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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

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Abstract

We have developed a sensitive, high-pressure liquid chromatographic–tandem mass spectrometric (LC/MS/MS) method for the simultaneous determination of didanosine (ddI) and stavudine (d4T) in human plasma, bronchoalveolar lavage fluid (BALF), alveolar cells (AC), peripheral blood mononuclear cells (PBMC), seminal plasma, cerebrospinal fluid (CSF), and tonsil tissue. Plasma, AC, PBMC and CSF were run with an isocratic HPLC method, while BALF supernatant, semen, and tonsil tissue utilized a gradient elution. Samples were prepared by solid phase extraction. Detection was by electrospray positive ionization with multiple reaction monitoring mode. The lower limits of quantitation for both ddI and d4T were 2.0 ng/ml in plasma; 0.5 ng/ml in CSF; 0.4 ng/ml in AC, PBMC, and BALF; 1.0 ng/ml in seminal plasma; and 0.01 ng/mg in tonsil tissue.

Keywords: Didanosine; Stavudine

1. Introduction

Didanosine (ddI), 2′,3′-dideoxyinosine (Fig. 1a), is a synthetic purine nucleoside analogue. Peak plasma concentrations (C_{max}) occur at 1–1.5 h and range from 1.2 to 2.4 mg/l following a 200 mg oral dose [1]. Stavudine (d4T), 2′,3′-didehydro-3′-deoxythymidine (Fig. 1b), is a synthetic thymidine nucleoside analogue. Its plasma C_{max} occurs at 0.5–1.0 h and range from 0.7 to 2.0 mg/l following a 40 mg oral dose [1]. Both ddI and d4T are active against human immunodeficiency virus (HIV), and are part of the regimen of drugs used in highly active antiretroviral therapy (HAART) [2].

There are several papers that describe ddI or/and d4T assay methods in human plasma [3–12]. Most of them utilize HPLC with UV detection. Recently, high-pressure liquid chromatographic–tandem mass spectrometric (LC/MS/MS) methods to determine ddI or d4T concentrations in human plasma have been reported [9–12]. However, no assay methods have been reported to detect ddI and d4T levels in other human matrices. Since HIV is found not only in blood but also in lymph nodes, brain and semen of infected male patients, there is a need for anti-HIV drug level information in these compartments [13]. Here, we describe an LC/MS/MS method for the simultaneous determination of ddI and d4T in human plasma, bronchoalveolar lavage fluid (BALF), alveo-

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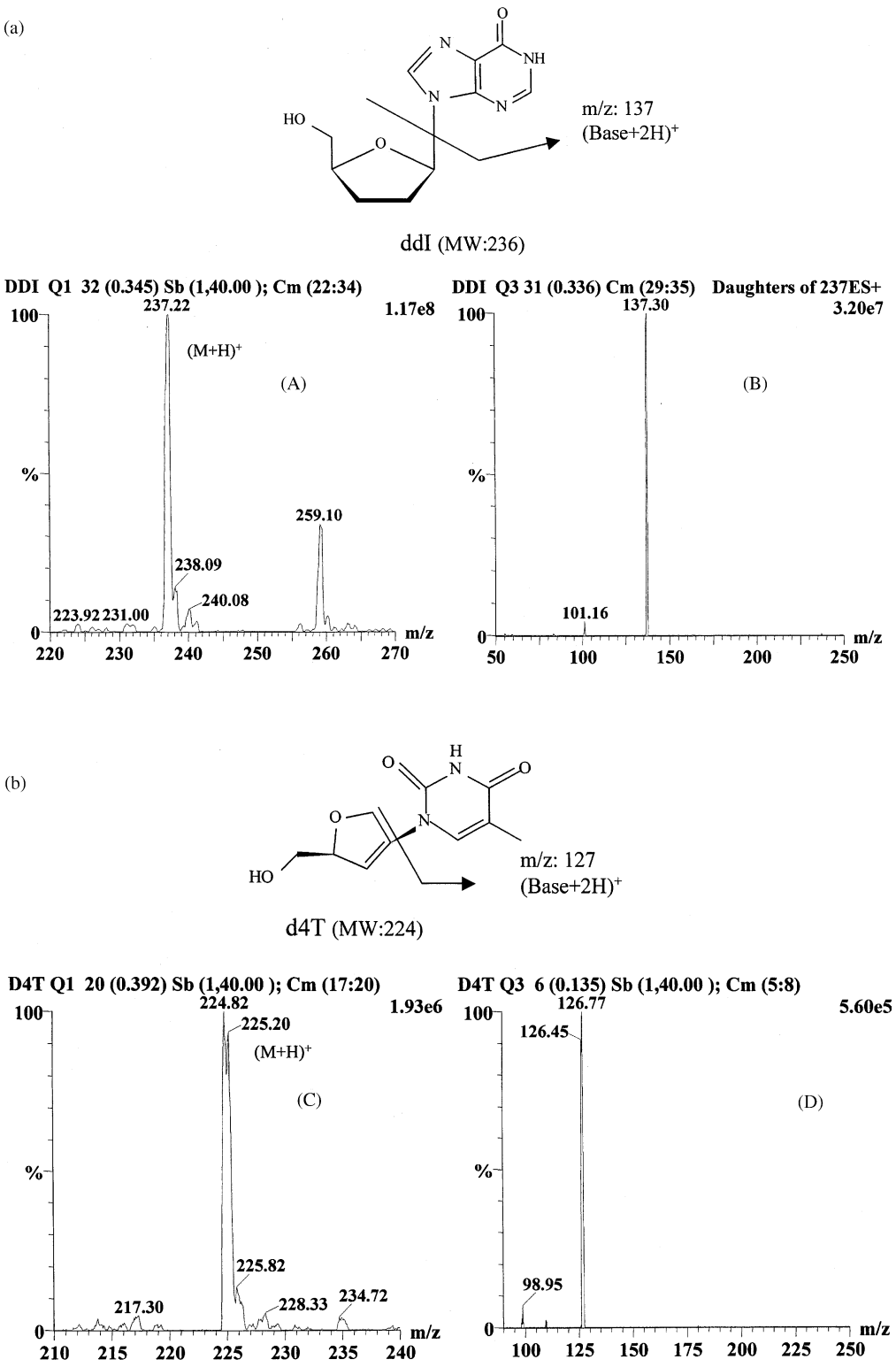

Fig. 1. Chemical structure and full mass scan of ddI (a, A); d4T (b, C); IS (c, E); without collision gas and their daughter ion scans: ddI (a, B); d4T (b, D); IS (c, F) with collision gas and collision energy at 10, 12 and 15 eV, respectively.

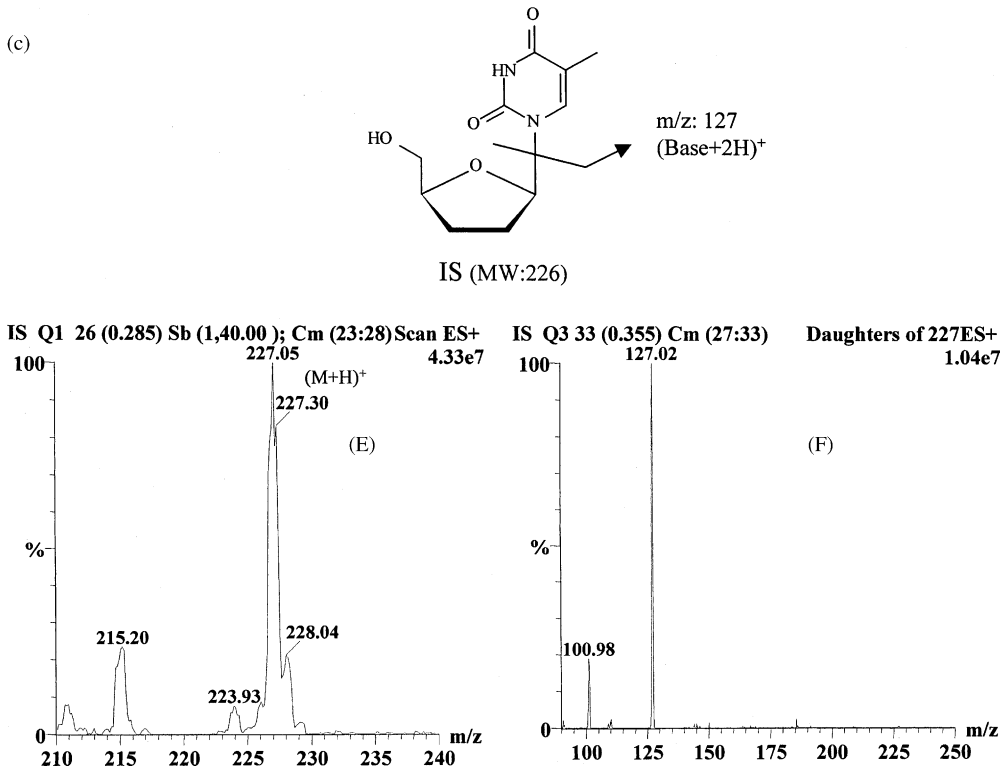

Fig. 1. (*Continued*).

lar cells (AC), peripheral blood mononuclear cells (PBMC), seminal plasma, cerebrospinal fluid (CSF), and tonsil tissue. These assays were utilized in a study of the distribution of ddI and d4T in these compartments, in HIV positive men on HAART therapy for 6 months.

2. Experimental

2.1. Chemicals

All solvents and chemicals were HPLC grade except ammonium formate, which was certified. Stock solutions (0.5 mg/ml) of ddI and d4T were prepared from reagent grade 2′,3′-dideoxyinosine (purity = 97.6%) and 2′,3′-didehydro-3′-deoxythymidine (purity = 99%) (Sigma, St. Louis, MO, USA) in 1:1 (v/v) acetonitrile–water. These solutions were pooled together and further diluted in 10 mM ammonium acetate adjusted to pH 7.0 with 1.25 N NaOH to produce working solutions of 10.0, 1.0 and 0.1 ng/μl. A 1 mg/ml stock solution of 3′-deoxythymidine for internal standard (IS) was also prepared in 1:1 (v/v) acetonitrile–water and diluted to 2.0 μg/ml in water as a working solution.

2.2. Chromatography

ddI and d4T were measured together, in all matrices, by a new LC/MS/MS technique. Plasma, CSF, AC and PBMC were run with an isocratic elution method. BALF supernatant, semen and tonsil tissue utilized a gradient HPLC method in order to remove late-eluted endogenous interference.

For the isocratic HPLC method, drug peaks were eluted from a Keystone, BDS C_{18} column (4.6 mm × 150 mm, 5 μm particle size) with mobile phase consisting of methanol–water (16:84, v/v), 0.05% trifluoracetic acid, and 1 mM ammonium formate, at a flow rate of 0.8 ml/min. Retention times for ddI, d4T and IS were 5.0, 5.6 and 7.0 min, respectively.

For the gradient elution, phase A: methanol–water (16:84, v/v) containing 0.05% TFA, 1 mM ammonium formate and phase B: methanol–water (80:20, v/v) containing 0.05% TFA, 1 mM ammonium formate were run as follows: from 0 to 3 min, the mobile phase consisted of 100% A. From 3 to 3.5 min, it changed with a linear gradient from 100% A to 100% B which remained until 5.0 min. From 5.0 to 5.5 min it changed with a linear gradient from 100% B to 100% A, then a 100% A mobile phase remained until the end of the run at 12 min. The flow rate was 0.8 ml/min. Retention times for ddI, d4T and IS were 1.7, 2.1 and 2.6 min, respectively.

2.3. Mass spectrometry

Peak detection was carried out on an LC/MS/MS Micromass Quattro LC using electrosray (ESI) positive mode. The MS/MS conditions were tuned by the continuing infusion of drug to the mass system and set as follows. (1) The multiple

reaction monitor (MRM) mode was set at 237.4 → 137.2 *m/z* for ddI, 225.0 → 127.0 *m/z* for d4T and 227.0 → 127.0 *m/z* for IS. (2) ESI/positive ionization was used with a flow rate of approximately 0.2 ml/min (a splitting ratio of 1:4 was applied to the mobile phase flow of 0.8 ml/min). (3) The desolvation and source block temperatures were set at 400 and 100 °C, respectively. (4) The sample cone voltages were set to 10 V for ddI, and 16 V for both d4T and 3′-deoxythymidine. (5) The collision energies were set to 12, 10 and 15 eV for ddI, d4T and 3′-deoxythymidine, respectively. (6) The collision gas used was ultra high purity grade argon (purity > 99.999%). The gas cell pressure was 1.8×10^{-3} mbar. A Digital P-2 2661 computer with Mass Lynx 3.5 Software was used for peak integration.

2.4. Sample preparation

2.4.1. Standard curves

Plasma standard curves were prepared by adding appropriate volumes of ddI/d4T working solutions to 0.5 ml blank plasma, to yield the following concentrations: 2, 4, 10, 20, 40, 100, 200, 400 ng/ml of ddI and d4T. Standard curves ranged from 1 to 400 ng/ml in seminal plasma, 0.4–200 ng/ml in BALF pellet and PBMC, 0.4–100 ng/ml in BALF supernatants, 0.5–100 ng/ml in CSF and 0.01–0.4 ng/mg in tonsil tissue. Standard curves were constructed by 1/*y* weighted least squares linear regression of ddI or d4T to IS peak area ratios versus the spiked concentrations.

2.4.2. Preparation of plasma, AC, PBMC and CSF standards and samples

AC were suspended in a measured volume of deionized water. PBMC were suspended in a measured volume of phosphate buffered saline so that a cell count could be performed on the suspension. The AC and PBMC cells were lysed on a Fisher 550 dismembrator. Standard curves and controls were prepared in a suspension of monocyte enriched human leukocytes, leukopak (Biological Specialty Corp., Colmar, PA, USA) which were further purified through ficoll-hypaque separation and sonicated.

Plasma and CSF were prepared with a solid phase extraction method utilizing a 3 ml Bond Elut C_8 extraction cartridge, 500 mg (Varian Associates Inc., Harbor City, CA 90710, USA): 50 μl of IS working solution was added to 0.5 ml of sample, this was placed on a conditioned cartridge (pre-rinsed with 3 ml of methanol followed by 3 ml of water), then washed with 2.5 ml of water, followed by 0.5 ml of acetonitrile, to remove the water from the column. The drugs were then eluted into a clean tube with 0.6 ml of acetonitrile. The eluent was evaporated to dryness under nitrogen at 37 °C. After reconstitution in 200 μl of mobile phase, the sample was injected into the LC/MS/MS. AC and PBMCs were prepared in the same way, but prior to loading onto the cartridge, 0.5 ml of acetonitrile was added to the sample. After vortexing for 30 s and centrifuging 5 min, at 3000 rpm, the supernatant was decanted into a clean tube. It was evaporated under nitrogen in a 37 °C water bath, to approximately 0.5 ml aqueous phase, and this was loaded onto the pre-conditioned Bond Elut cartridge.

2.4.3. Preparation of BALF supernatant, seminal plasma, and tonsil tissue

ddI and d4T were extracted from tonsil tissue by soaking a 10 mg piece of tissue in 0.5 ml of water for 24 h, in the refrigerator at 3 °C, then processing this extract. An aliquot of 1.0 ml of BALF supernatant, or 0.2 ml of semen or 0.4 ml of tonsil tissue extract were prepared for HPLC by a solid phase extraction method utilizing a 3 ml Waters Oasis HLB extraction cartridge (60 mg, Waters Corp., Milford, MA, USA). After mixing with 50 μl of IS working solution, the sample was placed onto a conditioned extraction cartridge (pre-rinsed with 1.0 ml of methanol, then 1.0 ml of water), then washed with 6 ml of water. The drugs were eluted into a clean tube with 0.5 ml of methanol. The eluant was evaporated to dryness under nitrogen, in a 37 °C water bath. After resuspension in 200 μl of mobile phase, 20 μl of the sample was injected into the LC/MS/MS.

2.4.4. Preparation of controls for method validation

Two sets of stock solutions were prepared, one used for spiking standards, the other for spiking controls. Spiked controls at two concentrations in plasma, leukopak, BALF, CSF, and seminal plasma were frozen at −80 °C and assayed weekly and monthly to assess frozen stability of the drugs. To assess inter-day reproducibility, standard curves and controls were analyzed on five different days. For intra-day reproducibility, six preparations of each control were analyzed with a standard curve.

2.5. Statistics

The statistical analysis was performed using the PROPHET Computer Resource [14]. Linearity (*r*), precision (coefficient of variation, i.e. CV), and percentage accuracy were calculated [15]. The lower limit of quantitation (LLOQ) was equivalent to the lowest point of the standard curve. Drug concentration in epithelial lining fluid were calculated using the urea diffusion method and AC concentrations were calculated using cell counts in BALF fluid as we have previously reported [16,17].

3. Results

Fig. 1(a)–(c) shows the full scan and daughter ion scan mass spectra of ddI, d4T and IS, respectively. Full scan mass spectra without collision gas shows the protonated molecular ions at *m/z* 237 (ddI), 225 (d4T) and 236 (IS). The major daughter ion of ddI with collision gas and collision energy of 10 eV is at *m/z* 137. It is produced from the protonation of the base and glycosidic bond cleavage with a hydrogen atom

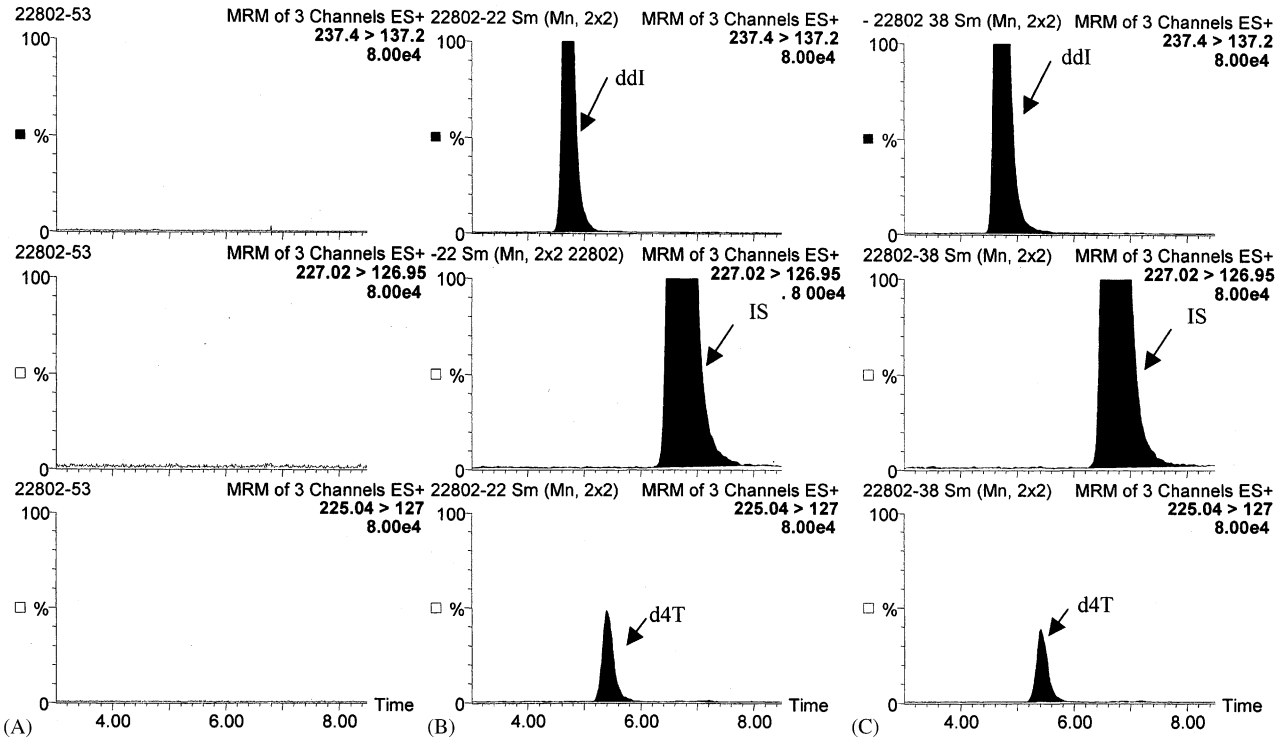

Fig. 2. Chromatograms of (A) blank plasma, (B) plasma standard with 20 ng/ml ddI and d4T, (C) study subject's plasma with a ddI concentration of 54.0 ng/ml and a d4T concentration of 17.9 ng/ml.

transferred from the sugar. The d4T and IS show similar fragments. This is consistent with other nucleoside analogs reported in the literature [18,19].

LC/MS/MS chromatograms of ddI, d4T and IS in human plasma and BALF supernatant are shown in Figs. 2 and 3. The chromatograms of all the other matrices were similar. The intra-day and inter-day validation data for each matrix are reported in Tables 1–6.

The LLOQ in plasma was 2.0 ng/ml. The mean (±S.D.) CVs of all the assays for intra-day and inter-day determinations together were 4.2 ± 2.59% (range 1.5–7.7%) for ddI and 10.5 ± 1.57% (range 9.0–12.7%) for d4T. The accuracy ranges for all determinations were −15.5 to 10% for ddI and −22.5 to 14.5% for d4T. The absolute recovery of ddI and d4T, that is, the amount of drug recovered from the matrix during the sample preparation, was evaluated by comparing three processed spiked samples with three unprocessed spiked samples at two concentrations. The absolute recoveries (mean ± S.D.) for ddI were 96 ± 9.6 and 106.7 ± 11.5% at 4 and 200 ng/ml, respectively. For d4T, they were 84.8 ± 3.6 and 84.3 ± 2.8% for 4 and 200 ng/ml, respectively.

The LLOQ in CSF was 0.5 ng/ml. The mean (±S.D.) CVs of all the assays for intra-day and inter-day determinations together were 9.3 ± 0.80% (range, 8.5–10.4%) for ddI and 8.9±2.14% (range 6.1–10.6%) for d4T. The accuracy ranges for all determinations were −12.5 to 20.0% for ddI and −15.0 to 20.0% for d4T.

Table 1
Assay precision and accuracy for ddI and d4T in plasma

Spiked concentration (ng/ml)	ddI			d4T		
	Measured concentration (ng/ml)	CV (%)	RE (%)[a]	Measured concentration (ng/ml)	CV (%)	RE (%)[a]
Intra-day[b] ($n = 6$)						
4.0	4.2	3.4	0.0 to 10.0	3.8	10.4	−17.5 to 10.0
200.0	188.0	1.5	−7.5 to −3.5	209.0	9.0	−7.0 to 14.5
Inter-day[c] ($n = 10$)						
4.0	4.1	4.2	−5.0 to 7.5	3.8	12.7	−22.5 to 10.0
200.0	199.2	7.7	−15.5 to 9.0	197.0	10.0	−15.5 to 12.0

[a] [(Measured concentration − spiked concentration)/spiked concentration] × 100.
[b] Six separately spiked samples at each concentration analyzed in the same run.
[c] On five different days, spiked samples at two concentrations analyzed in duplicate.

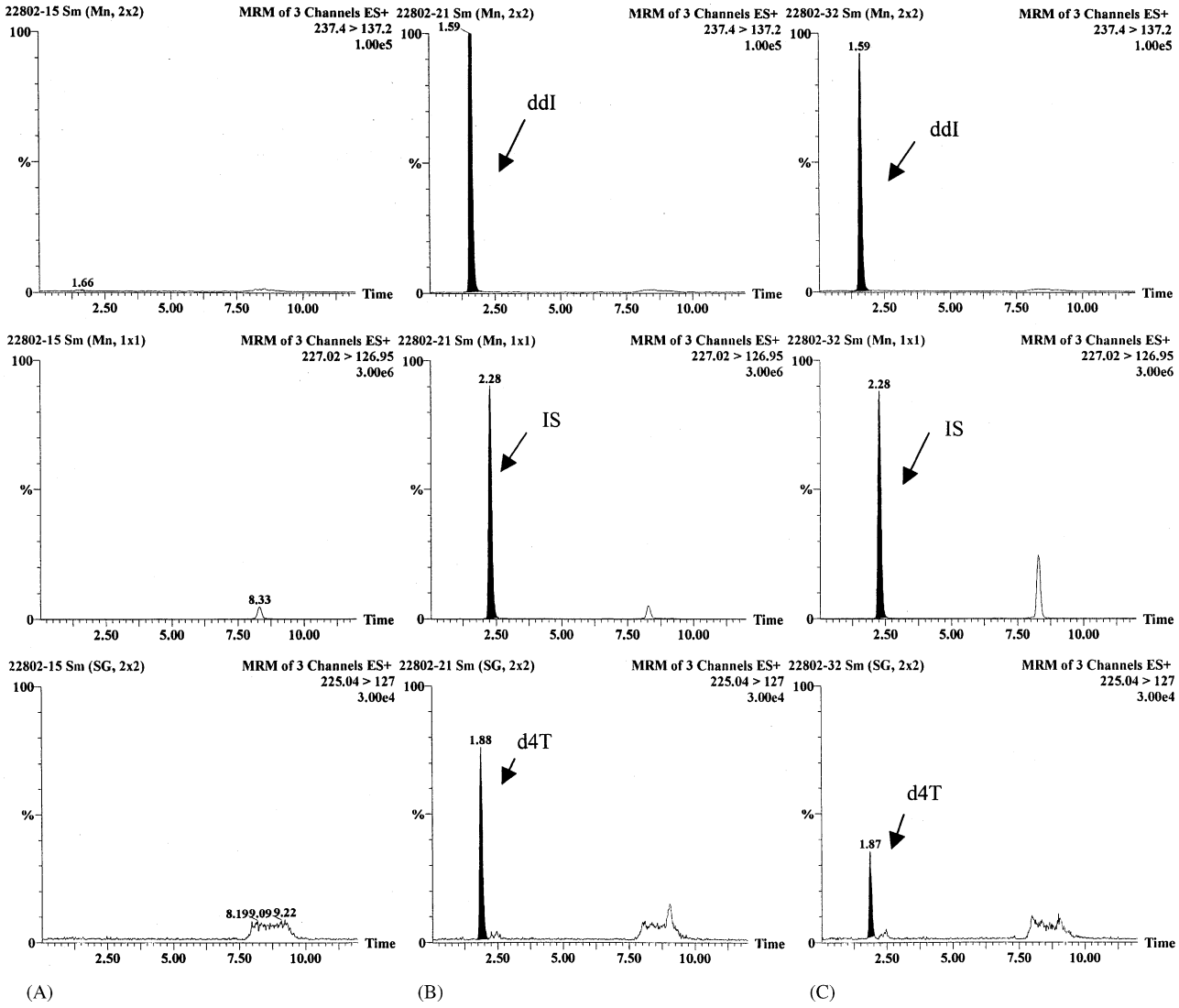

Fig. 3. Chromatograms of (A) blank BALF, (B) BALF standard with 4.0 ng/ml ddI and d4T, (C) study subject's BALF with a ddI concentration of 2.4 ng/ml and a d4T concentration of 1.7 ng/ml.

Table 2
Assay precision and accuracy for ddI and d4T in BALF supernatant

Spiked concentration (ng/ml)	ddI			d4T		
	Measured concentration (ng/ml)	CV (%)	RE (%)[a]	Measured concentration (ng/ml)	CV (%)	RE (%)[a]
Intra-day[b] ($n = 6$)						
4.0	4.3	1.7	5.0 to 10.0	4.0	2.5	−2.5 to 2.5
40.0	43.3	3.2	5.0 to 12.5	39.8	2.5	−2.5 to 2.5
Inter-day[c] ($n = 12$)						
4.0	4.5	5.8	0.0 to 22.5	4.2	5.3	−2.5 to 15.0
40.0	42.5	4.7	0.0 to 17.5	41.0	6.0	−7.5 to 15.0

[a] [(Measured concentration − spiked concentration)/spiked concentration] × 100.
[b] Six separately spiked samples at each concentration analyzed in the same run.
[c] On six different days, spiked samples at two concentrations analyzed in duplicate.

Table 3
Assay precision and accuracy for ddI and d4T in Alveolar cells

Spiked concentration (ng/ml)	ddI			d4T		
	Measured concentration (ng/ml)	CV (%)	RE (%)[a]	Measured concentration (ng/ml)	CV (%)	RE (%)[a]
Intra-day[b] ($n = 6$)						
4.0	4.4	6.1	−2.5 to 17.5	4.0	10.9	−15.0 to 15.0
100.0	100.3	5.0	−5.0 to 8.0	111.8	4.4	5.0 to 19.0
Inter-day[c] ($n = 10$)						
4.0	4.3	6.0	−2.5 to 17.5	4.4	6.2	−5.0 to 20.0
100.0	92.2	5.5	−17.0 to −1.0	98.0	5.7	−8.0 to 9.0

[a] [(Measured concentration − spiked concentration)/spiked concentration] × 100.
[b] Six separately spiked samples at each concentration analyzed in the same run.
[c] On five different days, spiked samples at two concentrations analyzed in duplicate.

Table 4
Assay precision and accuracy for ddI and d4T in seminal plasma

Spiked concentration (ng/ml)	ddI			d4T		
	Measured concentration (ng/ml)	CV (%)	RE (%)[a]	Measured concentration (ng/ml)	CV (%)	RE (%)[a]
Intra-day[b] ($n = 6$)						
4.0	4.5	6.1	5.0 to 22.5	4.2	6.8	−5.0 to 20.0
200.0	216.2	6.3	−2.0 to 16.0	199.7	6.9	−7.5 to 10.0
Inter-day[c] ($n = 12$)						
4.0	4.6	6.4	5.0 to 25.0	4.0	11.5	−15.0 to 20.0
200.0	220.9	8.9	−2.0 to 28.0	201.0	4.4	−5.5 to 10.0

[a] [(Measured concentration − spiked concentration)/spiked concentration] × 100.
[b] Six separately spiked samples at each concentration analyzed in the same run.
[c] On six different days, spiked samples at two concentrations analyzed in duplicate.

Table 5
Assay precision and accuracy for ddI and d4T in CSF

Spiked concentration (ng/ml)	ddI			d4T		
	Measured concentration (ng/ml)	CV (%)	RE (%)[a]	Measured concentration (ng/ml)	CV (%)	RE (%)[a]
Intra-day[b] ($n = 6$)						
2.0	2.1	9.1	−5.0 to 20.0	2.0	10.6	−15.0 to 10.0
40.0	40.7	10.4	−10.0 to 17.5	38.2	8.2	−15.0 to 7.5
Inter-day[c] ($n = 10$)						
2.0	2.1	8.5	−10.0 to 20.0	2.1	10.5	−15.0 to 20.0
40.0	39.2	9.1	−12.5 to 15.0	41.0	6.1	−5.0 to 12.5

[a] [(Measured concentration − spiked concentration)/spiked concentration] × 100.
[b] Six separately spiked samples at each concentration analyzed in the same run.
[c] On five different days, spiked samples at two concentrations analyzed in duplicate.

Table 6
Assay precision and accuracy for ddI and d4T in tonsil tissue

Spiked concentration (ng/mg)	ddI			d4T		
	Measured concentration (ng/mg)	CV (%)	RE (%)[a]	Measured concentration (ng/mg)	CV (%)	RE (%)[a]
Intra-day[b] ($n = 6$)						
0.050	0.054	8.8	−6.0 to 16.0	0.053	14.5	−20.0 to 22.0
0.300	0.308	6.4	−6.3 to 10.7	0.299	5.5	−7.3 to 8.0
Inter-day[c] ($n = 10$)						
0.050	0.056	5.4	2.0 to 22.0	0.053	7.0	−15.4 to 28.0
0.300	0.314	7.6	−12.3 to 17.3	0.296	7.0	−15.3 to 9.3

[a] [(Measured concentration − spiked concentration)/spiked concentration] × 100.
[b] Six separately spiked samples at each concentration analyzed in the same run.
[c] On five different days, spiked samples at two concentrations analyzed in duplicate.

Table 7
Measured concentrations of ddI and d4T in seven matrices in study subjects after 6 months of ddI and d4T treatment

Matrix	Subject #1		Subject #2		Subject #3	
	ddI concentration	d4T concentration	ddI concentration	d4T concentration	ddI concentration	d4T concentration
Plasma[a]	ND[c]	450.0	54.0	18.0	290.0	50.0
PBMC[a]	ND	ND	ND	427.0	ND	169.0
ELF[a]	ND	ND	ND	ND	ND	104.0
Alveolar cells[a]	ND	ND	ND	ND	ND	ND
Seminal plasma[a]	ND	2.0	205.0	67.0	884.0	142.0
CSF[a]	0.4	71.0	13.0	33.0	8.0	36.0
Tonsil tissue[b]	0.019	0.045	ND	ND	0.149	0.060

[a] Concentrations are in ng/ml.
[b] Concentrations are in ng/mg.
[c] ND: not detectable, <LLOQ.

The LLOQ in AC and PBMCs was 0.4 ng/ml. The mean (±S.D.) CVs of all the assays for intra-day and inter-day determinations together were 5.65±0.51% (range 5.0–6.1%) for ddI and 6.8 ± 2.84% (range 4.4–10.9%) for d4T. The accuracy ranges for all determinations were −17.0 to 17.5% for ddI and −15.0 to 20.0% for d4T.

The LLOQ in BALF supernatant was 0.4 ng/ml. The mean (±S.D.) CVs of all the assays for intra-day and inter-day determinations together were 3.85±1.79% (range 1.7–5.8%) for ddI and 4.08 ± 1.84% (range 2.5–6.0%) for d4T. The accuracy ranges for all determinations were 0–22.5% for ddI and −7.5 to 15.0% for d4T.

The LLOQ in semen was 1.0 ng/ml. The mean (±S.D.) CVs of all the assays for intra-day and inter-day determinations together were 6.93 ± 1.32% (range 6.1–8.9%) for ddI and 7.75 ± 2.97% (range 4.4–11.5%) for d4T. The accuracy ranges for all determinations were −2.0 to 28.0% for ddI and −15.0 to 20.0% for d4T.

The LLOQ in tonsil tissue was 0.01 ng/mg. The mean (±S.D.) CVs of all the assays for intra-day and inter-day determinations were 7.1 ± 1.47% (range 5.4–8.8%) for ddI and 9.88 ± 4.31% (range 5.5–14.5%) for d4T. The accuracy ranges for all determinations were −12.3 to 22.0% for ddI and −20.0 to 28.0% for d4T.

3.1. Stability

Stability studies were performed at two concentrations in plasma, semen, CSF, BALF supernatant and alveolar cells. When stored at −80 °C, neither ddI nor d4T showed any degradation in these matrices for at least 3 months.

3.2. Clinical samples

Table 7 summarizes the concentrations of ddI and d4T measured in plasma, epithelial lining fluid (ELF), alveolar cells, PBMC, seminal plasma, CSF, and tonsil tissue, in three study subjects who participated in a pharmacokinetic and virologic analysis of HIV sanctuary sites in HIV-positive patients receiving HAART. The ELF concentrations are calculated from the concentration of antibiotic measured in the BALF supernatant. We have previously described our techniques for measurement of pulmonary pharmacokinetics of antibiotics in plasma, ELF and AC [16,17]. These patients were taking Videx, 200 mg b.i.d., Zerit, 40 mg b.i.d and Crixivan, 800 mg t.i.d. for 6 months.

4. Discussion

ddI and d4T are nucleoside analogues. Their high polarities prevent them from being extracted by organic solvents. Two sample preparation methods have been reported for ddI and d4T in human plasma [3–12]. One is ultrafiltration; the other is solid phase extraction (SPE). We chose SPE as the sample preparation, it is easier to concentrate the drug from the matrices than with other methods. With SPE treatment, good absolute recoveries were obtained for both ddI and d4T in different matrices.

Since ddI and d4T are hydrophilic, the mobile phase must contain a high proportion of aqueous solvent in order to retain the drugs on the C_{18} column. We first developed an isocratic elution for analyzing ddI and d4T in human plasma, CSF and alveolar cells, which resulted in linear standard curves and good reproducibility. However, when this method was applied to BALF supernatant and seminal plasma, the results were inconsistent. We first tested different SPE conditions for these two matrices, without significant improvement. We noticed that with repeated injections of the same BALF supernatant extraction sample, the peak response at the fifth injection was several times lower than the previous injections. To confirm that the ionization suppression was due to some interference eluted from the column, we connected the split to a UV detector and monitored by UV and MS/MS simultaneously. We found that when the MRM response was reduced at the fifth injection, a big UV peak was observed at the same time (Fig. 4). To eliminate the interference eluted from the column, we changed the isocratic elution to a gradient elution. After the elution of ddI, d4T and IS, the column was washed with mobile phase B which contains a high percentage of methanol. In order to shorten the run time, we replaced the 150 mm column with

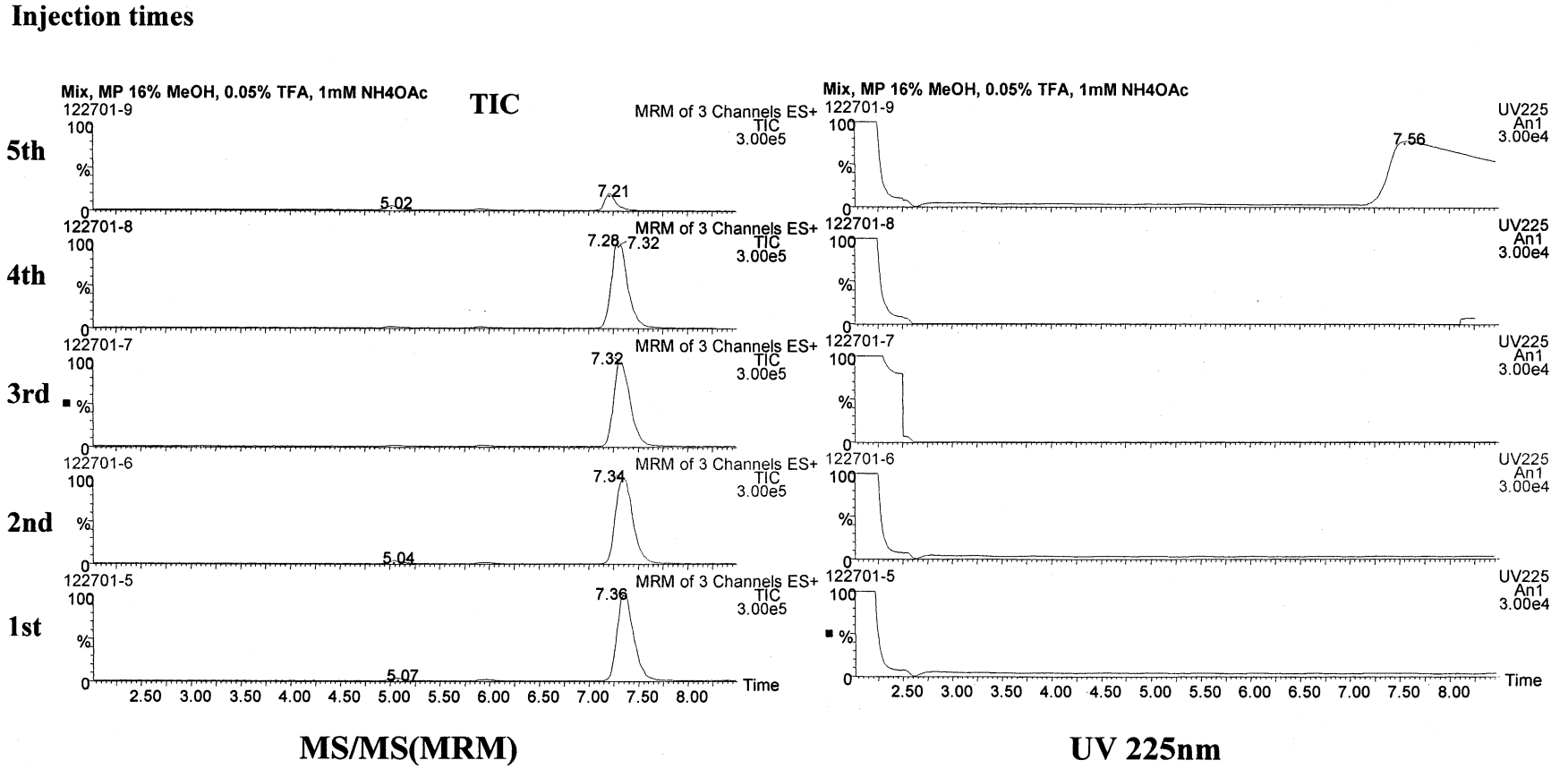

Fig. 4. Chromatograms (ddI, d4T and IS) of five injections of the same BALF supernatant sample extract with simultaneous detection by MS/MS and UV, using isocratic elution. The large peak detected by UV in the fifth injection, corresponds to the mass ionization suppression.

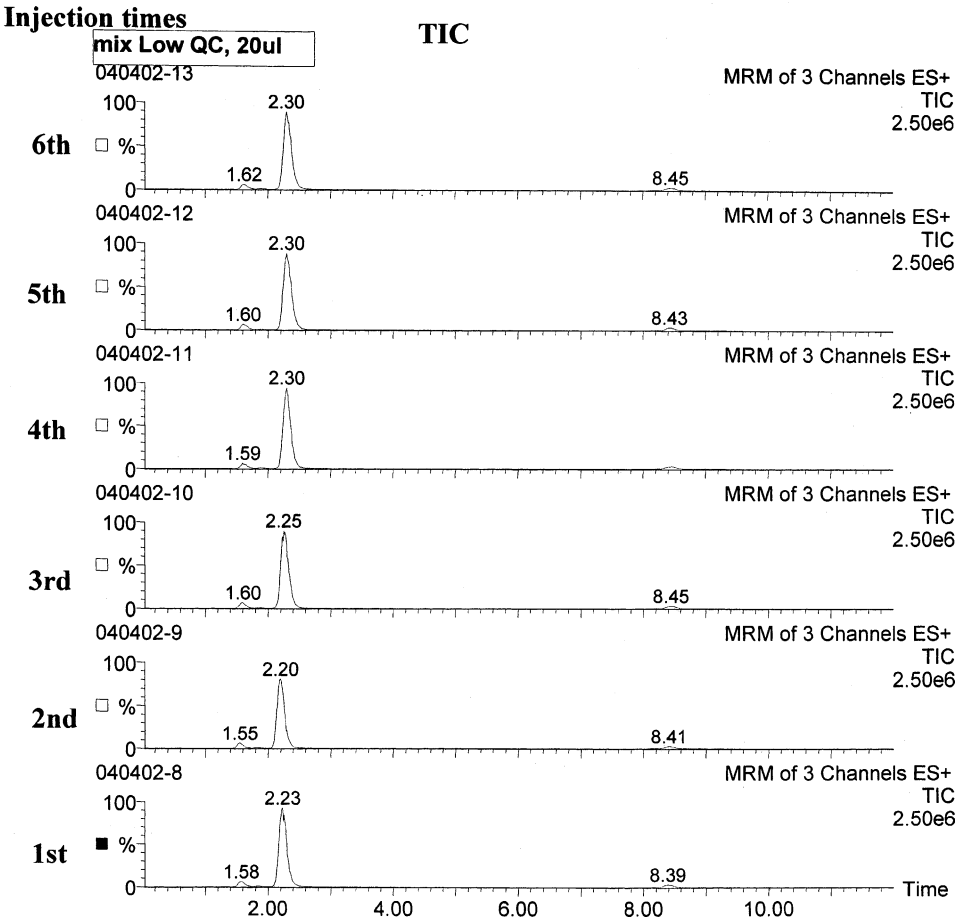

Fig. 5. Chromatograms of six injections of the same BALF supernatant sample extract showing good reproducibility using gradient elution.

a 50 mm column. Fig. 5 shows that good reproducibility was obtained with the gradient elution when six injections of the same BALF supernatant sample were made. Tables 2 and 4 summarize the ddI and d4T assay precision and accuracy in BALF supernatant and seminal fluid matrices. We also compared the UV peaks eluted with mobile phase B in human plasma, BALF supernatant and seminal plasma samples and found that human plasma had a much smaller UV peak than BALF supernatant and seminal fluid. This supported the observation that seminal plasma and BALF supernatant have more matrix effect than human plasma.

Because of its high sensitivity and selectivity, LC/MS/MS is widely used to determine drug concentrations in biological matrices. However, the matrix effect sometimes affects the assay results, especially with ESI ionization [20,21]. Most interference peaks in biological matrices were eluted with the front in reversed phase chromatography. However, since ddI and d4T are very polar compounds, a high aqueous mobile phase has to be used resulting in some interfering peaks being eluted later, which might cause the ionization suppression of the next injections. By using gradient elution, this problem can be avoided.

5. Conclusion

We have developed a sensitive, LC/MS/MS assay that provides specific, sensitive determinations of ddI and d4T simultaneously, in seven matrices. Sample preparation is performed by solid phase extraction for all the matrices. The limit of quantitation of the assays in plasma and CSF are lower than those reported in other HPLC methods. Assays in ELF, AC, seminal plasma and tonsil tissue have not been reported.

This method is being used to support a study of the distribution of ddI and d4T in the seven compartments presented in this report.

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