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Simultaneous determination of abacavir and zidovudine from rat tissues using HPLC with ultraviolet detection

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Abstract

A simple high-performance liquid chromatography (HPLC) method has been developed and validated for the simultaneous determination of abacavir and zidovudine (AZT) in rat plasma, amniotic fluid, fetal, and placental tissues. Extraction of abacavir, AZT, and the internal standard, azidouridine (AZDU) in amniotic fluid was carried out by protein precipitation. Extraction from plasma, fetal and placental homogenates was achieved by using a salting out technique. Chromatographic separation was performed using a C_8 column (150 mm × 4.6 mm, 5 μm). The mobile phase consisted of 12% acetonitrile in 25 mM sodium phosphate buffer (adjusted to pH 7 with sodium hydroxide) for the fetus, placenta, plasma and amniotic fluid samples at a flow rate of 0.8 mL/min. The method was validated over the range from 0.05 to 50 μg/mL for both abacavir and AZT in the four biological matrices. The absolute recovery of abacavir ranged from 79 to 94%, while AZT recoveries ranged from 79 to 90% in the different biological matrices. The internal standard recovery ranged from 90 to 92%. Acceptable intra- and inter-day assay precision (<10% R.S.D.) and accuracy (<10% error) were observed over 0.05–50 μg/mL for all four matrices.

Keywords: Abacavir; Zidovudine; Validation; Bioanalytical; HPLC

1. Introduction

In 2005, an estimated 700,000 children were newly infected with HIV worldwide and 540,000 children died from AIDS [1]. Vertical transmission of HIV may occur early or late in pregnancy, during birth or postnatally through breastfeeding. In the absence of preventative measures, about 35% of children born to HIV-positive women will contract the virus. With the advent of potent antiretroviral therapies, a great reduction in morbidity and mortality has been achieved. Combination therapy or highly active antiretroviral therapy, generally consisting of two nucleoside reverse transcriptase inhibitors (NRTIs) and a non-nucleoside reverse transcriptase inhibitor (NNRTI) or protease inhibitor (PI), is currently the recommended standard treatment of HIV-infected non-pregnant adults in industrialized countries [2]. In resource-poor settings, the approach to antiretroviral prophylaxis of vertical transmission of HIV is dramatically different in that the goal is to obtain effective, shorter, and less expensive antiretroviral regimens [3,4].

Zidovudine (AZT) belongs to the NRTI class of antiretroviral agents and was the first anti-HIV agent to be approved by the FDA for the treatment of HIV-1 infection. In 1994, results from the PACTG protocol 076 clinical trial demonstrated that short-course AZT therapy reduced the maternal–fetal transmission of HIV-infected pregnant women from 26 to 8% [5]. As resistance to zidovudine begins to increase, emphasis has shifted to combination therapies. Combination therapies involving AZT can further reduce vertical transmission to less than 2% [6].

Abacavir is a novel nucleoside reverse transcriptase inhibitor (NRTI) used for the treatment of HIV and is a synthetic analogue of guanine. It differs structurally from other NRTIs in that it is a carbocyclic nucleoside analogue rather than a dideoxynucleoside analogue. Abacavir sulfate was approved by the FDA in 1998 for use in combination with other antiretroviral agents for the treatment of HIV-1 infection in adults and children [7]. It is also prescribed in combination with lamivudine (3TC) and AZT, and marketed as Trizivir® for use in combination with a non-nucleoside reverse transcriptase inhibitor or a protease inhibitor.

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Abacavir is classified by the FDA as a pregnancy category C drug, meaning that animal studies have shown an adverse effect on the fetus and there are no well-controlled studies in humans, but potential benefits may warrant use of this drug in pregnant women despite potential risks [8].

While the use of combinations of antiviral drugs is becoming increasingly common, the impact of such combination therapies on placental transport is largely unknown. A series of studies have reported the lack of interaction between several anti-HIV drugs, suggesting passive diffusion as the primary mechanism of placental transfer [9,10]. However, the combination of abacavir and AZT was not studied. Recent studies of other antiviral compounds found substantial interactions between antiviral compounds in placental transport [11,12]. Continued study of these compounds is necessary to gain further understanding of the mechanism of placental transport for this important class of therapeutic agents.

Due to ethical concerns, pregnant women are excluded from clinical trials, making it difficult to study placental and fetal distribution in humans [13]. Therefore, an animal model must be utilized that will provide clinically useful information. The pregnant rat model has been proved successful for the investigation of the basic mechanisms involved in placental transfer of nucleoside analogs due to the structural similarities between rat and human placenta [14]. The large litter size allows for serial sampling, providing a complete concentration versus time profile. The pregnant rat model has been utilized in maternal–fetal drug transfer studies of a variety of compounds, including nucleoside analogs [12,15–21].

Several HPLC methods have been developed for abacavir and AZT analysis [22–25]. However, none of these methods deal with the analysis of these compounds from complex matrices such as maternal plasma, amniotic fluid, placental and fetal homogenates. Also, some of the methods use long run times and large sample volumes. This report describes the development and validation of a rapid HPLC assay for the simultaneous determination of abacavir and AZT in pregnant rat plasma, amniotic fluid, fetal and placental tissues. This method will be used in support of a comparative pharmacokinetic study to investigate the impact of abacavir and AZT combination therapy on placental transport.

2. Experimental

2.1. Chemicals and reagents

Abacavir was obtained from GlaxoSmithKline. AZT and internal standard, 3′-azido-3′-deoxythymidine (AZDU), were obtained from Sigma (St. Louis, MO). HPLC-grade acetonitrile, sodium phosphate monobasic, and ammonium sulfate were obtained from Fisher Scientific (Fair Lawn, NJ, USA).

2.2. Preparation of stock and standard solutions

Stock solutions of 1.0 mg/mL abacavir, AZT, and AZDU were individually prepared in deionized water. Standard solutions of abacavir and AZT were prepared by mixing and diluting the appropriate amounts from the individual stock solutions. The final concentrations of the standard solutions were 500, 250, 50, 25, 5, 2.5 and 0.5 μg/mL. A 25 μg/mL standard solution of AZDU was prepared with deionized water from the 1.0 mg/mL stock. Precision and accuracy standards with concentrations of 400, 35, 2, and 0.5 μg/mL were also prepared in the same manner. Stock solutions were kept refrigerated when not in use and replaced on a bi-weekly basis. Fresh standard solutions were prepared for each day of analysis or validation.

2.3. Chromatographic system

The chromatographic analyses were performed using an HPLC system consisting of an Agilent 1100 Series components including a quaternary pump, degasser, autosampler, and variable-wavelength UV detector (Palo Alto, CA, USA). Chromatographic separations were achieved using an Agilent Eclipse XDB C_8 column (150 mm × 4.6 mm, 5 μm) (Palo Alto, CA, USA) with a Phenomenex Security Guard C_{18} guard column (Torrance, CA, USA).

2.4. Chromatographic conditions

The mobile phase used for all biological matrices was 12% acetonitrile in 25 mM sodium phosphate buffer (adjusted to pH 7 with sodium hydroxide). The mobile phase flow rate was 0.8 mL/min and the detection wavelength was set at 270 nm. Under the chromatographic conditions described, abacavir, AZT and AZDU eluted at 8.3, 13.5, and 5.7 min, respectively.

2.5. Calibration curves

Blank plasma, amniotic fluid, placenta, and fetal tissue were collected from untreated anesthetized animals. The placental and fetal tissues were homogenized with two volumes of deionized water (w/v) using an Ultra-Turbax T8 tissue grinder (IKA Labortechnik, Germany). Plasma, placenta and fetus calibration points were prepared by spiking 100 μL of the biological matrices with 10 μL of each abacavir-AZT and AZDU standard solution. Amniotic fluid calibration points were prepared by spiking 50 μL of the biological matrix with 5 μL of each abacavir-AZT and AZDU standard solution. The calibration curves of all four matrices were in the range of 0.05–50 μg/mL, with an internal standard concentration in each sample of 2.5 μg/mL. After each matrix was spiked, it was subject to further sample preparation before analysis.

2.6. Precision and accuracy

This method was validated using four QC points for each calibration curve. Five replicates of each QC point were analyzed every day to determine the intra-day accuracy and precision. This process was repeated three times over 3 days in order to determine the inter-day accuracy and precision. The concentrations of the QC points for all four matrices were 0.05, 0.2, 3.5, and 40 μg/mL.

2.7. Sample preparation

Amniotic fluid samples were prepared with protein precipitation. After spiking, samples were vortexed briefly and 10 μL of 2 M perchloric acid was added. Samples were vortexed and centrifuged for 10 min at 10,000 rpm. The supernatant was removed and the pellet was discarded. Plasma, and placental and fetal tissues were extracted using a salting out technique. Three hundred microliters of saturated ammonium sulfate solution and 400 μL acetonitrile were added to 100 μL of the biological matrices containing the analytes. The samples were vortexed and centrifuged at 10,000 rpm for 10 min. The upper organic layer was then evaporated to dryness in a vacuum centrifuge and the residues reconstituted in 100 μL deionized water. An injection volume of 20 μL was used for all samples.

2.8. Sample collection

The use of animals in this study was approved by the University of Georgia Animal Use and Care Committee. The rats were housed one animal per cage in the College of Pharmacy animal facility (AALAC accredited). The environment was controlled with daily feedings of standard chow pellets and water ad libitum.

Timed pregnant female Sprague–Dawley rats (Harlan, Indianapolis, IN, USA) weighing an average of 325 g were used. On day 19 of gestation rats were anesthetized using ketamine:acepromazine:xylazine (50:3.3:3.4 mg/kg) injected intramuscularly. A cannula was placed in the right jugular vein and a laparotomy was performed to allow concurrent serial sampling of blood and the fetal sac, each containing a fetus, placenta and amniotic fluid. The rats were administered an i.v. bolus dose of abacavir (25 mg/kg) and AZT (25 mg/kg) dissolved in 0.1N NaOH in physiological saline (pH 7.4) via the jugular cannula. Individual blood and fetal sac samples were collected at 5, 15, 30, 45, 60, 90, 120, 180, 240, 300, and 360 min after dosing and stored on ice until processed. Blood samples were collected in heparinized tubes and centrifuged at 10,000 rpm for 10 min to enable plasma collection. Placental and fetal tissue samples were homogenized in two volumes

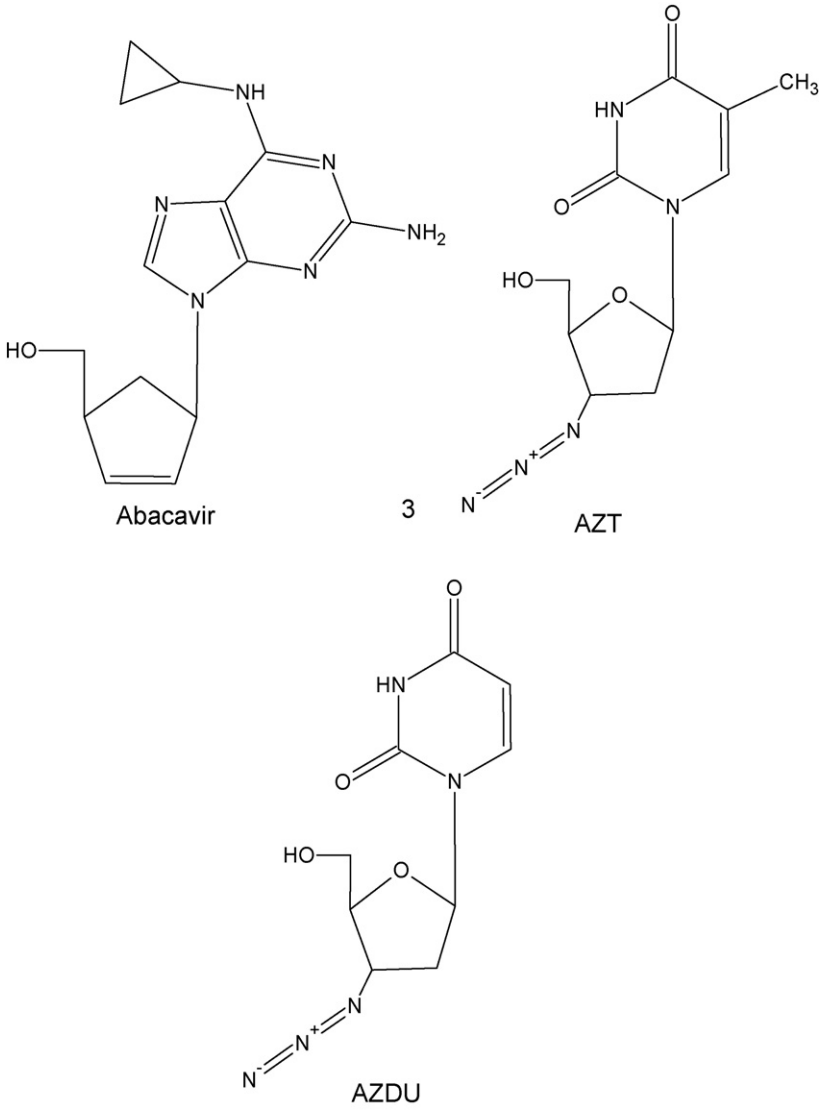

Fig. 1. Chemical structures of abacavir, zidovudine (AZT), and azidouridine (AZDU).

of deionized water. All samples were stored at −20 °C until analysis.

3. Results and discussion

The chemical structures for abacavir, AZT and AZDU are shown in Fig. 1. Due to the differences in pK_a values of the two analytes, it was necessary to maintain a pH value that was fairly neutral. Therefore, separation of the compounds from endogenous compounds was explored using various ratios of acetonitrile and buffer adjusted to pH 7. Baseline resolution was achieved at 12% acetonitrile in buffer for plasma, amniotic fluid, fetal and placental samples. Fig. 2a–d shows chromatographs of spiked abacavir and AZT (5 μg/mL) with the internal standard, AZDU. Several liquid–liquid extraction and protein precipitation methods were investigated for the extraction of abacavir, AZT, and the internal standard, AZDU, from the different biological matrices. The complexity of the biological matrices limited the use of available techniques for the extraction of abacavir and AZT due to the wide variety of endogenous substances. Therefore, sample clean-up played a critical role in eliminating the overlapping of endogenous substances with peaks of interest. For plasma, fetal and placental homogenates, a salting out technique using saturated ammonium sulfate solution

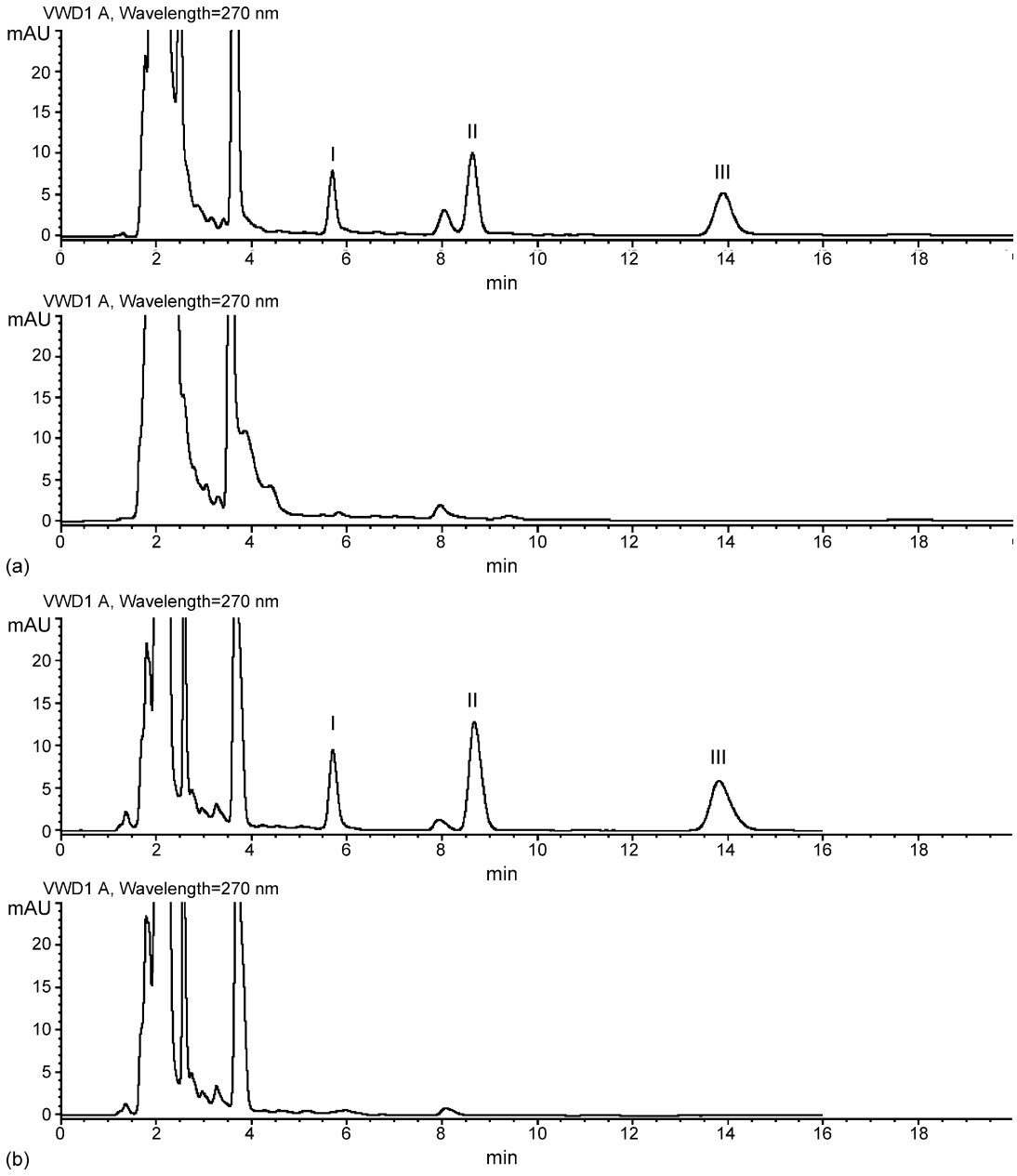

Fig. 2. (a) Chomatographs of maternal plasma spiked with 5 μg/mL abacavir (II) and AZT (III) and 2.5 μg/mL (AZDU (I) (top) and blank maternal plasma (bottom)). (b) Chromatographs of amniotic fluid spiked with 5 μg/mL abacavir (II) and AZT (III) and 2.5 μg/mL AZDU (I) (top) and blank amniotic fluid (bottom). (c) Chromatographs of fetal homogenate spiked with 5 μg/mL abacavir (II) and AZT (III) and 2.5 μg/mL AZDU (I) (top) and blank fetal homogenate (bottom). (d) Chromatographs of placental homogenate spiked with 5 μg/mL abacavir and AZT and 2.5 μg/mL AZDU (I) (top) and blank placental homogenate (bottom).

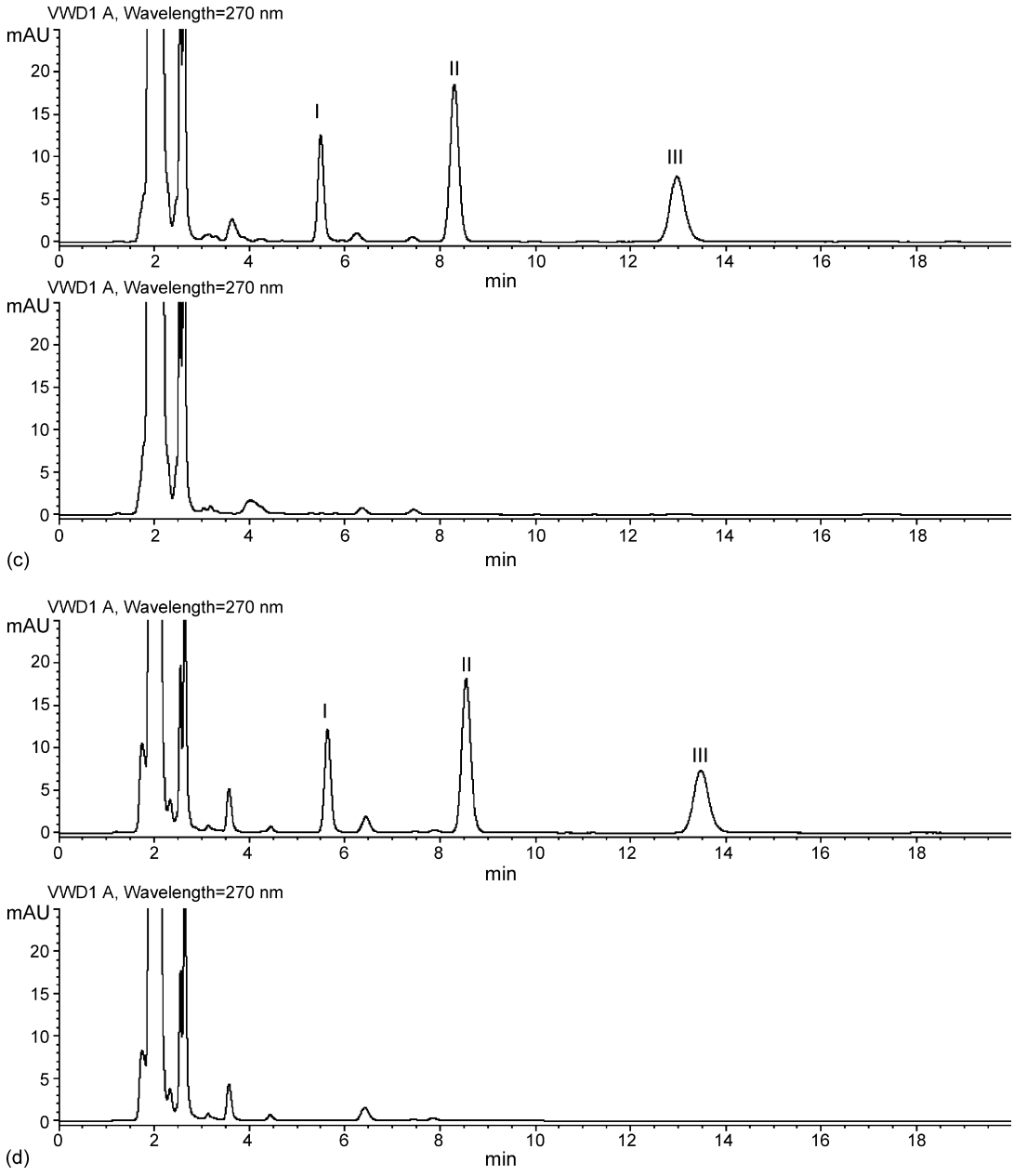

Fig. 2. (*Continued*).

and acetonitrile provided the best extraction technique. This technique has been successfully used in the sample preparation of other antiviral compounds from the pregnant rat [25]. For amniotic fluid, acid precipitation using perchloric acid achieved satisfactory separation of the analytes from the biological content.

The calibration curves for each day of validation and analysis showed acceptable linear response ($R^2 > 0.999$) through a range of 0.05–50 μg/mL. Linear regression equations were generated with JMP IN statistical software using a $1/x$-weighting scheme for each day of validation. Calibration curves for the different matrices are displayed in Table 1. The range of concentrations was sufficient for use in calculating abacavir and AZT levels from samples taken from rats that were dosed with 25 mg/kg abacavir and 25 mg/kg AZT.

The extraction efficiencies for abacavir, AZT, and AZDU from various matrices are expressed in terms of absolute recovery. The recoveries are shown in Table 2. The absolute recoveries were calculated by comparing the peak areas of spiked plasma, amniotic fluid, fetal and placental homogenate samples to the corresponding peak areas of the untreated standard solutions ($n = 15$). Abacavir recoveries ranged from 79 to 94%, while AZT recoveries ranged from 79 to 90% in the different biological matrices. The internal standard recovery ranged from 90 to 92%.

Intra-day ($n = 5$) and inter-day ($n = 15$) precision and accuracy were calculated for each matrix over 3 days. Intra-day precision and accuracy were calculated from the measurement of five samples at each QC point on 3 separate days. Inter-day precision and accuracy were calculated from the pooled data from the 3 days.

Table 1
Linear regression equations generated from validation data for each matrix; slope ± S.D., intercept ± S.D., and correlation coefficient ± S.D.

Analyte	Matrix	Slope	Intercept	R^2
ABC	Plasma	0.344 ± 0.030	0.013 ± 0.004	0.99959 ± 0.00053
	Amniotic fluid	0.390 ± 0.031	0.011 ± 0.011	0.99943 ± 0.00010
	Placental homogenate	0.494 ± 0.021	0.005 ± 0.003	0.99971 ± 0.00022
	Fetal homogenate	0.494 ± 0.019	0.006 ± 0.002	0.99962 ± 0.00051
AZT	Plasma	0.309 ± 0.027	−0.004 ± 0.002	0.99916 ± 0.00051
	Amniotic fluid	0.305 ± 0.032	0.002 ± 0.007	0.99906 ± 0.00052
	Placental homogenate	0.310 ± 0.029	0.003 ± 0.003	0.99927 ± 0.00088
	Fetal homogenate	0.325 ± 0.024	−0.001 ± 0.000	0.99931 ± 0.00031

$n = 3$, for each matrix.

Table 2
Absolute recoveries of ABC, AZT, and AZDU from plasma, amniotic fluid, placenta, and fetus ($n = 15$)

Analyte	Concentration	Plasma	Amniotic fluid	Placenta	Fetus
ABC	40	86.0 ± 3.6	78.5 ± 2.6	94.1 ± 3.5	89.6 ± 4.3
	3.5	84.3 ± 2.4	92.5 ± 2.8	89.5 ± 5.3	89.7 ± 5.5
	0.2	87.8 ± 6.5	85.6 ± 5.7	82.3 ± 4.2	92.3 ± 4.2
	0.05	90.8 ± 5.1	90.1 ± 5.4	87.5 ± 3.6	92.1 ± 4.9
AZT	40	87.3 ± 3.8	79.3 ± 2.7	80.7 ± 5.7	80.1 ± 3.8
	3.5	83.2 ± 2.4	88.6 ± 2.9	78.6 ± 4.8	82.7 ± 5.3
	0.2	84.7 ± 9.2	90.4 ± 4.9	85.5 ± 4	88.9 ± 5.7
	0.05	90.4 ± 6.1	89.1 ± 5.3	80.3 ± 4.7	83.2 ± 4.7
AZDU	2.5	92.3 ± 2.1	90.1 ± 1	91.7 ± 1.6	90.7 ± 1.5

Four QC points of concentration 0.05 μg/mL (limit of quantitation, LOQ), 0.2, 3.5 and 40 μg/mL were selected to validate the method. Intra-day precision (% R.S.D.) and accuracy (% error) for abacavir ranged from 0.23 to 9.86% and from 0.19 to 8.11%, respectively, while for AZT it ranged from 0.07 to 9.36% and from 0.01 to 8.12%, respectively. Inter-day precision and accuracy for abacavir ranged from 0.87 to 6.49% and from 0.10 to 6.20%, respectively, while for AZT it ranged and from 0.36 to 7.79% and from 0.14 to 8.69%, respectively. This validation data are compiled in Tables 3 and 4.

Table 3
Intra-day ($n = 5$) and inter-day ($n = 15$) precision (% R.S.D.) and accuracy (% error) measured for QC points for abacavir from plasma, amniotic fluid, placental and fetal tissues

T.C.	Day 1			Day 2			Day 3			Inter-day		
	E.C.	R.S.D.	Error	E.C.	R.S.D.	Error	E.C.	R.S.D.	Error	E.C.	R.S.D.	Error
Plasma												
40	42.6	6.52	6.48	41.1	3.69	2.79	38.3	0.73	4.37	40.7	6.20	1.65
3.5	3.59	2.60	6.01	3.51	1.72	0.19	3.31	1.78	5.40	3.47	5.00	0.87
0.2	0.19	4.92	6.88	0.18	2.17	8.11	0.20	3.86	0.36	0.19	5.78	4.22
0.05	0.050	0.47	2.64	0.051	7.51	3.04	0.051	6.07	1.89	0.051	5.49	1.80
Amniotic fluid												
40	37.8	5.56	3.53	38.8	3.10	2.63	38.8	3.06	2.54	38.4	3.91	2.99
3.5	3.42	2.31	3.07	3.44	1.60	3.22	3.41	2.43	2.07	3.43	2.11	2.65
0.2	0.19	5.66	1.78	0.20	0.72	3.14	0.20	0.29	3.26	0.20	2.03	3.80
0.05	0.053	5.28	7.16	0.052	3.32	5.16	0.053	5.76	3.06	0.052	4.79	5.10
Placenta												
40	40.2	0.41	2.12	38.1	4.68	1.10	39.7	0.77	2.05	39.3	1.68	2.85
3.5	3.53	0.97	2.82	3.63	3.98	3.48	3.37	3.77	2.89	3.51	0.39	4.36
0.2	0.18	8.31	0.93	0.19	3.70	3.65	0.19	5.96	3.41	0.19	5.99	3.44
0.05	0.049	2.00	2.30	0.048	3.24	1.90	0.054	8.24	6.04	0.051	1.00	6.49
Fetus												
40	40.5	1.26	1.06	39.9	0.23	3.38	39.7	0.72	2.39	40.0	0.10	2.44
3.5	3.53	1.11	0.53	3.26	6.81	2.84	3.41	2.51	2.71	3.40	2.73	4.02
0.2	0.19	0.40	1.25	0.19	4.19	0.62	0.18	9.86	2.87	0.19	4.81	4.53
0.05	0.049	2.80	3.12	0.051	1.92	2.19	0.048	3.24	5.49	0.049	1.37	4.29

T.C. denotes theoretical concentration and E.C. denotes experimental concentration.

Table 4
Intra-day ($n = 5$) and inter-day ($n = 15$) precision (% R.S.D.) and accuracy (% error) measured for four QC points for AZT from plasma, amniotic fluid, placental, and fetal tissues

T.C.	Day 1			Day 2			Day 3			Inter-day		
	R.S.D.	Error	E.C.	R.S.D.	Error	E.C.	R.S.D.	Error	E.C.	R.S.D.	Error	E.C.
Plasma												
40	41.8	4.42	4.48	40.6	2.91	1.55	37.4	1.35	6.44	39.9	5.65	0.14
3.5	3.29	3.67	6.02	3.25	1.54	7.12	3.39	1.56	3.05	3.31	2.95	5.40
0.2	0.19	5.38	3.63	0.20	1.66	1.58	0.19	1.92	4.63	0.19	3.45	3.28
0.05	0.054	2.30	8.71	0.054	2.03	9.11	0.053	2.82	6.91	0.054	2.40	8.24
Amniotic fluid												
40	39.2	2.08	3.59	38.7	3.14	2.66	38.9	2.85	2.38	38.9	2.69	2.75
3.5	3.44	1.66	3.13	3.49	0.40	1.89	3.38	3.52	1.41	3.43	1.86	2.50
0.2	0.20	0.46	2.90	0.20	0.27	3.93	0.19	3.81	2.37	0.20	1.33	3.46
0.05	0.053	7.04	3.08	0.052	4.44	8.18	0.054	8.12	1.61	0.053	6.53	4.91
Placenta												
40	39.0	2.53	2.63	39.1	2.15	2.88	38.9	2.66	2.40	39.0	2.44	2.46
3.5	3.46	1.17	1.08	3.56	1.92	3.76	3.43	1.85	3.25	3.49	0.36	3.23
0.2	0.18	7.72	5.43	0.21	5.16	5.60	0.19	4.48	9.36	0.20	2.34	8.69
0.05	0.051	2.00	4.78	0.053	6.28	6.43	0.054	0.64	7.67	0.051	2.97	6.39
Fetus												
40	40.0	0.01	1.42	40.7	1.81	4.12	43.7	5.09	7.88	40.6	5.92	1.51
3.5	3.51	0.28	1.38	3.51	0.20	7.15	3.36	6.62	0.07	3.46	3.63	1.06
0.2	0.20	1.78	2.33	0.20	0.55	4.38	0.20	2.93	3.93	0.20	7.79	0.88
0.05	0.051	2.20	4.82	0.052	4.20	5.33	0.054	0.67	9.21	0.053	5.64	5.39

T.C. denotes theoretical concentration and E.C. denotes experimental concentration.

Stability testing was performed for abacavir, AZT, and AZDU at the concentration level of 0.2 μg/mL. Spiked matrix samples (20 samples) were subjected to three consecutive freeze/thaw cycles over a period of 4 days. Five samples were extracted and analyzed as described before. The remaining spiked matrix samples were stored at −20 °C. Each of the following 3 consecutive days, spiked matrix samples were thawed, and 5 more were extracted and analyzed. The day-to-day measured peak areas of abacavir, AZT, and AZDU were compared and the results listed in Table 5. The % R.S.D. between the average peak areas of abacavir, AZT, and AZDU each day was less than 15%. The stability of extracted matrix samples in the autosampler was also evaluated. At time 0, three samples of each matrix were injected onto the HPLC column and analyzed. In another 24 h, the same samples from each matrix were injected again. The peak areas for abacavir, AZT and AZDU in each injection were compared.

Table 5
Results of freeze/thaw stability of abacavir, AZT, and internal standard, AZDU, in maternal plasma, amniotic fluid, placenta, and fetus, represented by area ± S.D.

	Maternal plasma	Amniotic fluid	Placenta	Fetus
Abacavir				
Day 1	9.56 ± 0.98	10.8 ± 0.93	10.58 ± 1.45	9.16 ± 1.59
Day 2	9.94 ± 10.8	10.22 ± 1.39	8.22 ± 1.11	9.8 ± 0.76
Day 3	9.16 ± 0.69	9.18 ± 0.59	9.66 ± 1.29	8.24 ± 1.17
Day 4	9.98 ± 0.66	9.4 ± 0.32	10.54 ± 1.74	9.26 ± 0.36
% R.S.D.	8.8	8.1	14.3	10.6
AZT				
Day 1	5.28 ± 0.87	5.02 ± 0.7	3.66 ± 0.69	4.34 ± 0.42
Day 2	5.96 ± 0.74	5.62 ± 0.04	4.68 ± 0.49	5.66 ± 0.38
Day 3	5.06 ± 0.75	5.04 ± 0.32	5.52 ± 0.44	5.38 ± 0.42
Day 4	4.72 ± 0.48	5.04 ± 0.51	4.78 ± .2	5.56 ± 0.31
% R.S.D.	13.4	7.6	9.8	7.3
AZDU				
Day 1	94.78 ± 9.94	107.96 ± 3.8	98.56 ± 11.21	111.54 ± 14.08
Day 2	85.18 ± 5.9	107.3 ± 6.43	76.12 ± 16.71	104.38 ± 5.07
Day 3	99.22 ± 7.57	105.46 ± 4.71	72.9 ± 5.33	103.18 ± 5.81
Day 4	99.58 ± 4.77	112.02 ± 2.46	78.4 ± 13.63	109.64 ± 4.24
% R.S.D.	7.2	4.0	14.4	6.8

($n = 5$) of each day and % R.S.D. of the area between days.

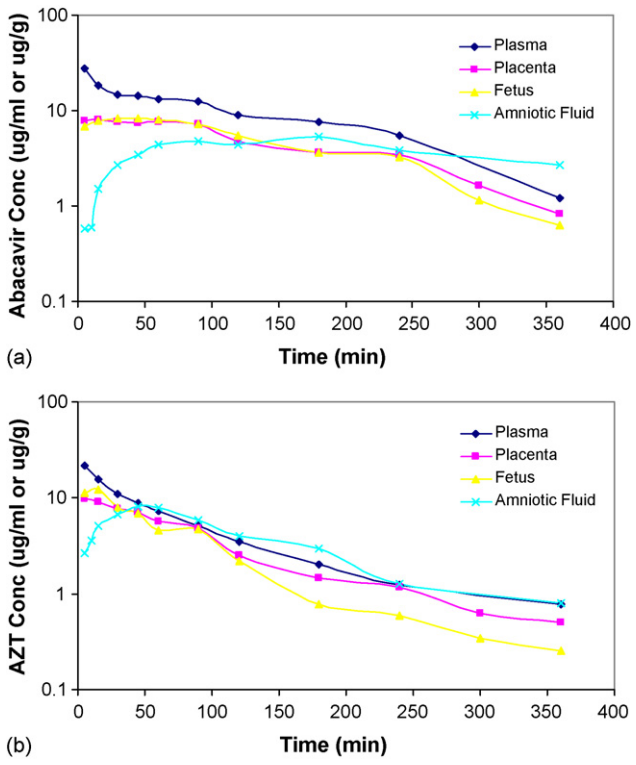

Fig. 3. Concentration vs. time profiles of abacavir (a) and AZT (b) in maternal plasma, amniotic fluid, placenta, and fetus after 25 mg/kg i.v. bolus doses of abacavir and AZT.

The % R.S.D. between samples was less than 13% for all three compounds and no obvious decline in peak areas between each injection was observed.

To demonstrate the utility of this assay, a pregnant rat was given an i.v. bolus dose of abacavir and AZT (25 mg/kg each). Plasma, amniotic fluid, placental and fetal tissues were processed and analyzed as mentioned. A calibration curve from each matrix was prepared on the day of analysis to calculate the concentrations of abacavir and AZT present in the real samples. Before analysis, each sample collected from the dosed pregnant rat was spiked to yield a concentration of 2.5 μg/mL of the internal standard. In addition, real samples were spiked with the appropriate amounts of deionized water (10 μL for plasma, fetal, and placental homogenates, 5 μL for amniotic fluid) to account for the addition of abacavir and AZT in the calibration points. The sample peak area ratios of abacavir and AZT to the internal standard were used to calculate the concentrations of abacavir and AZT in each sample. The concentration–time profiles of abacavir and AZT in all matrices are shown in Fig. 3. Plasma data was analyzed using a two-compartment model using WinNonlin (Pharsight, Mountain View, CA, USA). For abacavir, estimates for half-life ($t_{1/2}$), volume of distribution at steady state (V_{ss}), and clearance (CL) in maternal plasma were 213.4 min, 1.46 L/kg, and 5.83 mL/min kg, respectively. For AZT, estimates for $t_{1/2}$, V_{ss}, and CL in maternal plasma were 85.7 min, 1.74 L/kg, and 17.17 mL/min kg. These values were in close agreement with earlier reported data for abacavir and AZT [12,26].

4. Conclusions

A sensitive and accurate method was developed and validated for the quantification of abacavir and AZT in rat maternal plasma, amniotic fluid, placental and fetal tissues. The use of acid precipitation and salting out techniques provided an inexpensive and convenient method of sample preparation. This method yielded high recoveries, good linearity, and precision and accuracy in the range of 0.05–50 μg/mL. This method will be useful for pharmacokinetic studies to investigate the fetal and maternal disposition of abacavir and AZT in the pregnant rat.

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