Journal of Chromatography A, 1216 (2009) 3546–3552

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Screening and monitoring antiretrovirals and antivirals in the serum of acquired immunodeficiency syndrome patients by micellar liquid chromatography

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ARTICLE INFO

Article history:
Available online 31 January 2009

Keywords:
Antiretrovirals
Antivirals
HIV
Screening
Monitoring
Micellar liquid chromatography
Direct injection
Hospitals

ABSTRACT

Thirteen different antiretrovirals are commonly used in hospital protocols for suppressing the activity of the human immunodeficiency virus (HIV) and associated opportunistic diseases in patients with acquired immunodeficiency syndrome (AIDS). In this work, three micellar mobile phases are recommended for screening these substances, using UV detection, and the process can be performed in less than 18 min. The first mobile phase (sodium dodecyl sulphate or SDS 50 mM) is used for the group consisting of acyclovir, didanosine, ganciclovir, stavudine and zidovudine. The second mobile phase (SDS 120 mM/4.5% propanol) is used for the group containing abacavir, lamivudine, nevirapine, valaciclovir and zalcitabine, whereas the third mobile phase (SDS 150 mM/5% pentanol) is used for efavirenz, indinavir and ritonavir. The use of micellar liquid chromatography (MLC) as an analytical tool allows serum samples to be injected directly. The method was validated over the range of 0–10 $\mu g\,mL^{-1}$. The limits of detection (signal-to-noise ratio of 3), which ranged from 6 to 30 $ng\,mL^{-1}$, were adequate for monitoring these substances. Intra- and inter-day relative standard deviations of the assay were below 3% for all compounds. The recoveries in spiked serum samples were in the 89.5–104.4% range. The method can be applied to the screening, monitoring and control of patients' treatment with antiretrovirals and antivirals.

1. Introduction

Twenty-six years ago, reports were published on fatal cases of rare opportunistic infections. The disease, which was caused by the acquired immunodeficiency virus 1 (HIV-1), then became known as acquired immunodeficiency syndrome (AIDS) [1–4]. Various attempts have been undertaken since then to find a cure for the disease, but with limited success. In 1996, highly active antiretroviral therapy (HAART) was introduced, with impressive clinical results [5–8]. Generally speaking, HAART regimes (Fig. 1) contain two nucleoside reverse transcriptase inhibitors (NRTIs), such as abacavir, didanosine, emtricitabine, lamivudine, stavudine, tenofovir, zalcitabine and zidovudine, one non-nucleoside reverse transcriptase inhibitor (NNRTI), such as nevirapine and efavirenz, or protease inhibitors (PI), such as amprenavir, atazanavir, fosamprenavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir and tipranavir [9]. On the other hand, due to the fact that viral infection with cytomegalovirus is one of the most serious problem for patients with AIDS, the drugs acyclovir, ganciclovir and valaciclovir are also coadministered to these patients [10].

Therapeutic drug monitoring (TDM) involves taking a blood sample to measure the amount of a particular PI and/or NNRTI. Most experts believe that measuring the levels of NRTIs will be of little value, as they block HIV inside the cell. Drug levels found in blood might not necessarily compare to those inside cells. TDM may be particularly useful for protease inhibitors, as their levels in blood can vary greatly from one individual to another because there are differences in how people break down (metabolise) these drugs. Ensuring that people stay within a "therapeutic range" may greatly improve the likelihood of a lasting anti-HIV response. TDM may also help determine the proper dose of a drug for a particular person [11]. Moreover, studies have proved the existence of a series of relations between plasmatic levels of drug and (a) significantly increased levels of fasting triglyceride and cholesterol from continued use of lopinavir/ritonavir; (b) renal disorders in the case of indinavir; and (c) liver toxicity for nevirapine. No such relationships were established with other conditions, such as exanthem in the case of NNRTIs or hyperlipidemia [12–14].

On the other hand, the lack of compliance to HAART is the first cause of therapeutic failure. The virological characteristics of the HIV are such that when there are subtherapeutic levels of antiretroviral drugs, the virus can multiply and develop resistance, which is why a level of treatment compliance above 95% is necessary [15–17]. In addition, poor compliance is associated to a

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doi:10.1016/j.chroma.2009.01.082

poor immunological response [18] and a greater risk of mortality [15,19].

Specific and sensitive analytical methods are needed for simultaneously determining serum concentrations of as many HIV medications as possible. A clinician can use such methods to provide valuable information about several aspects of patient treatment, including malabsorption, drug interactions, fulfilment of the treatment and individual drug pharmacokinetics, as well as therapeutic drug level monitoring [20–22].

Therefore, an analytical method for their determination in serum on a routine basis could constitute a useful clinical tool. In this regard, analytical methods have already been described to quantify single [23,24] and combined [20–22,25–27] anti-HIV agents in human serum. Furthermore, each method (individual or simultaneous) involves a previous sample preparation procedure: liquid–liquid [22,23] or solid–liquid extraction [20,21,26,27] or deproteinisation [24,25]. Such methods increase the difficulty, in both time and costs, of quantifying all the drugs taken by one single HIV-infected patient undergoing multiple therapy with drugs from different therapeutic classes. In addition, many methods use HPLC instruments together with either column-switching techniques [26] or mass spectroscopy [28,29], which are not commonly available in conventional hospital laboratories.

Micellar liquid chromatography (MLC) is an alternative to these methods [30] for drug determination in physiological fluids. The use of surfactants in direct injection is much less complex. The sodium dodecyl sulphate (SDS) micelles tend to bind proteins competitively, thereby releasing protein-bound drugs. Therefore, the drugs are free in the stationary phase, whereas the proteins, rather than precipitating in the column, are solubilised and elute with or shortly after the solvent front. MLC has recently proved itself to be a useful technique in the control of diverse groups of substances in serum with direct injection of the samples, such as vitamins [31], antiepileptic drugs [32], benzodiazepines [33], barbiturates [34] and stimulants [35]. Finally, compared to other eluents, the micellar mobile phases [36,37] are less flammable, inexpensive, non-toxic, biodegradable, and can co-solubilise hydrophobic and hydrophilic analytes in complex matrices like serum.

This paper describes the studies carried out to develop and validate a fast, simple method for monitoring 13 antiretrovirals and antivirals used in the treatment of patients with AIDS, using three mobile phases that contained sodium dodecyl sulphate alone or with propanol, butanol or pentanol and with direct injection of human serum.

2. Experimental

2.1. Chemicals and reagents

The antivirals studied were: abacavir sulphate, valaciclovir hydrochloride (Glaxo Smith Kline, Brentford, UK), acyclovir sodium, ganciclovir sodium (Roche Farma, Barcelona, Spain), didanosine, efavirenz, indinavir sulphate, lamivudine, nevirapine anhydrous, zalcitabine and zidovudine (Filaxis, Córdoba, Argentina), ritonavir (Abbot Laboratories, North Chicago, IL, USA) and stavudine (Bristol Myers Squibb, New York, NY, USA). Sodium dodecyl sulphate, or SDS, 1-propanol, 1-butanol, 1-pentanol, disodium hydrogenphosphate, sodium dihydrogenphosphate, hydrochloric acid and sodium hydroxide were obtained from Merck (Darmstadt, Germany). Stock solutions and mobile phases were prepared in ultrapure water (Simplicity UV, Millipore Molsheim, France).

The blood samples used for spiking were taken from five healthy volunteers; these samples were centrifuged at 3000 rpm for 10 min and serum was finally separated and either used immediately or frozen and stored at −20 °C. The antivirals, the antiretrovirals and the blood samples were provided by the La Plana Hospital in Vila-real and the General Hospital in Castelló, after consent had been obtained from the Ethical Committee and from patients. Serum solutions were injected into the chromatography system with no treatment other than filtration. The solutions and the mobile phases were filtered through 0.45 μm nylon membranes (Micron Separations, Westboro, MA, USA).

2.2. Instrumentation

The chromatographic system used for the optimisation procedure and for method validation was an Agilent Technologies Model 1100 (Palo Alto, CA, USA). It was equipped with a quaternary pump, an autosampler with 2 mL vials fitted with a Rheodyne valve (Fitatu, CA, USA) and a diode array detector (range 190–700 nm). A Kromasil 100 C18 column (5 μm particle size, 250 mm × 4.6 mm I.D.) from Scharlab (Barcelona, Spain) thermostated at 25 °C was used in the separations. The flow rate and injection volume were 1 mL min^{-1} and 20 μL, respectively. The detection wavelengths were 260 nm for acyclovir, didanosine, ganciclovir, stavudine and zidovudine (group A) and abacavir, lamivudine, nevirapine, valaciclovir and zalcitabine (group B), and 214 nm for efavirenz, indinavir and ritonavir (group C). The signal was acquired by a personal computer connected to the chromatograph by means of a Hewlett-Packard Chemstation. When the mobile phase changed, a period of 30 min was required to reequilibrate the column and to obtain a stable baseline.

A GLP 22 potentiometer (Crison, Barcelona, Spain) equipped with a combined Ag/AgCl/glass electrode was used to measure pH values. The balance used was a Mettler-Toledo AX105 Delta-Range (Greifensee, Switzerland).

2.3. Preparation of solutions, samples and mobile phases

For optimisation studies, stock solutions of each antiretroviral or antiviral were prepared in methanol–water 5:95 (v/v). Working solutions were prepared by diluting these stock solutions in mobile phase. All stock and working solutions stored at +4 °C were stable for at least 3 months, which was confirmed by measuring the chromatographic signal. For preparation of the serum sample, 0.5 mL of serum was diluted with an appropriate amount (0.01–1 mL) of the stock solutions and mobile phase (final volume of 5 mL) and injected into the chromatograph with no pretreatment other than filtration.

The micellar mobile phase was prepared using SDS, which was buffered with disodium hydrogenphosphate/sodium dihydrogenphosphate 10 mM at pH 7, and lastly 1-propanol, 1-butanol or 1-pentanol was added to achieve the desired concentration.

Solutions of potential interfering drugs were prepared from clear filtered extracts of the pharmaceutical formulations. These solutions were prepared in methanol–water 5:95 (v/v) and diluted with mobile phase before injection into the MLC system at concentrations of 2 μg mL^{-1}.

3. Results and discussion

3.1. Optimisation strategy and mobile phase selection

In order to find the best mobile phase composition that allows the 13 antiretrovirals and antivirals considered in this study to be analysed simultaneously, each of them was injected in mobile phases at pH 7 containing SDS (mM)/modifier (%): SDS/pentanol (50/1, 50/5, 75/3, 100/3, 125/3, 150/1, 150/5); SDS/butanol (50/1, 50/7, 75/4.5, 100/4, 125/4.5, 150/1, 150/7); SDS/propanol (50/2.5, 50/12.5, 75/7.5, 100/7.5, 125/7.5, 150/2.5, 150/12.5) and finally, SDS (mM) pure micellar phase (50, 125, 100, 125 and 150). The

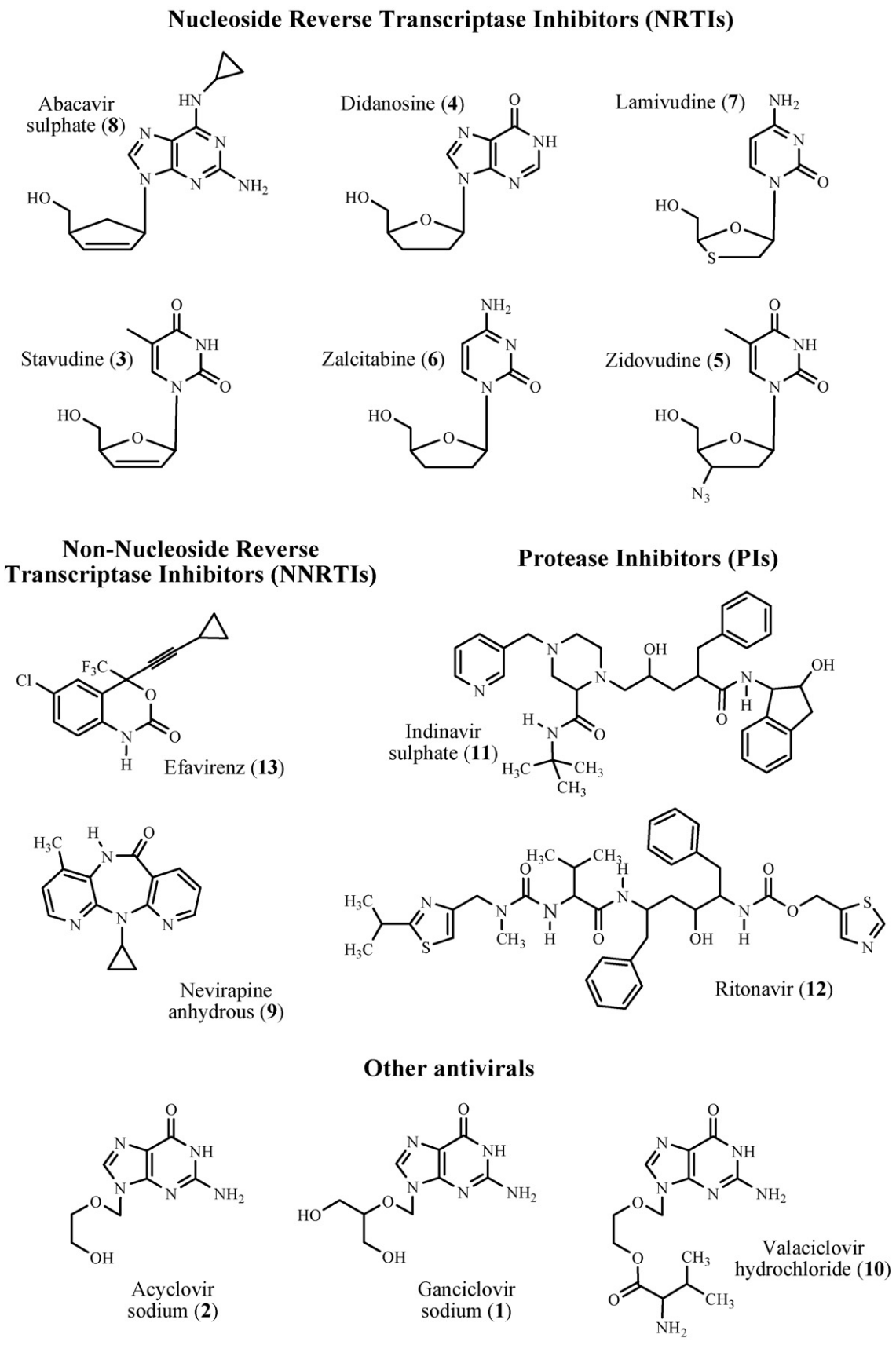

Fig. 1. Chemical structures of the investigated drugs.

peaks obtained in these mobile phases allowed us to obtain measurements of the chromatographic parameters for each antiretroviral and antiviral: time retention (t_R), capacity factor (k), efficiency (N) and asymmetry factor (B/A). These data were used together with a mathematical model and an interpretative optimisation procedure to predict the behaviour of the peaks corresponding to the antiretrovirals and antivirals [38]. For all the antiretrovirals studied behaviour observed was: retention factors and efficiencies decreases when the concentration of the surfactant increases, but when the concentration of modifier increase, the retention factor decrease but the efficiency increase.

When attempting to establish an optimisation criterion, the possibility of using the same mobile phase to determine the 13 antiretrovirals and antivirals is impossible because of the overlapping that occurs between some of the compounds: abacavir and stavudine, and zidovudine or indinavir with valaciclovir. Since many of the drugs share certain structural characteristics and have very similar physicochemical properties, a good resolution was not obtained between all of them and thus the decision was taken to form groups under the criteria of maximum resolution and efficiency and minimum analysis time.

In liquid chromatography, interpretive optimisation strategies are more efficient and reliable than sequential approaches. The methodology followed by chromatographers can be mimicked by these strategies, which can be assisted by computer simulation, thus reducing the amount of time and effort required. MLC is capable of predicting the retention of compounds using simple equations. Different mathematical models can be used to describe the retention of analytes. The model employed in this work was the following Eq. (1) [36]:

$$k = \frac{K_{AS}(1/1 + K_{AD}\varphi)}{1 + K_{AM}[(1 + K_{MD}\varphi)/(1 + K_{AD}\varphi)][M]} \quad (1)$$

where $[M]$ and φ are the concentrations of surfactant and modifier; $K_{AS}, K_{AM}, K_{MD}, K_{SD}, K_{AD}$ and K_{AD} correspond to the equilibria between the solute (A) in the stationary phase (S), micelle (M), or bulk water (φ). This equation was non-linearly fitted according to the Powell method [37] using the retention data from injections ($n = 3$) of the drug solutions in several mobile phases. To avoid inconsistent results, the experimental design should have at least one mobile phase more than there are parameters in the equation; in this case this consisted of seven mobile phases as described earlier. The prediction capacity of the mathematical model was evaluated as the differences between the experimental and predicted capacity factors for the antiaretrovirals, expresed as the relative global error [38]. Using Eq. (1) and the mathematical treatment described here, the relative global error in the prediction of retention factors was below 2% for the 13 substances studied. Adjusting the coefficients that were calculated to Eq. (1) for each substance allows the mobile phase composition to be predicted for any desired retention time and also provides a simple way to optimise the separation of mixtures. It must be noted that, in the analysis of physiological fluids, the retention time of the endogenous compounds and the protein band at the head of the chromatogram should be considered when selecting the mobile phase.

Table 1
Mean values ($n = 3$) of retention time (t_R), capacity factor (k), efficiencies (N) and asymmetry factor (B/A) for the substances that were studied.

Compound	t_R	k	N	B/A
Abacavir	4.1	1.75	5,200	0.73
Acyclovir	2.8	1.09	8,800	0.73
Didanosine	6.4	3.8	10,900	0.85
Efavirenz	1.7	1.06	6,350	0.81
Ganciclovir	2.4	0.74	8,700	0.77
Indinavir	8.8	5.0	5,350	0.77
Lamivudine	2.8	0.83	9,500	0.71
Nevirapine	7.1	3.7	6,500	0.76
Ritonavir	9.9	6.4	3,400	0.78
Stavudine	4.1	2.0	8,700	0.76
Valaciclovir	8.2	4.4	9,300	0.76
Zalcitabine	2.5	0.62	8,800	0.71
Zidovudine	7.8	5.5	11,150	0.75

The optimization procedure, which maximized the resolution in the separation of the drugs, was used to select the most suitable mobile phases. The mobile phases allowing for the separation and quantification of the components in each group in an acceptable analysis time will be useful to analyse the serum samples.

The resolution diagram data (results unshown) are obtained by the option *Evaluate grid* in the Michrom program [36–38]. This option calculates the resolution matrix. This matrix may contain one, two or three experimental factors. In the present case, two experimental factors (surfactant and modifier) were studied and thus two-dimensional matrices were obtained. The maximal resolution value found is given during the calculation process, together with the composition of the corresponding mobile phase. Once the calculation of the matrix has finished, the active mobile phase for the simulations and other calculations will be the optimal phase that was found. The grid thus calculated has the extension GRD and contains all the information about the series needed to build the diagrams. The grids could be exported to allow retrieval by other programs such as *Surfer* (RockWare Europe, Cureglia, Switzerland).

After this mathematical treatment of the data for all the substances, the mobile phases chosen were: SDS 50 mM for acyclovir, didanosine, ganciclovir, stavudine and zidovudine; SDS 120 mM/4.5% propanol for abacavir, lamivudine, nevirapine, valaciclovir and zalcitabine, and SDS 150 mM/5% pentanol for efavirenz, indinavir and ritonavir. Using these mobiles phases and under the criteria of maximum resolution and efficiency–minimum analysis

Table 2
Calibration with 6 points ($n = 6$) and each point is the mean of three injections ($m = 3$): slope, intercept, regression coefficient (r), limit of detection (LOD, 3 s criterion) and quantification (LOQ, 10 s criterion) for the antiretrovirals and antivirals that were studied.

Compound	Slope	Intercept[a]	r	LOD (ng mL^{-1})	LOQ (ng mL^{-1})
Abacavir	35.4 ± 0.5	3.3 ± 2.1	0.9993	150	450
Acyclovir	66.2 ± 0.7	8.3 ± 4.5	0.9995	60	180
Didanosine	38.2 ± 0.2	2.2 ± 0.2	0.9998	100	300
Efavirenz	55.3 ± 0.4	−9.4 ± 2.2	0.9995	80	240
Ganciclovir	64.6 ± 0.5	3.7 ± 5.1	0.9995	55	150
Indinavir	29.1 ± 0.4	−3.3 ± 5.8	0.9995	165	480
Lamivudine	35.5 ± 0.1	7.1 ± 1.8	0.9999	90	280
Nevirapine	30.2 ± 0.1	−5.6 ± 5.5	0.9997	120	360
Ritonavir	42.5 ± 0.5	12.2 ± 0.5	0.9995	75	210
Stavudine	51.1 ± 0.3	−4.4 ± 10.2	0.9995	65	200
Valaciclovir	48.1 ± 0.3	5.2 ± 3.3	0.9996	68	200
Zalcitabine	45.9 ± 0.4	−4.5 ± 1.1	0.9999	70	210
Zidovudine	42.2 ± 0.2	−6.4 ± 9.2	0.9997	72	220

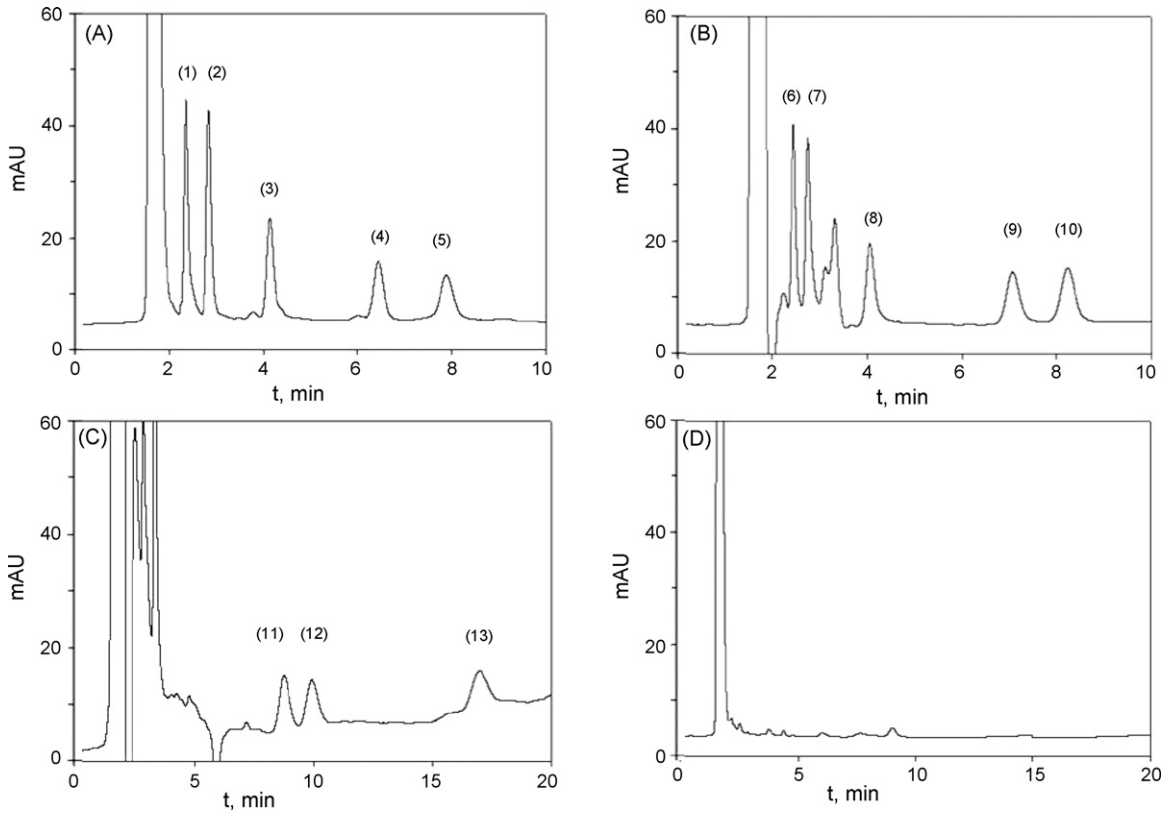

Fig. 2. Chromatograms showing spiked serum samples (A–C) and a serum blank (D). In (A) the mobile phase was SDS 50 mM and substances are (1) ganciclovir, (2) acyclovir, (3) stavudine, (4) didanosine, (5) zidovudine at 2 μg mL^{-1} for each antiretroviral or antiviral drugs; retention times: 2.3, 2.8, 4.1, 6.4 and 7.9, respectively. In (B) the mobile phase was SDS 120 mM/4.5% propanol, and the substances: (6) zalcitabine, (7) lamivudine, (8) abacavir, (9) nevirapine, (10) valaciclovir at 2 μg mL^{-1} for each antiretroviral or antiviral drugs, retention times: 2.4, 2.8, 4.1, 7.1 and 8.2, respectively. In (C) the mobile phase was SDS 150 mM/5% pentanol, and the substances (11) indinavir, (12) ritonavir, (13) efavirenz at 4 μg mL^{-1} for each antiretroviral drugs, retention times: 8.8, 9.9 and 17.0, respectively.

time, the values of the parameters for each antiviral are summarised in Table 1. All the asymmetry factors are around 0.75 and most of the efficiency values are above 6000, the maximum efficiency being 11 127 for zidovudine.

3.2. Method validation

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

The standard curves for all compounds were satisfactorily described by unweighted least-square linear regression. Calibration curves were constructed using the areas of the chromatographic peaks (triplicate injections) obtained at eight different concentrations, in the 0–10 μg mL^{-1} range for all the compounds, in the line of real samples. This interval is adequate for this drugs, in which peak-valley concentrations are in the 0.5–5 μg mL^{-1} range. The detection limit (LOD) of a method, is the lowest antiretroviral concentration in serum, that produces a response that is detectable above the noise level of the system, typically taken as being three times the noise level (3 s *criterion*). The quantification limit (LOQ) of a method, is the lowest antiretroviral concentration in serum, that can be identified and quantitatively measured, typically taken as being 10 times the noise level (10 s *criterion*). Table 2 shows the slopes, intercepts and regression coefficients ($r > 0.999$) of the calibration curves, the LODs (using the 3 s *criterion*) and LOQs (with the 10 s *criterion*) determined after measurements in 20 injections of a serum blank. The results of LOD and LOQ were based on the standard deviation of the response, and on the slope of a specific

Table 3
Recovery ± SD (%, $n = 5$) in spiked serum samples: c_1 and c_2 were 1 and 5 μg mL^{-1} for all substances except for efavirenz, indinavir and ritonavir (2 and 4 μg mL^{-1}).

Compound	c_1	c_2
Abacavir	97.9 ± 0.40	96.7 ± 1.1
Acyclovir	94.0 ± 4.1	98.6 ± 0.10
Didanosine	102.4 ± 1.6	101.2 ± 0.40
Efavirenz	101.4 ± 0.40	100.0 ± 0.88
Ganciclovir	104.1 ± 0.65	104.0 ± 1.5
Indinavir	84.5 ± 28.1	94.1 ± 3.9
Lamivudine	101.7 ± 0.85	97.4 ± 0.50
Nevirapine	97.4 ± 0.15	100.5 ± 0.40
Ritonavir	99.2 ± 4.2	104.4 ± 0.30
Stavudine	101.0 ± 0.75	100.2 ± 0.45
Valaciclovir	95.9 ± 1.1	96.3 ± 0.85
Zalcitabine	96.4 ± 1.2	92.2 ± 0.10
Zidovudine	95.6 ± 0.10	96.5 ± 0.10

Table 4
Determination of antivirals and antiretrovirals in the serum of HIV patients.

Patient	Compound	MLC (ng mL^{-1}; $n = 3$)	HPLC[a] (ng mL^{-1}; $n = 3$)
1	Zidovudine	708 ± 11	690 ± 18
	Ritonavir	627 ± 9	620 ± 12
2	Zidovudine	581 ± 15	565 ± 25
	Ritonavir	455 ± 5	441 ± 10
3	Efavirenz	347 ± 11	325 ± 10
	Lamivudine	1007 ± 5	998 ± 8
	Stavudine	871 ± 8	860 ± 15
4	Efavirenz	462 ± 7	448 ± 16
	Lamivudine	873 ± 8	869 ± 9
	Stavudine	794 ± 18	781 ± 8

[a] Lamivudine and stavudine were determined using the method proposed in citation of Ref. [21]; zidovudine, ritonavir and efavirenz were determined with the method of citation of Ref. [27].

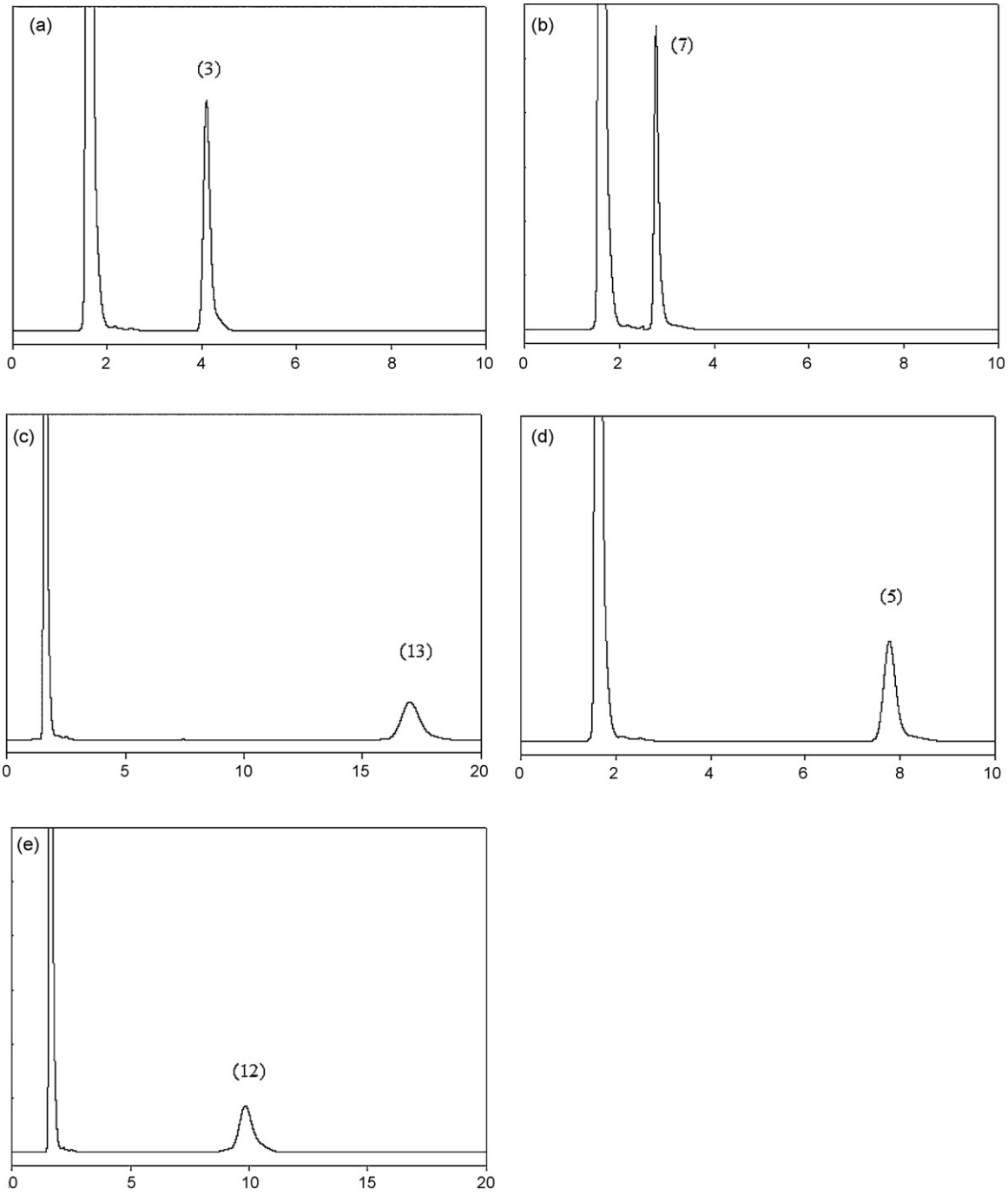

Fig. 3. Chromatograms showing the screening of real serum samples for two patients of AIDS—first patient: chromatograms (a), (b) and (c) show the separation of stavudine (3), lamivudine (7) and efavirenz (13), with mobiles phases A, B and C, respectively. For the second patient, chromatograms (d) and (e) show the separation of zidovudine (5), and ritonavir (12), with mobile phases A and C, respectively.

calibration curve containing the substance. The LOD and LOQ of each antiretroviral and antiviral ranged from 6.0 to 30.2 ng mL^{-1} and 20.1 to 100.5 ng mL^{-1}, respectively, and were therefore comparable to (or were even better than) reported methods. These values were good enough to monitor the compounds under study in serum matrices.

3.2.2. Precision

Precision, defined as the relative standard deviation, was determined by intra- and inter-day assays. These were determined at low, medium and high concentrations, according to the calibration curve ranges (Table 2). The intra-day variability of the assay was tested on 20 samples ($n = 20$) over three different concentrations for each compound for 20 separate days in a 4-month period; inter-day variability was tested using the same procedure. The method proved to be precise and the intra-day results ranged from 0.17 to 1.89% and inter-day values ranged from 0.21 to 2.98%.

3.2.3. Selectivity

An interfering drug is defined as a molecule which exhibits a retention time close to 0.3 min with the substances studied here. The drugs that are most commonly prescribed together with the antiretrovirals and antivirals are: acebutolol, acetylprocainamide, acetaminophen, acetylsalycilic acid,

amiodarone, amoxicillin, ampicillin, atenolol, azithromycin, bromazepam, caffeine, captopril, carbamazepine, ciprofloxacin, clonazepam, chloramphenicol, cocaine, codeine, corticosterone, desipramine, dexamethasone, diazepam, diltiazem, doxycycline, ephedrine, flunitrazepam, furosemide, gentamicin, hydrocortisone, imipramine, levofloxacine, lidocaine, loratadine, medazepam, methotrexate, nicotine, nifedipine, norfloxacin, oxazepam, phenylefrine, propranolol, quinidine, sulphametoxazole, sulphathiazole, tetracycline, theophylline and valproic acid. In our laboratory, 1 $\mu g\,mL^{-1}$ of these substances were injected in the three mobile phases recommended for monitoring and quantifying antiretrovirals and antivirals. In these studies and under the criteria previously defined, none of these substances were seen to interfere in the determination of the antiretrovirals and antivirals studied in this work using the recommended micellar liquid chromatography conditions. Finally, after injection of real serum samples in the three mobile phases recommended in this work, and comparison with blank serum samples and spiked serum samples (containing the standard solutions of the 13 drugs), no metabolites or impurities of the drugs could be detected in the chromatograms.

3.2.4. Analysis of spiked and real serum samples

The recoveries of all drugs were estimated by comparing peak areas in extracted spiked drug-free serum with those of standard solutions. MLC is a reliable way to eliminate material from serum, with satisfactory recovery rates (range from 92.2 to 104.4%), except for the relatively low recovery for indinavir (range from 84.5 to 94.1%). Table 3 summarises the recoveries from serum samples at two different spiking levels. Fig. 2 shows chromatograms of spiked serum corresponding to the three groups of substances. In the first case, an endogenous peak at a t_R of less than 2 min (Fig. 2A) does not interfere with the peaks of the antiretrovirals and antivirals. In the second group, in addition to this peak, there are other small peaks eluting between lamivudine and abacavir (Fig. 2B) that do not interfere with these compounds. Finally, in the third group, we can observe peaks in/with the solvent front, which do not interfere with their determination either. Some of the changes observed in Fig. 2A–C are due to the fact that throughout the chromatograms some changes in wavelength are included in the chromatographic method.

Finally, the assay was used for the determination of these substances in HIV serum patients and results are shown in Table 4 and Fig. 3.

4. Conclusions

The MLC assay described here offers an accurate, precise and highly reproducible procedure for the direct monitoring of 13 antiretroviral and antiviral drugs from different therapeutic groups in serum. Thanks to the absence of interferences, the methods are specific and reliable. This method does not require complex procedures such as sample extraction and/or sample cleaning. Chromatographic analyses require only 9, 9 and 18 min for the three recommended mobile phases, respectively. In addition, this method needs only simple chromatographic apparatus that is available in any hospital laboratory. Such monitoring is very important and useful to verify that correct dosages have been established, and also because patients' responses due to subtherapeutic and toxic concentrations are similar. These conditions make this method suitable for rapid monitoring and quantifying of antiretrovirals and antivirals.

Acknowledgements

This work was supported by the project P1-1B2006-12 of *Fundació Caixa de Castelló-Bancaixa-Universitat Jaume I* and CTQ2007-64473/BQU of *MEC*. M.A.R. is also grateful to this foundation for the grant.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2009.01.082.

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