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Determination of antiretroviral agents in human serum by capillary electrophoresis

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Abstract

In this work, a simple and rapid electrokinetic chromatography method for the simultaneous separation of different protease inhibitors (indinavir, ritonavir, saquinavir, nelfinavir), nucleoside reverse transcriptase inhibitors (stavudine, zidovudine, didanosine) and non-nucleoside reverse transcriptase inhibitors (nevirapine, efavirenz) was developed. The analyses were performed in a 75 μm i.d. uncoated fused-silica capillary with 48.5 cm length (effective length of 40 cm) using a running buffer consisting of 20 mmol L^{-1} sodium dodecyl sulfate, 10 mmol L^{-1} sodium tetraborate, 30% acetonitrile and 5% ethanol. Samples were injected hydrodynamically by applying 50 mbar pressure during 6 s. All analytes were separated within 10 min with a voltage of 20 kV. The proposed method was validated for zidovudine, didanosine and efavirenz in human serum. Serum samples were prepared using a solid-phase extraction procedure (Waters® Oasis HLB cartridges). For quantitative purposes, stavudine was chosen as the internal standard (IS). Method validation parameters were determined revealing good migration time repeatability (<0.7% RSD) and peak area repeatability (<1.2% RSD). Intra- and inter-day precisions were less than 1.7% and 4.4% RSD, respectively. Matrix matching analytical curves for each drug were linear in the 1.0–20.0 μg mL^{-1} interval ($r > 0.998$). Limits of detection (LOD) were in range of 0.3–0.5 μg mL^{-1}. The extraction recoveries were higher than 90% with exception of efavirenz, which was 77.4%. Based on the performance characteristics, the proposed method was found suitable for the determination of zidovudine, didanosine and efavirenz in serum samples.

Keywords: Micellar electrokinetic chromatography; Antiretroviral agents; Human immunodeficiency virus

1. Introduction

The acquired immunodeficiency syndrome (AIDS) was first recognized in 1981, and the human immunodeficiency virus (HIV), the causative agent of AIDS, was identified in 1983 [1]. Currently, HIV/AIDS is the fourth greatest cause of death worldwide. It is estimated that 40 million people are infected with HIV and 22 million have died of the disease [2].

For a decade, nucleoside analogue reverse transcriptase inhibitors (NRTIs), such as zidovudine, didanosine, zalcitabine, stavudine and abacavir, were the only drugs available to treat HIV-1 infection. This unsatisfactory situation was dramatically changed with the introduction of two additional drug classes: protease inhibitors (PIs) that include saquinavir, ritonavir, indinavir, nelfinavir and amprenavir and non-nucleoside reverse transcriptase inhibitors (NNRTIs), such as efavirenz, nevirapine and delavirdine. Standard therapy consists of two nucleoside analogues in combination with either a protease inhibitor or a non-nucleoside reverse transcriptase inhibitor [3,4]; the triple combination therapy using these antiretroviral drugs was termed highly active antiretroviral therapy.

Therapeutic monitoring of these drugs is recommended in order to avoid or to delay the occurrence of viral resistance, to assess the usually underestimated non-adherence to treatment and to study drug interactions. Up to now, high-performance

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liquid chromatography (HPLC) is the most widely adopted technique for the quantitative determination of protease and reverse transcriptase inhibitors in biological fluids [5–9]. However, this method has presented some practical disadvantages: long analysis time (20–55 min), extensive sample preparation, large solvent volumes and complicated system operation and maintenance.

Capillary electrophoresis (CE) has emerged in recent years as a powerful analytical technique for the separation of a large variety of substances, including pharmaceutical compounds [10,11]. CE possesses many unique advantages, such as small sample volumes, high separation efficiency, low operating and consumable costs, low consumption of reagents, short analysis time and easy conditioning of column. In the present literature, only a few publications describe the separation and determination of antiretroviral drugs using CE. Chelyapov et al. [12] developed a method for the quantitative determination of 4 PIs in deproteinized serum samples, using formic acid containing acetonitrile as electrolyte. Analysis time of 15 min and adequate sensitivity (62.5 ng mL^{-1}) are a few characteristics of their proposed method. Zeemann et al. [13] studied the separation of five PIs using an electrolyte containing phosphoric acid and hexadimethrin bromide (HDB), an electroosmotic flow (EOF) modifier, which allowed the establishment of a strong cathodic EOF. All five PIs were separated within 5 min. In latter study, the separation of 11 antiretroviral drugs in 8 min was achieved when a mixture containing phosphoric acid, acetonitrile and sodium polyanethol sulfonate (SPAS), an EOF modifier, was used as electrolyte [14]. More recently, the authors proposed a method where 15 drugs were separated in approximately 10 min using as electrolyte phosphoric acid containing SPAS, acetonitrile, ethanol and sodium dodecyl sulfate, SDS [15]. The separation of Zeeman et al., although impressive, does not seem useful for validation purposes and routine use in clinical laboratories or quality control of pharmaceutical formulations. System peaks and baseline perturbations in the vicinity of the solvent peak would compromise the quantitative determination of at least four drugs: didanosine, estavudine, zidovudine, ritonavir and, possibly, amprenavir. Moreover, as the authors showed the use of low pH electrolytes may be detrimental due to the fact that some analytes exhibit limited pH stability. Up to now, only Fan and Stewart [16] developed and validated method to determine serum concentration of anti-HIV drug mixtures using alkaline conditions (borate/phosphate buffer and SDS). Despite the method's excellent performance, only four drugs were evaluated.

In this work, a reliable, simple and rapid electrokinetic chromatographic (EKC) method was developed. This method employs an alkaline buffer, allowing simultaneous separation of nine anti-HIV drugs with baseline resolution. Since for most therapeutic studies only three drugs have to be monitored simultaneously, the proposed method was validated for zidovudine, didanosine and efavirenz in human serum (three routinely prescribed drugs of the highly active antiretroviral therapy).

2. Experimental

2.1. Instrumentation

All experiments were conducted in a capillary electrophoresis system (Agilent Technologies, model HP 3D CE, Palo Alto, CA, USA), equipped with a diode array detector set at 200 nm and a temperature control device maintained at 25 °C. Data acquisition and treatment software was supplied by the manufacturer (HP ChemStation, rev A.06.01). Fused-silica capillaries (Polymicro Tecnologies, Phoenix, AZ, USA) with dimensions 48.5 cm total length, 40 cm effective length and 75 μm i.d. × 375 μm o.d. were used. Samples were injected hydrodynamically, 50 mbar pressure (1 mbar = 100 Pa) during 6 s. The instrument was operated under positive polarity (injection end of the capillary). A constant voltage of 20 kV was used for all experiments.

2.2. Chemical and reagents

All reagents and solvents were of analytical grade and used with no further purification. Indinavir sulfate (IDV), nelfinavir mesylate (NFV), saquinavir (SQV), ritonavir (RTV), zidovudine (AZT), stavudine (D4T), didanosine (DDI) and nevirapine (NVP) were kindly donated by Cristália Produtos Químicos e Farmacêuticos LTDA (São Paulo, Brazil) and efavirenz (EFV) was kindly supplied by Instituto de Tecnologia de Fármacos–Fundação Oswaldo Cruz (Rio de Janeiro, Brazil). Sodium dodecyl sulfate (SDS) was purchased from Riedel-de Haën (Seelze, Germany). Sodium tetraborate was obtained from Aldrich (Milwaukee, WI, USA). Water was purified by deionization (Milli-Q, Millipore Corp., Bedford, MA, USA). Water® Oasis HLB 3cc cartridges were purchased from Waters Corporation (Milford, MA, USA). Drug-free human serum was obtained from the Faculdade de Ciências Farmacêuticas–USP (São Paulo, Brazil).

2.3. Analytical procedure

The electrolyte solution was prepared fresh daily. At the beginning of each day, the fused-silica capillary was conditioned by flushing with a 1 mol L^{-1} NaOH solution (5 min), followed by a 5 min flush of deionized water and electrolyte solution (40 min). In between runs, the capillary was rinsed with 0.1 mol L^{-1} NaOH for 3 min, followed by fresh electrolyte solution (3 min).

2.4. Preparation of standards

Individual stock solutions of all nine antiretroviral agents were prepared by dissolving appropriate amounts in methanol to a final concentration of 1000 μg mL^{-1}. All stock solutions were stored at −20 °C. Working solutions were prepared fresh daily by diluting appropriately the stock solutions with 9 mmol L^{-1} SDS solution.

2.5. Sample preparation procedure

For serum preparation, a procedure described on the literature was used [16]. Water® Oasis HLB cartridges were conditioned with methanol, followed by deionized water. After conditioning, the cartridges were loaded with 1 mL of the spiked serum samples. The cartridges were washed with 1 mL 10:90 (v/v) methanol–water. Sample was eluted from the cartridge with 1 mL methanol. The solvent was evaporated to dryness under nitrogen and the residue was dissolved in 1 mL of SDS solution (9 mmol L^{-1}) prior to analysis.

2.6. Analytical curve

Standard curves were prepared by spiking drug-free serum with appropriate aliquots of the stock standard solution of drugs and a fixed aliquot of an internal standard (IS) (stavudine, 5 μg mL^{-1}). The range of standard concentrations tested were from 1.0 to 20.0 μg mL^{-1} for zidovudine, didanosine and efavirenz. All standard solutions related to the analytical curve were processed as described in section 2.5. Each solution was injected in triplicate. Peak area ratios (drug/stavudine) were plotted against the respective concentration of each drug.

2.7. Recovery

The accuracy of the procedure was assessed by performing recovery experiments. Three different concentration levels of the drugs and a fixed concentration of internal standard were added to serum samples. The samples were extracted as described in Section 2.5. The recovery was calculated by comparing peak area ratios of the samples after extraction with peak area ratios of standard solutions at the same concentration.

3. Results and discussion

3.1. Optimization of the separation conditions

The structures of the anti-HIV drugs under investigation in this work are depicted in Fig. 1. The simultaneous separation of all nine anti-HIV drugs is not possible by free solution CE over the practical pH range from pH 2.5 to 9.0. This is because at low pH values only the drugs that exhibit at least one amino group are expected to be in the cationic form, while those with nitrogen vicinal to carbonyl group remains neutral. On the other hand, none of the drugs present acidic groups and only a few present acidic proton from phenol, lactam and imide moieties, which can be dissociate at high pH values generating anionic species. Thus, the separation of all drugs simultaneously would be successfully approached only by interaction with a secondary phase.

In this work, several electrolyte systems were studied to achieve the best resolution, highest sensitivity and shortest analysis time for the analysis of nine anti-HIV drugs. Electrolyte systems comprising of sodium tetraborate and SDS were chosen to start the optimization procedure. Initial results have shown that insufficient resolution occurred with electrolyte systems containing only tetraborate and SDS (Fig. 2A). As a consequence, additional modifiers were required. A dramatic improvement concerning selectivity, resolution, and separation efficiency can be reached if organic solvents, such as methanol, ethanol and acetonitrile or mixtures thereof, are added to the electrolyte [17].

The effect of organic solvents on the separation was first studied using methanol in the range of 5–15%, with constant electrolyte concentration, pH and applied voltage (10 mmol L^{-1} tetraborate, 30 mmol L^{-1} SDS, pH 9.5, +20 kV, fused-silica capillary 48.5 cm total length, 40 cm effective length, and 75 μm i.d. versus 375 μm o.d.). With increasing methanol concentration, an improvement of resolution was observed. However, no further improvement was observed for NFV, EFV and SQV above 15% methanol (Fig. 2B). According to the literature, acetonitrile has already proven to be a suitable solvent for separations of antiretroviral drugs and the selectivity of the system can be further altered when small amounts of ethanol are added [15]. The effect of acetonitrile concentration on the separation performance was evaluated at a fixed ethanol concentration. Fig. 3A depicts effective mobility curves as a function of acetonitrile concentration for constant electrolyte composition, pH and applied voltage (10 mmol L^{-1} tetraborate, 30 mmol L^{-1} SDS, 5% ethanol, pH 9.5, +20 kV). At 10% acetonitrile the co-elution of RTV, SQV, NFV and EFV was observed. At 20% acetonitrile the resolution of NFV and RTV increased significantly, but NVP could not be separated from the EOF peak. It was also observed that the effective mobilities of IDV, RTV, NFV, SQV and EFV decreased in acetonitrile concentrations above 10%, indicating a change in the distribution ratios of solutes between the buffer and the micellar phase. Regarding the mobilities of NVP, DDI, D4T and AZT it was observed a decrease at 20% acetonitrile, however, a slightly increase was occurred when acetonitrile concentration surpassed 20%. Generally, the use of high concentrations of organic solvent are not recommended because above 20% organic solvent micelle structure is disrupted [18]. However, at 30% acetonitrile some discrimination of the drugs was observed, indicating that interactions between the drugs and monomeric SDS or aggregates take place.

The concentration of the ethanol was studied over the range of 0–10% for fixed amount of acetonitrile. Fig. 3B depicts effective mobility curves as a function of ethanol concentration for constant electrolyte composition, pH and applied voltage (10 mmol L^{-1} tetraborate, 30 mmol L^{-1} SDS, 30% acetonitrile, pH 9.5, +20 kV. In the absence of ethanol, co-elution of the pairs RTV,AZT and EFV,NFV occurred. At 10% ethanol the separation of the pairs DDI,SQV and NFV,EFV was not possible. It is worth mentioning that for some drugs, changes in the elution order were observed with increasing organic solvent concentration. It

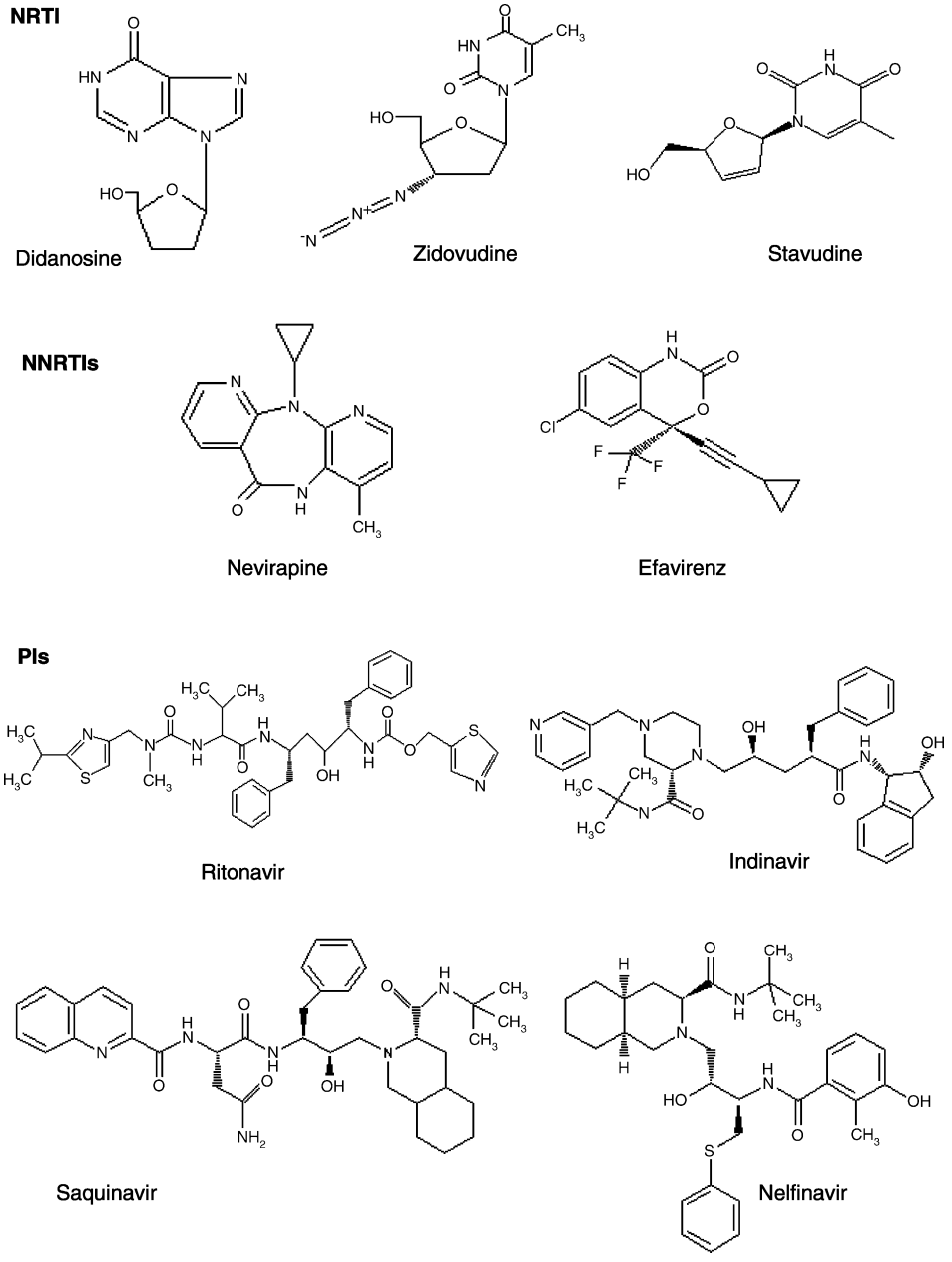

Fig. 1. Chemical structures of anti-HIV drugs.

was found that a 30% acetonitrile and 5% ethanol mixture allowed almost complete resolution of all peaks.

The effect of SDS concentration on the separation of the drugs in 10 mmol L^{-1} tetraborate, 30% acetonitrile and 5% ethanol at +20 kV was further investigated. Fig. 3C shows the effective mobilities of the drugs as a function of SDS concentration (0–40 mmol L^{-1}). As it can be seen, NFV, EFV, D4T, AZT and DDI were completely separated when SDS is absent. In fact under this pH condition, DDI (p*K*a 8.7) migrates as negatively charged species, however, NFV, EFV, D4T and AZT only behave as negatively charged species due to their interaction with SDS (drugs were dissolved in 9 mmol L^{-1} SDS prior to analysis, see Section 2.5). With increasing SDS concentration, the mobilities of NVP, D4T, AZT and DDI were only slightly modified, whereas IDV, RTV, NFV, SQV and EFV were significantly increased, which can be explained by the increased number of SDS micelles in the medium. Concentrations above 20 mmol L^{-1} were detrimental to resolution, a partial co-elution of the AZT,NFV pairs at 30 mmol L^{-1} and AZT,RTV pairs at 40 mmol L^{-1} was observed. It was also observed that for higher SDS concentrations changes in selectivity occurs. A SDS concentration of 20 mmol L^{-1} was selected because it represented a compromise between baseline resolution and analysis time. The separation of nine antiretroviral agents under investigation at the optimized conditions is shown in Fig. 4.

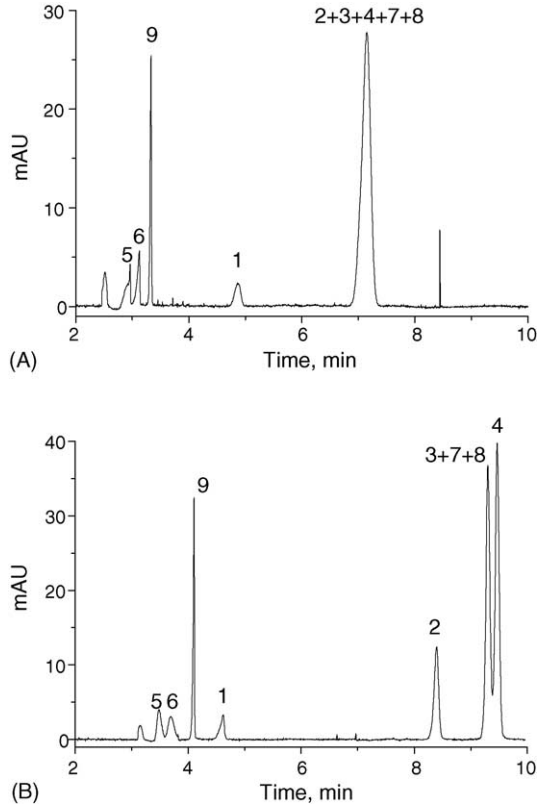

Fig. 2. Optimization of electrolyte composition. Concentration of each standard drug: 25 μg mL^{-1} prepared in 9 mmol L^{-1} SDS. Separation conditions: fused-silica capillary 75 μm i.d., 375 μm o.d., 48.5 cm total length (40 cm to detector); separation voltage: 20 kV; hydrodynamic injection: 3 s at 50 mbar; temperature 25 °C, detection at 200 nm. (A) electrolyte: 10 mmol L^{-1} tetraborate and 30 mmol L^{-1} SDS, pH 9.5; (B) electrolyte: 10 mmol L^{-1} tetraborate, 30 mmol L^{-1} SDS and 15% methanol, pH 9.5. Peak identification: (1) Nevirapine; (2) Indinavir; (3) Ritonavir; (4) Saquinavir; (5) Stavudine (6) Zidovudine,(7) Nelfinavir; (8) Efavirenz and (9) Didanosine.

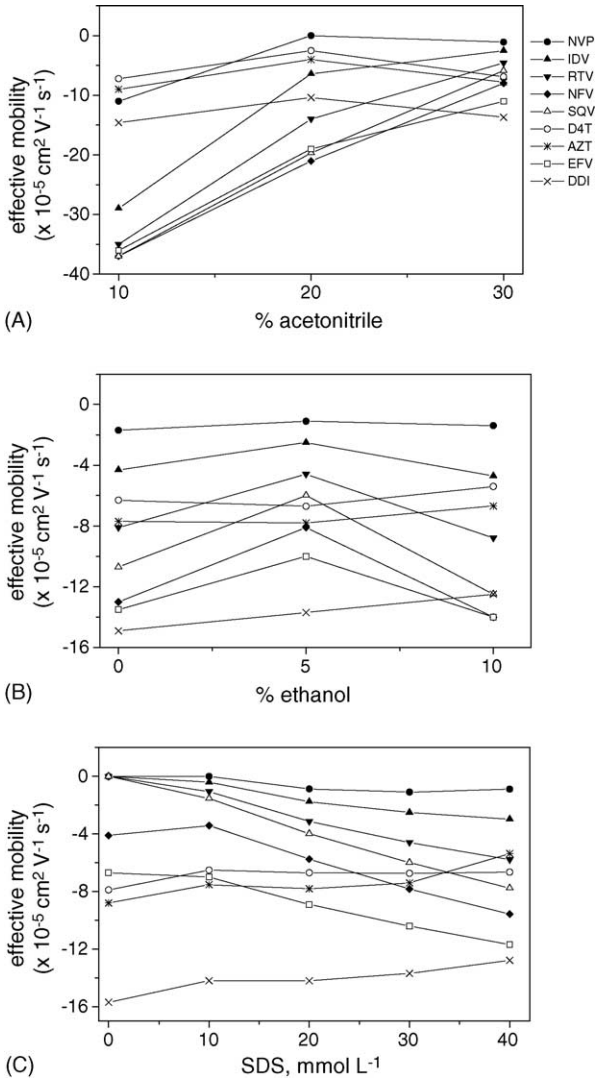

Fig. 3. Dependence of effective mobilities on the concentration of (A) acetonitrile; (B) ethanol and (C) SDS in the electrolyte. Standard drugs were prepared in 9 mmol L^{-1} SDS Separation conditions: fused-silica capillary 75 μm i.d., 375 μm o.d., 48.5 cm total length (40 cm to detector); separation voltage: 20 kV; hydrodynamic injection: 3 s at 50 mbar; temperature 25 °C, detection at 200 nm. (A) 10 mmol L^{-1} tetraborate, 30 mmol L^{-1} SDS and 5% ethanol, pH 9.5. (B) 10 mmol L^{-1} tetraborate, 30 mmol L^{-1} SDS and 30% acetonitrile, pH 9.5. (C) 10 mmol L^{-1} tetraborate, 30% acetonitrile and 5% ethanol, pH 9.5.

As the use of an internal standard is recommended for quantitative analysis to improve injection precision [19,20], several antiretroviral agents were investigated as internal standard for validation procedures of AZT, DDI and EFV. Stavudine was found to be a suitable candidate since its migration time was close to the drugs of interest and its peak presented an acceptable profile.

3.2. Method validation

As part of method validation for the CE system, linearity, precision, accuracy, limit of detection and limit of quantification were evaluated [21–23].

3.3. Precision

Relative peak area and relative migration time repeatability for 10 consecutive injections of a standard solution containing 20 μg mL^{-1} of each drug and 5 μg mL^{-1} of internal standard was estimated. The results are given in Table 1. RSDs were better than 0.7% for relative migration time and

Table 1
Analytical performance of the method regarding precision

Antiretroviral agent	MT (min)	RMT, RSD (%)	RPA, RSD (%)
Zidovudine	6.58	0.24	0.64
Efavirenz	6.94	0.64	1.1
Didanosine	8.99	0.32	0.86

MT, migration time; RMT, relative migration time (MT drug/MT stavudine); RPA, relative peak area (PA drug/PA stavudine); RSD, relative standard deviation (10 consecutive injections of standard solution at 10 μg mL^{-1} of each drug).

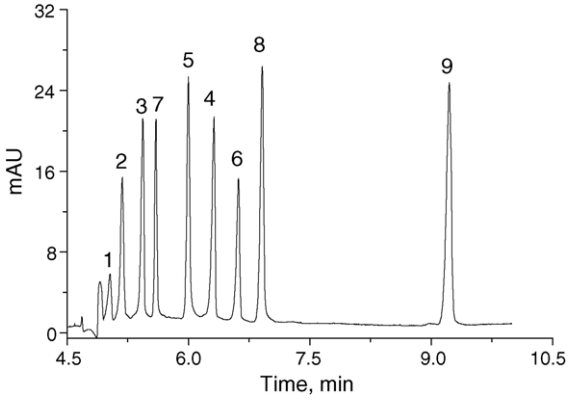

Fig. 4. Separation of a standard mixture under optimized conditions. Concentration of each standard drug: 25 μg mL^{-1} prepared in 9 mmol L^{-1} SDS. Separation conditions: fused-silica capillary 75 μm i.d., 375 μm o.d., 48.5 cm total length (40 cm to detector); separation voltage: 20 kV; hydrodynamic injection: 3 s at 50 mbar; temperature 25 °C, detection at 200 nm; electrolyte: 10 mmol L^{-1} tetraborate, 20 mmol L^{-1} SDS, 30% acetonitrile and 5% ethanol, pH 9.5. Peak labels as in Fig. 2.

better than 1.2% for relative peak area, establishing therefore the method instrumental precision.

The intra-day precision was determined by assaying three control samples at low, medium and high concentrations for each drug in triplicate injections in the same day. For inter-day precision determination, samples were analyzed in three different days. The results are summarized in Table 2. The method showed good intra- and inter-day precision with RSDs ranging from 0.28 to 1.7% and from 0.30 to 4.4%, respectively.

3.4. Linearity, limit of detection (LOD) and limit of quantification (LOQ)

Matrix matching analytical curves of relative peak areas (drug/IS) versus concentration consisted of five points and three replicate injections of standards at each concentration level were performed. The analytical curve equations and the limits of detection and quantification are presented in Table 3. The results showed good linearity over the concentration range from 1.0 to 20.0 μg mL^{-1} ($r > 0.998$). The method limits of detection and quantification for the drugs under investigation were in the range of 0.3–0.5 μg mL^{-1} and 1.0–1.8 μg mL^{-1}, respectively. The criterion used to determine the LOD and LOQ was based on signal-to-noise ratio 3:1 and 10:1, respectively [22,23].

Table 3
Statistical parameters of the matrix matching analytical curves and estimates of limits of detection (LOD) and quantification (LOQ) for the proposed method

Antiretroviral agent	Analytical curve equation[a]	R	LOD[b] (μg mL^{-1})	LOQ[c] (μg mL^{-1})
Zidovudine	$Y = 0.137X + 0.0495$	0.9992	0.5	1.8 (0.8)[d]
Efavirenz	$Y = 0.28X - 0.0805$	0.9989	0.3	1.0 (1.5)[d]
Didanosine	$Y = 0.268X + 0.0626$	0.9999	0.4	1.2 (0.9)[d]

[a] Drugs concentration interval from 1.0 to 20 μg mL^{-1}; based on relative peak area.
[b] S/N = 3.
[c] S/N = 10.
[d] RSD, $n = 3$.

3.5. Accuracy

Accuracy was calculated as the percentage recovery of known amounts of analyte added to blank serum matrices [22]. The extraction recoveries were >90% with exception of EFV, which was >77.4%. The internal standard was extracted with a recovery of 88.5% (Table 4).

3.6. Method application

Fig. 5 shows the electropherogram of a blank serum sample spiked with AZT, DDI, EFV as the active components of therapy and D4T as internal standard. The expected plasma concentrations for each antiretroviral drug as compiled from the literature are summarized in Table 5 [24]. Limits of quantification (LOQ) established by our proposed methodology are within the expected plasmatic therapeutic concentration, which demonstrates that the proposed method is suitable for therapeutic drug monitoring.

Table 2
Intra- and inter-day precision

Antiretroviral agent	Concentration (μg mL^{-1})	Intra-day[a] RSD (%)	Inter-day[b] RSD (%)
Zidovudine	2.00	0.47	3.8
	10.0	0.43	1.8
	20.0	0.28	1.5
Efavirenz	2.00	1.7	1.8
	10.0	0.59	2.5
	20.0	1.7	0.60
Didanosine	2.00	1.7	4.4
	10.0	0.45	1.2
	20.0	0.59	0.30

[a] Based on $n = 3$.
[b] Based on $n = 9$.

Table 4
Recovery study

Antiretroviral agents	Added (μg mL^{-1})	Found (μg mL^{-1})	Recovery (%)
Zidovudine	2.00	1.95	97.3 ± 0.49
	10.0	9.72	97.2 ± 0.45
	20.0	20.9	104.7 ± 0.40
Efavirenz	2.00	1.62	81.1 ± 1.6
	10.0	7.74	77.4 ± 0.46
	20.0	16.9	84.8 ± 1.5
Didanosine	2.00	1.88	94.0 ± 1.6
	10.0	9.75	97.5 ± 0.48
	20.0	19.0	95.0 ± 0.57
Stavudine[a]	5.00	4.69	88.5 ± 1.2

[a] Internal standard.

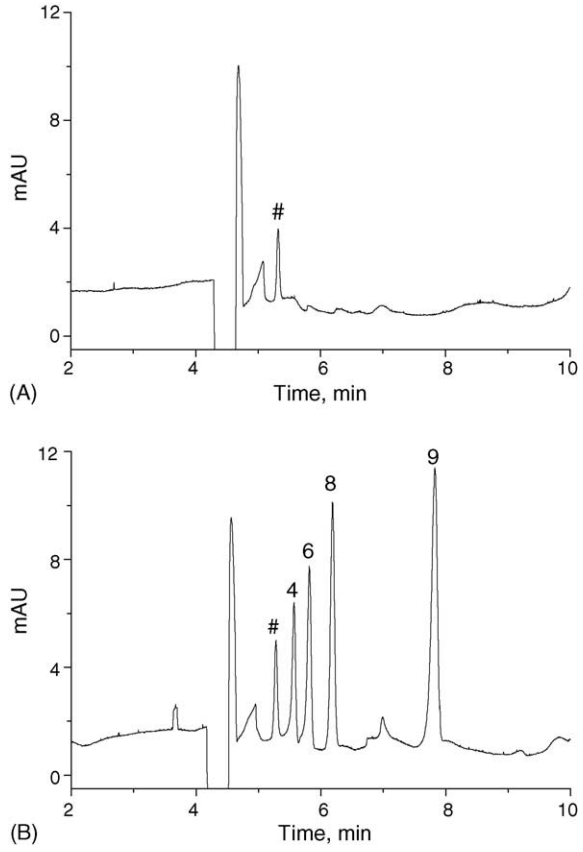

Fig. 5. Electropherograms of serum samples: (A) blank human serum and (B) human serum spiked with 10 μg mL^{-1} of each drug (zidovudine, efavirenz and didanosine) and 5 μg mL^{-1} of internal standard (stavudine) prepared in 9 mmol L^{-1} SDS. Electrophoretic conditions as in Fig. 4 except for injection: 6 s × 50 mbar. The peak # is an unknown serum component.

Table 5
Expected plasma concentrations for each of the antiretroviral agents [24]

	C_{max}[a]	C_{min}
NRTIs		
Didanosine	1.2–2.4 μg mL^{-1}	0.1–0.3 μg mL^{-1}
Lamivudine	1.4–1.8 μg mL^{-1}	0.1–1.0 μg mL^{-1}
Stavudine	0.7–2 μg mL^{-1}	0.02 μg mL^{-1}
Zalcitabine	<0.025 μg mL^{-1}	undetectable
Zidovudine	1.8 μg mL^{-1}	<0.02 μg mL^{-1}
NNRTIs		
Efavirenz	1.8–5.8 μg mL^{-1}	n.a.
Delavirdine	16 μg mL^{-1}	3–8 μg mL^{-1}
Nevirapine	20 μg mL^{-1}	3 μg mL^{-1}
PIs		
Indinavir	10 μg mL^{-1}	0.1 μg mL^{-1}
Nelfinavir	4 μg mL^{-1}	1 μg mL^{-1}
Ritonavir	14 μg mL^{-1}	1 μg mL^{-1}
Saquinavir	250–500 ng mL^{-1}	15–40 ng mL^{-1}

C_{max}, maximum concentration; C_{min}, minimum concentration; n.a., not available.

[a] C_{max} is achieved 0.5–1.5 h after oral administration for most NRTIs; from 2 to 4 h for most NNRTIs and PIs.

The occurrence of two zidovudine metabolites (3′ amino-3′ deoxythymidine and glucuronide G-AZT) has been reported [25,26]. There is some concern that they could interfere with the assay, but as the maximal concentrations found for the metabolites in plasma samples are in the range of 0.1–0.2 μg mL^{-1} (10-fold lower than the parent drug), we believe that they would not be detected by the proposed method. However, the electropherograms of samples from patients receiving AZT should be cautiously examined, when sample preconcentration strategies are attempted, to avoid misidentification of AZT metabolites with other inhibitors. For DDI and EFV no relevant metabolites have been described [27,28].

4. Conclusion

A rapid, simple and reliable analytical method for determination of nine antiretroviral agents has been developed. Method validation parameters were established for three drugs (zidovudine, didanosine and efavirenz) revealing good migration time repeatability and area repeatability, excellent linearity and adequate accuracy. In addition, the proposed method exhibited limits of quantification which allows examination of serum concentration during therapeutic drug monitoring.

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