal, Analysis of anti-retroviral drugs in biological matrices for therapeutic drug monitoring.*Journal of Food and Drug Analysis* 14 (2006):99–119.
7. J. Lai, J. Wang, and Z. Cai, Nucleoside reverse transcriptase inhibitors and their phosphorylated metabolites in human immunodeficiency virus-infected human matrices. *Journal of Chromatography B* 868 (2008):1–12.
8. C. Saka, Review of analytical methods for identification and determination of triptans. *Critical Reviews in Analytical Chemistry* 39 (2009):32–42.
9. F. Esther and C. Tibor, Relationship between the hydrophobicity and specific hydrophobic surface area of pesticides determined by high-performance liquid chromatography compared with reversed-phase thin-layer chromatography. *Journal of Chromatography A* 771 (1997):105–109.
10. B. Dogan-Topal, S. A. Ozkan, and B. Uslu, Simultaneous determination of abacavir, efavirenz and valganciclovir in human serum samples by isocratic HPLC-DAD detection. *Chromatographia* 66 (2007):25–30.
11. S. B. Kappelhoff, H. Rosing, A. D. R. Huitema, and J. H. Beijnen, Simple and rapid method for the simultaneous determination of the non-NRTIs efavirenz and nevirapine in human plasma using liquid chromatography. *Journal of Chromatography B* 792 (2003):353–362.
12. M. Bouley, C. Brıere, C. Padoın, O. Petıtjean, and M. Tod, 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. *Therapeutic Drug Monitoring* 23 (2001):56–60.
13. J. M. Poirier, P. Robidou, and P. Jaillon, 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. *Therapeutic Drug Monitoring* 24 (2002):302–311.
14. M. Boffito, J. Tija, H. E. Reynolds, P. G. Hoggard, S. Bonora, G. Di Perri, and D. J. Back, Simultaneous determination of

rifampicin and efavirenz in plasma.*Therapeutic Drug Monitoring* 24 (2002):670–674.
15. P. D. Walson, S. Cox, I. Utkin, N. Gerber, L. Crim, M. Brady, and K. Koranyi, Clinical use of a simultaneous HPLC assay for indinavir, saquinavir, ritonavir and nelfinavir in children and adults. *Therapeutic Drug Monitoring* 25 (2003):650–656.
16. J. A. H. Droste, C. P. W. G. M., Verweıj-Van Wıssen, and D. M. Burger, 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. *Therapeutic Drug Monitoring* 25 (2003):393–399.
17. K. Tıtıer, F. Lagrange, F. Pehourcq, L. Edno-Mcheık, N. Moore, and M. Molımard, 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. *Therapeutic Drug Monitoring* 24 (2002):417–424.
18. O. Tribut, C. Arvieux, C. Michelet, J. M. Chapplain, H. Allain, and D. Bentué-Ferrer, 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. *Therapeutic Drug Monitoring* 24 (2002):554–562.
19. O. Tribut, M. C. Verdier, C. Arvieux, H. Allain, C. Michelet, and D. Bentue-Ferrer, Simultaneous quantitative assay of atazanavir and 6 other HIV protease inhibitors by isocratic reversed-phase liquid chromatography in human plasma. *Therapeutic Drug Monitoring* 27 (2005):265–269.
20. M. Takahasıı, M. Yoshıda, T. Okı, N. Okumura, T. Suzukı, and T. Kaneda. Conventional HPLC method used for simultaneous determination of the seven HIV protease inhibitors and nonnucleoside reverse transcription inhibitor efavirenz in human plasma. *Biological & Pharmaceutical Bulletin* 28 (2005):1286–1290.
21. M. Schuman, S. Schneider, C. Omes, R. Wennig, L. Fundira, J.-C. Tayari, and V. Arendt, HPLC analysis of generic anti-retroviral drugs purchased in Rwanda. *Bulletin de la Société des Sciences Médicales du Grand-Duché de Luxembourg* 3 (2005):317–325.
22. Y. Alnouti, C. A. White, and M. G. Bartlett, Simultaneous determination of zidovudine and lamivudine from rat plasma, amniotic fluid and tissues by HPLC. *Bıomedıcal Chromatography* 18 (2004):641–647.
23. J. Donnerer, M. Kronawetterc, A. Kapperc, I. Haasb, and H. H. Kesslerb, Therapeutic drug monitoring of the HIV/AIDS drugs abacavir, zidovudine, efavirenz, nevirapine, indinavir, lopinavir, and nelfinavir. *Pharmacology* 69 (2003):197–204.
24. V. Bezy, P. Morin, P. Couerbe, G. Leleu, and L. Agrofoglio, Simultaneous analysis of several anti-retroviral nucleosides in rat-plasma by high-performance liquid chromatography with UV using acetic acid/hydroxylamine buffer test of this new volatile medium-pH for HPLC–ESI-MS/MS. *Journal of Chromatography B* 821 (2005) 132–143.
25. N. L. Rezk, R. R. Tidwell, and A. D. M. Kashuba, 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 UV absorbance detection. *Journal of Chromatography B* 774 (2002):79–88.
26. N. L. Rezk, R. R. Tidwell, and A. D. M. Kashuba, Simultaneous determination of six HIV nucleoside analogue reverse transcriptase inhibitors and nevirapine by liquid chromatography with ultraviolet absorbance detection. *Journal of Chromatography B* 791 (2003):137–147.
27. N. L. Rezk, R. R. Tidwell, and A. D. M. Kashuba, High-performance liquid chromatography assay for the quantification of HIV protease inhibitors and non-nucleoside reverse transcriptase inhibitors in human plasma. *Journal of Chromatography B* 805 (2004):241–247.
28. N. L. Rezk, R. D. Crutchley, and A. D. M. Kashuba, Simultaneous quantification of emtricitabine and tenofovir in human plasma using high-performance liquid chromatography after solid phase extraction. *Journal of Chromatography B* 822 (2005):201–208.
29. N. L. Rezk, R. D. Crutchley, R. F. Yeh, and A. D. M. Kashuba, Full validation of an analytical method for the HIV-protease inhibitor atazanavir in combination with 8 other anti-retroviral agents and its applicability to therapeutic drug monitoring. *Therapeutic Drug Monitoring* 28 (2006):517–525.
30. N. L. Rezk, M. F. Abdel-Megeed, and A. D. M. Kashuba, Development of a highly efficient extraction technique and specific multiplex assay for measuring anti-retroviral drug concentrations in breast milk. *Therapeutic Drug Monitoring* Volume 29 (2007):429–436.
31. H. Rebiere, B. Mazel, C. Civade, and P. A. Bonnet, Determination of 19 anti-retroviral agents in pharmaceuticals or suspected products with two methods using high-performance liquid chromatography. *Journal of Chromatography B* 850 (2007):376–383.
32. S. O. Choi, N. L. Rezk, and A. D. Kashuba, High-performance liquid chromatography assay for the determination of the HIV-protease inhibitor tipranavir in human plasma in combination with nine other anti-retroviral medications. *Journal of Pharmaceutical and Biomedical Analysis* 43 (2007):1562–1569.
33. S. Notari, A. Bocedi, G. Ippolito, P. Narciso, L. P. Pucillo, G. Tossini, R. P. Donnorso, F. Gasparrini, and P. Ascenzi, Simultaneous determination of 16 anti-HIV drugs in human plasma by high-performance liquid chromatography. *Journal of Chromatography B* 831 (2006):258–266.
34. U. S. Justesen, C. Pedersen, and N. A. Klitgaard, 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 *Journal of Chromatography B* 783 (2003):491–500.
35. R Verbesselt, E. Van Wijngaerden, and J. De Hoon, Simultaneous determination of 8 HIV protease inhibitors in human plasma by isocratic high-performance liquid chromatography with combined use of UV and fluorescence detection: Amprenavir, indinavir, atazanavir, ritonavir, lopinavir, saquinavir, nelfinavir. *Journal of Chromatography B* 845 (2006):51–60.
36. E. Marchei, L. Valvo, R. Pacıfıcı, M. Pellegrını, G. Tossını, and P. Zuccaro, Simultaneous determination of zidovudine and nevirapine in human plasma by RP-LC. *Journal of Pharmaceutical and Biomedical Analysis* 29 (2002):1081–1088.
37. P. Leibenguth, C. Le Guellec, J. M. Besnier, F. Bastides, M. Mace, M. L. Gaudet, E. Autret-Leca, and G. Paintaud, Therapeutic drug monitoring of HIV protease inhibitors using high-performance liquid chromatography with ultraviolet or photodiode array detection. *Therapeutic Drug Monitoring* 23 (2001):679–688.

38. J. Faux, N. Venisse, G. Le Moal, A. Dupuis, and S. Bouquet, Simultaneous determination of six HIV protease inhibitors, one metabolite, and two non-nucleoside reverse transcriptase inhibitors in human plasma by isocratic reversed-phase liquid chromatography after solid-phase extraction. *Chromatographia* 58 (2003):421–426.
39. H. Yamada, H. Kotakı, N. Tetsuya, and I. Aikichi, Simultaneous determination of the HIV protease inhibitors indinavir, amprenavir, saquinavir, ritonavir and nelfinavir in human plasma by high-performance liquid chromatography. *Journal of Chromatography B* 755 (2001):85–89.
40. M. L. Turner, K. Reed-Walker, J. R. King, and E. P. Acosta, Simultaneous determination of nine anti-retroviral compounds in human plasma using liquid chromatography. *Journal of Chromatography B* 784 (2003):331–341.
41. M. Sarasa-Nacenta, Y. Lopez-Pua, J. Mallolas, L. J. Blanco, M. J. Gatell, and X. Carne, Simultaneous determination of the HIV-protease inhibitors indinavir, amprenavir, ritonavir, saquinavir and NFV in human plasma by reversed-phase high-performance liquid chromatography. *Journal of chromatography B* 757 (2001):325–332.
42. R. D. Tarinas, G. Tápanes, and J. P. Ferrer, Validation of high-performance liquid chromatography methods for determination of zidovudine, stavudine, lamivudine and indinavir in human plasma. *Farmacia Hospitalaria* 31 (2007):243–247.
43. V. Albert, P. Modamio, C. F. Lastra, and E. L. Mariño, Determination of saquinavir and ritonavir in human plasma by reversed-phase high-performance liquid chromatography and the analytical error function. *Journal of Pharmaceutical and Biomedical Analysis* 36 (2004):835–840.
44. S.-M. Hsıeh, H.-Y. Yu, and S.-C. Chang, Simultaneous determination of indinavir, ritonavir and saquinavir in plasma by high-performance liquid chromatography. *Journal of the Formosan Medical Association* 103 (2004):191–195.
45. D. Kuschak, S. Mauss, G. Schmutz, and B. Gantke, Simultaneous determination of the new HIV protease inhibitor lopinavir (ABT 378) and of indinavir(1), amprenavir, saquinavir, ritonavir (ABT 538)(2) and nelfinavir(3) in human plasma by gradient HPLC. *Clinical Laboratory* 47 (2001):471–478.
46. Y. Hırabayashı, K. Tsuchıya, S. Kımura, and S. Oka, Simultaneous determination of six HIV protease inhibitors (amprenavir, indinavir, lopinavir, nelfinavir, ritonavir and saquinavir), the active metabolite of nelfinavir (M8) and non-nucleoside reverse transcriptase inhibitor (efavirenz) in human plasma by high-performance liquid chromatography. *Biomedical Chromatography* 20 (2006):28–36.
47. M. Xu, C. A. White, and M. G. Bartlett, Simultaneous determination of zalcitabine and stavudine in maternal plasma, amniotic fluid, placental, and fetal tissues using reversed phase on silica liquid chromatography. *Journal of Liquid Chromatography & Related Technologies* 31 (2008):482–496.
48. V. Bezy, P. Morin, P. Couerbe, G. Leleu, and L. A. Agrofoglio, Determınatıon of several nrtıs in plasma by HPLC usıng a polar embeddıng reversed statıonary phase. *Antiviral Therapy* 8 (2003):423–424.
49. C. P. W. G. M. Verweıj-Van Wıssen, R. E. Aarnoutse, and D. M. Burger, 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. *Journal of Chromatography B* 816 (2005):121–129.
50. P. Djurdjevıc, A. Laban, S. Markovıc, and M. Jelıkıc-Stankov, Chemometric optimization of a RP-HPLC method for the simultaneous analysis of abacavir, lamivudine, and zidovudine in tablets. *Analytical Letters* 37 (2004):2649–2667.
51. A. Savaser, S. Goraler, A. Tasöz, B. Uslu, H. Lıngeman, and S. A. Özkan, Determination of abacavir, lamivudine and zidovudine in pharmaceutical tablets, human serum and in drug dissolution studies by HPLC. *Chromatographia* 65 (2007):259–265.
52. R. L. Summer, C. A. Whıte, and M. G. Bartlett, Simultaneous determination of abacavir and zidovudine from rat tissues using HPLC with ultraviolet detection. *Journal of Chromatography B* 850 (2007):45–52.
53. S. Anbazhagan, N. Indumathy, P. Shanmugapandıyan, and K. S. Seshaıah, Simultaneous quantification of stavudine, lamivudine and nevirapine by UV spectroscopy, reverse phase HPLC and HPTLC in tablets. *Journal of Pharmaceutical and Biomedical Analysis* 39 (2005):801–804.
54. M. Sarkar, S. Khandavıllı, and R. Panchagnula, Development and validation of RP-HPLC and ultraviolet spectrophotometric methods of analysis for the quantitative estimation of anti-retroviral drugs in pharmaceutical dosage forms. *Journal of Chromatography B* 830 (2005):349–354.
55. C. Marzolini, A. Béguin, A. Telenti, A. Schreyer, T. Buclin, J. Biollaz, and L. A. Decosterd, Determination of lopinavir and nevirapine by high-performance liquid chromatography after solid-phase extraction: Application for the assessment of their transplacental passage at delivery. *Journal of Chromatography B* 774 (2002):127–140.
56. N. Kapoor, S. Khandavilli, and R. Panchagnula. Simultaneous determination of lamivudine, stavudine and nevirapine in antiretroviral fixed dose combinations by high performance liquid chromatography. *Analytica Chimica Acta* 570 (2006):41–45.
57. N. Pai and A. D. Desai, Simultaneous reverse phase HPLC estimation of some anti-retroviral drugs from tablets. *Ýndian Journal of Pharmaceutical Sciences* 69 (2007):118–120.
58. A. D'Avolio, L. Baietto, M. Siccardi, M. Sciandra, M. Simiele, V. Oddone, S. Bonora, and G. D. Peri, An HPLC-PDA method for the simultaneous quantification of the HIV integrase inhibitor raltegravir, the new nonnucleoside reverse transcriptase inhibitor etravirine, and 11 other anti-retroviral agents in the plasma of HIV-infected patients. *Therapeutic Drug Monitoring* 30 (2008):662–669.
59. Y. Usamı, T. Ok, M. Naka, M. Sagısaka, and T. Kaneda, A simple HPLC method for simultaneous determination of lopinavir, ritonavir and efavirenz. *Chemical and Pharmaceutical Bulletin* 51 (2003):715–718.
60. B. Dogan-Topal, S. A. Ozkan, and B. Uslu, Simultaneous determination of abacavir, efavirenz and valganciclovir in human serum samples by Isocratic HPLC-DAD detection. *Chromatographia* 66 (2007):25–30.
61. A. Checa, R. Oliver, S. Hernández-Cassou, and J. Saurina, Reversed-phase liquid chromatographic method with spectrophotometric detection for the determination of anti-retroviral drugs. *Analytica Chimica Acta* 616 (2008):85–94.

62. K. Keil, V. A. Frerichs, R. DiFrancesco, and G. Morse, Reverse phase high-performance liquid chromatography method for the analysis of amprenavir, efavirenz, indinavir, lopinavir, nelfinavir and its active metabolite (M8), ritonavir, and SQV in heparinized human plasma. *Therapeutic Drug Monitoring* 25 (2003):340–346.
63. B. Uslu and S. A. Özkan, Determination of lamivudine and zidovudine in binary mixtures using first derivative spectrophotometric, first derivative of the ratio-spectra and high-performance liquid chromatography–UV methods. *Analytica Chimica Acta* 466 (2002):175–185.
64. G. Ramachandran, A. K. Hemanthkumar, V. Kumaraswami, and S. Swaminathan, A simple and rapid liquid chromatography method for simultaneous determination of zidovudine and nevirapine in plasma. *Journal of Chromatography B* 843 (2006):339–344.
65. V. A. Simon, M. D. Thiam, and L. C. Lipford, Determination of serum levels of thirteen human immunodeficiency virus-suppressing drugs by high-performance liquid chromatography. *Journal of Chromatography A* 913 (2001):447–453.
66. D. R. Weller, R. C. Brundage, H. H. Balfour, and H. E. Vezina, An isocratic liquid chromatography method for determining HIV non-nucleoside reverse transcriptase inhibitor and protease inhibitor concentrations in human plasma. *Journal of Chromatography B* 848 (2007):369–373.
67. W. V. Caufield and J. T. Stewart, Determınatıon of zidovudine and levofloxacin in human plasma by reversed phase HPLC and solıd phase extractıon. *Journal of Liquid Chromatography & Related Technologies* 25 (2002):1791–1805.
68. S. D. Brown, C. A. White, and M. G. Bartlett, HPLC determination of acyclovir and zidovudine in maternal plasma, amniotic fluid, fetal, and placental tissues using ultra-violet detection. *Journal of Lıquıd Chromatography & Related Technologıes* 25 (2002):2857–2871.
69. J. Ray, E. Pang, and D. Carey, Simultaneous determination of indinavir, ritonavir and lopinavir (ABT 378) in human plasma by high-performance liquid chromatography. *Journal of Chromatography B* 775 (2002):225–230.
70. E. M. Donato, C. L. Dıas, R. C. Rossı, R. S. Valente, P. E. Froehlıch, and A. M. Bergold, LC method for studies on the stability of lopinavir and ritonavir in soft gelatin capsules. *Chromatographia* 63 (2006):437–443.
71. S. Colombo, A. Beguin, A. Telenti, J. Biollaz, T. Buclin, B. Rochat, and L. A. Decosterd, Intracellular measurements of anti-HIV drugs indinavir, amprenavir, saquinavir, ritonavir, nelfinavir, LPV, atazanavir, efavirenz and nevirapine in peripheral blood mononuclear cells by liquid chromatography coupled to tandem mass spectrometry. *Journal of Chromatography B* 819 (2005):259–276.
72. N. Mesplet, Y. Saito, P. Morin, and A. A. Luigi, HPLC and mass spectrometry analysis of the enzymatic hydrolysis of anti-HIV pronucleotide diastereomers. *Bioorganic Chemistry* 31 (2003):237–247.
73. Y. Alnouti, S. R. Lewis, C. A. White, and M. G. Bartlett, Simultaneous determination of zidovudine and lamivudine from rat tissues by liquid chromatography/tandem mass spectrometry. *Rapid Communications in Mass Spectrometry* 19 (2005):503–508.
74. P. Vıllanı, M. Feroggıo, L. Gıanellı, A. Bartolı, M. Montagna, R. Maseratı, and M. B. Regazzı, Anti-retrovirals: Simultaneous determination of five protease inhibitors and three nonnucleoside transcriptase inhibitors in human plasma by a rapid high-performance liquid chromatography-mass spectrometry assay. *Therapeutic Drug Monitoring* 23 (2001):456–499.
75. B. Fan, M. G. Bartlett, and J. T. Stewart, Determination of lamivudine/stavudine/efavirenz in human serum using liquid chromatography/electrospray tandem mass spectrometry with ionization polarity switch. *Biomedical Chromatography* 16 (2002):383–389.
76. Y. Huang, E. Zurlinden, E. Lin, X. Li, J. Tokumoto, J. Golden, A. Murr, J Engstrom, and J. Conte, 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. *Journal of Chromatography B* 799 (2004):51–61.
77. A. D'Avolio, M. Sciandra, M. Siccardi, L. Baietto, R. D. Gonzalez, S. Bonora, and G. Di Perri. A new assay based on solid-phase extraction procedure with LC-MS to measure plasmatic concentrations of tenofovir and emtricitabine in HIV infected patients. *Journal of Chromatographic Science* 46 (2008):524–528.
78. R. C. E. Estrela, F. S. Ribeiro, B. V. Seixas, and G. Suarez-Kurtz, Determination of lopinavir and ritonavir in blood plasma, seminal plasma, saliva and plasma ultra-filtrate by liquid chromatography/tandem mass spectrometry detection. *Rapid Communications in Mass Spectrometry* 22 (2008):657–664.
79. M. Ehrhardt, M. Möck, W. E. Haefelı, G. Mıkus, and J. Burhenne, Monitoring of LPV and RTV in peripheral blood mononuclear cells, plasma, and ultrafiltrate using a selective and highly sensitive LC/MS/MS assay. *Journal of Chromatography B*. 850 (2007):249–258.
80. K. M. Rentsch, Sensitive and specific determination of eight anti-retroviral agents in plasma by high-performance liquid chromatography–mass spectrometry. *Journal of Chromatography B* 88 (2003):339–350.
81. R. Nirogi, G. Bhyrapuneni, V. Kandikere, K. Mudigonda, P. Komarneni, R. Aleti, and K. Mukkanti, Simultaneous quantification of a non-nucleoside reverse transcriptase inhibitor efavirenz, a nucleoside reverse transcriptase inhibitor emtricitabine and a nucleotide reverse transcriptase inhibitor tenofovir in plasma by liquid chromatography positive ion electrospray tandem mass spectrometry. *Biomedical Chromatography* 23 (2009):371–381.
82. A. Volosov, C. Alexander, L. Tıng, and S. J. Soldın, Simple rapid method for quantification of anti-retrovirals by liquid chromatography: Tandem mass-spectrometry. *Clinical Biochemistry* 35 (2002):99–103.
83. A. K. Ghoshal and S. J. Soldin, Improved method for concurrent quantification of anti-retrovirals by liquid chromatography–tandem mass spectrometry. *Therapeutic Drug Monitoring* 25 (2003):541–543.
84. W. Gao, T. Kishida, K. Kimura, M. Kageyama, M. Sumi, Y. Yoshikawa, N. Shibata, and K. Takada, Sensitive and simultaneous determination of HIV protease inhibitors in rat biological samples by liquid chromatography-mass spectrometry. *Biomedical Chromatography* 16 (2002):267–273.
85. J. E. Vela, L. Y. Olson, A. Huang, A. Fridland, and A. S. Ray, Simultaneous quantitation of the nucleotide analog adefovir, its phosphorylated anabolites and 2′-deoxyadenosine triphosphate by ion-pairing LC/MS/MS. *Journal of Chromatography B* 848 (2007):335–343.

86. K. M. Crommentuyn, H. Rosing, L. G. Nan-Offeringa, M. J. Hillebrand, A. D. Huitema, and J. H. Beijnen. Rapid quantification of HIV protease inhibitors in human plasma by high-performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry. *Journal of Mass Spectrometry* 38 (2003):157–66.
87. K. M. L. Crommentuyn, H. Rosing, M. J. X. Hillebrand, A. D. R. Huitema, and J. H. Beijnen, 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. *Journal of Chromatography B* 804 (2004):359–367.
88. E. Gangl, I. Utkin, N. Gerber, and P. Vouros, Structural elucidation of metabolites of ritonavir and indinavir by liquid chromatography–mass spectrometry. *Journal of Chromatography A* 974 (2002):91–101.
89. J. Chı, A. L. Jayewardene, J. A. Stone, T. Motoya, and F. T, Aweeka, Simultaneous determination of five HIV protease inhibitors nelfinavir, indinavir, ritonavir, saquinavir and amprenavir in human plasma by LC/MS/MS. *Journal of Pharmaceutical and Biomedical Analysis* 30 (2002):675–684.
90. R. L. Divi, D. R. Doerge, N. C. Twaddle, M. E. Shockley, M. C. St. Claire, J. W. Harbaugh, S. W. Harbaugh, and M. C. Poirier, Metabolism and pharmacokinetics of the combination zidovudine plus lamivudine in the adult erythrocebus patas monkey determined by liquid chromatography-tandem mass spectrometric analysis. *Toxicology and Applied Pharmacology* 226 (2008):206–211.
91. H. Pelerin, S. Compain, X. Duval, F. Gimenez, H. Benech, and A. Mabondzo, Development of an assay method for the detection and quantification of protease and non-nucleoside reverse transcriptase inhibitors in plasma and in peripherical blood mononuclear cells by liquid chromatography coupled with ultraviolet or tandem mass spectrometry detection. *Journal of Chromatography B* 819 (2005):47–57.
92. V. A. Frerichs, R. DiFrancesco, and G. D. Morse, Determination of protease inhibitors using liquid chromatography-tandem mass spectrometry. *Journal of Chromatography B* 787 (2003):393–403.
93. I. Van Den Broek, R. W. Sparıdans, A. D. R. Huıtema, J. H. M. Schellens, and J. H. Beıjnen, Development and validation of a quantitative assay for the measurement of two HIV-fusion inhibitors, enfuvirtide and tifuvirtide, and one metabolite of enfuvirtide (M-20) in human plasma by liquid chromatography-tandem mass spectrometry. *Journal of Chromatography B* 837 (2006):49–58.
94. L. D. Wıllıams, L. S. Von Tungeln, F. A. Beland, and D. R. Doerge, 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. *Journal of Chromatography B* 798 (2003):55–62.
95. P. G. Wang, J. S. Weı, G. Kım, C. Mın, and T. El-Shourbagy, Validation and application of a high-performance liquid chromatography-tandem mass spectrometric method for simultaneous quantification of lopinavir and ritonavir in human plasma using semi-automated 96-well liquid-liquid extraction. *Journal of chromatography A* 1130 (2006):302–307.
96. T. Koal, M. Sibum, E. Koster, K. Resch, and V. Kaever, Direct and fast determination of anti-retroviral drugs by automated online solid-phase extraction-liquid chromatography-tandem mass spectrometry in human plasma. *Clinical Chemical Laboratory Medicine* 44 (2006):299–305.
97. B. H. Jung, N. L. Rezk, A. S. Bridges, A. H. Corbett, and A. D. M. Kashuba, Simultaneous determination of 17 anti-retroviral drugs in human plasma for quantitative analysis with liquid chromatography–tandem mass spectrometry. *Biomedical Chromatography* 21 (2007):1095–1104.
98. A. D'Avolıo, M. Sıccardı, M. Scıandra, B. Lorena, S. Bonora, L. Trentını, and G. Dı Perrı, HPLC-MS method for the simultaneous quantification of the new HIV protease inhibitor darunavir, and 11 other anti-retroviral agents in plasma of HIV-infected patients. *Journal of Chromatography B* 859 (2007):234–240.
99. H. E. Reynolds, J. F. Tjia, S. E. Gibbons, S. H. Khoo, and D. J. Back, Simultaneous determination of four HIV protease inhibitors by HPLC-MS/MS for use in a therapeutic drug monitoring service. *British Journal of Clinical Pharmacology* 52 (2001):481.
100. N. L. Rezk, N. White, A S. Bridges, M. F. Abdel-Megeed, T. M. Mohamed, S. S. Moselhy, and A. D. Kashuba, Studies on anti-retroviral drug concentrations in breast milk: validation of a liquid chromatography-tandem mass spectrometric method for the determination of 7 anti-human immunodeficiency virus medications. *Therapeutic Drug Monitoring* 30 (2008):611–619.
101. N. A. Gomes, V. V. Vaidya, A. Pudage, S. S. Joshi, and S. A. Parekh, Liquid chromatography–tandem mass spectrometry (LC–MS/MS) method for simultaneous determination of tenofovir and emtricitabine in human plasma and its application to a bioequivalence study. *Journal of Pharmaceutical and Biomedical Analysis* 48 (2008):918–926.
102. R. ter Heine, C. G. Alderden-Los, H. Rosing, M. J. Hillebrand, E. C. van Gorp, A. D. Huitema, and J. H. Beijnen, Fast and simultaneous determination of darunavir and eleven other antiretroviral drugs for therapeutic drug monitoring: Method development and validation for the determination of all currently approved HIV protease inhibitors and non-nucleoside reverse transcriptase inhibitors in human plasma by liquid chromatography coupled with electrospray ionization tandem mass spectrometry. *Rapid Communications in Mass Spectrometry* 21 (2007):2505–2519.
103. R. Ter Heinea, H. Rosinga, E. C. M. van Gorpb, J. W. Mulderb, W. A. van der Steegb, J. H. Beijnena, and A. D. R. Huitemaa, Quantification of protease inhibitors and non-nucleoside reverse transcriptase inhibitors in dried blood spots by liquid chromatography–triple quadrupole mass spectrometry. *Journal of Chromatography B* 867 (2008):205–212.
104. T. Le Saux, S. Chhun, E. Rey, O. Launay, L. Weiss, J.-P. Viard, G. Pons, and V. Jullien, Quantification of seven nucleoside/nucleotide reverse transcriptase inhibitors in human plasma by high-performance liquid chromatography with tandem mass-spectrometry. *Journal of Chromatography B* 865 (2008):81–90.
105. L. Dickinson, L. Robinson, J. Tjia, S. Khoo, and D. Back, Simultaneous determination of HIV protease inhibitors APV, atazanavir, indinavir, lopinavir, nelfinavir, RTV and saquinavir in human plasma by high-performance liquid chromatography-tandem mass spectrometry. *Journal of Chromatography B* 829 (2005):82–90.