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ORIGINAL RESEARCH

Simultaneous determination of zidovudine and lamivudine from rat plasma, amniotic fluid and tissues by HPLC

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ABSTRACT: A sensitive HPLC method has been developed and validated for the simultaneous quantification of zidovudine (AZT) and lamivudine (3TC) in rat plasma, amniotic fluid and placental and fetal tissues. Samples were processed by solid-phase extraction using C_2 cartridges. Chromatography was performed using a phenyl column (5 μm, 150 × 2 mm i.d.) under a flow rate of 0.2 mL/min. The mobile phase consisted of 8% acetonitrile in 5 mM 1-heptane sulfonic acid dissolved in 30 mM ammonium formate buffer (pH 3.3). The method was validated in the range 0.25–50 μg/mL for both 3TC and AZT in the four biological matrices. Finally, the method was applied to a study involving fetal transport of co-administration of these compounds in a pregnant rat. Copyright © 2004 John Wiley & Sons, Ltd.

KEYWORDS: zidovudine; lamivudine; ion-pairing chromatography; validation; bioanalytical

INTRODUCTION

Multi-drug therapy has become the standard treatment for acquired immunodeficiency syndrome (AIDS) (Clercq *et al*., 2002). This situation is imposed by the need to delay the development of resistance by the human immunodeficiency virus (HIV), the causative virus of AIDS, to single anti-HIV drugs and to minimize potential dose-dependent side effects (Beach, 1998). The current typical regimen for treating HIV infection is to use a combination of at least three drugs, a practice known as ‘highly active antiretroviral therapy’ (HAART) (Gallant, 2002).

All anti-HIV drugs can be classified into three categories: (i) nucleoside reverse transcriptase inhibitors (NRTIs), including zidovudine, lamivudine, didanosine, zalcitabine, abacavir and stavudine; the active triphosphate metabolites of NRTIs act as chain terminators at the substrate-binding site of the viral enzyme reverse transcriptase. (ii) Non-nucleoside reverse transcriptase inhibitors (NNRTIs), including nevirapine, delavirdine and efavirenz; NNRTIs interact with the viral reverse transciptase enzyme at a non-substrate binding site. (iii) Protease inhibitors (PIs), including saquinavir, ritonavir, indinavir, nelfinavir, amprenavir and lopinavir; PIs inhibit the HIV enzyme protease, which causes the production of immature viruses incapable of spreading to new uninfected cells (Beach, 1998). Most of the HAART regimens include at least two NRTIs, such as AZT and 3TC, in combination with another NNRTI, such as nevirapine, or a PI, such as indinavir (Gallant, 2002). The use of AZT and 3TC together has become so common that these two compounds are now formulated in the same tablet and sold under the trade name Combivir™.

The use of HAART has increased survival and improved quality of life for AIDS patients (Gallant, 2002). This positive outcome results from extensive understanding of the pharmacological, toxicological and pharmacokinetic profiles of all individual anti-HIV agents in HIV-infected individuals. Unfortunately, less is known about the behavior of these drugs in pregnant women infected with HIV. Pregnant women are a group of special interest among AIDS patients because of vertical transmission of HIV to fetuses. Vertical transmission can take place before labor, during labor or by breast-feeding after delivery (JUNP on HIV/AIDS, 1996). Worldwide vertical transmission of HIV is reaching epidemic proportions with over 600,000 infected infants being born every year (Mofenson and Mclntyre, 2000).

In pregnant women, the ultimate goal of therapy is to decrease the viral load and suppress viral replication in the fetal compartment as well as the maternal compartment. A better understanding of the kinetics of drug transport across the placenta would help in achieving this goal. The acquisition of this data has become more important with the recent report of significant drug–drug interactions in the placental transport of the antiviral nucleosides acyclovir and zidovudine (Brown *et al*., 2003). Therefore, more

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Abbreviations used: AZT, zidovudine; HAART, highly active antiretroviral therapy; NRTI, nucleoside reverse transcriptase inhibitors; NNRTI, non-nucleoside reverse transcriptase inhibitors; PI, protease inhibitor; 3TC, lamivudine.

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understanding of the consequences of drug–drug interactions between the individual agents in HAART on the transport of these drugs across the placenta would aid in achieving therapeutic levels in the fetal compartment.

To investigate the kinetic profiles of 3TC and AZT transport across the placenta, an appropriate animal model is needed where the results can be successfully extrapolated to humans. Pregnant rats are an appropriate model because of the anatomical resemblance between the rat and human placenta and because both placentas belong to the same hemochorial type (Faber and Thornburg, 1983; Knipp *et al*., 1999).

In order to build a kinetic profile with reliable parameters that accurately describe the kinetics of drug transport across the placenta, a reliable, accurate, sensitive and rugged analytical method is needed to analyze 3TC and AZT in the plasma amniotic fluid, placenta and fetal tissues of the rat. Several HPLC-UV and LC-MS methods are available in the literature for the simultaneous quantification of AZT and 3TC in human plasma (Kenney *et al*., 2000; Pereira *et al*., 2000; Fan and Stewart, 2002). All these methods utilize solid-phase extraction (SPE) using different cartridges for the extraction of 3TC and AZT from the biological matrix. Extraction methods that are optimized for less complex matrices, such as plasma or urine, are frequently not robust enough for direct transfer to tissues.

This paper reports an efficient and reproducible HPLC method using ultraviolet detection. This method has been validated for quantifying AZT and 3TC from maternal plasma, amniotic fluid, fetal and placental tissues collected during a maternal–fetal drug transfer study. The assay reported here is the first to report quantitation of these commonly co-administered compounds from such complex tissue matrices. This method requires small plasma and amniotic fluid volumes in order to maximize the number of pharmacokinetic time points that can be collected during the animal experiment. This study utilized the pregnant rat model where all samples of the four biological matrices were collected at various time-points to get a complete profile of the drug distribution across the placenta.

EXPERIMENTAL

Chemicals and reagents. Albuterol (internal standard) and 1-heptane sulfonic acid were purchased from Sigma (St Louis, MO, USA). HPLC-grade acetonitrile and methanol were obtained from Fisher Scientific (Fair Lawn, NJ, USA). Ammonium formate was obtained from Aldrich (Milwaukee, WI, USA). Syringe filters (0.22 μm) were purchased from XPERTEK (St Louis, MO, USA). Syringes (1 mL) were obtained from Becton Dickinson Co. (Franklin Lakes, NJ, USA). BOND ELUT-C2, silica, cyano and phenyl cartridges were purchased from Varian (Harbor City, CA, USA). C_8, C_{18} and Oasis HLB cartridges were obtained from Waters (Milford, MA, USA).

Instrumentation. All chromatographic analysis were performed using a HP 1090 series II HPLC system (Agilent, Wilmington, DE, USA) with an external Gilson Model 117 UV detector (Middleton, WI, USA). All data were analyzed using Turbochrom 4.21 software (PerkinElmer, Torrance, CA, USA). A YMC phenyl column (5 μm, 150 × 2 mm i.d., Waters, Milford, MA, USA) equipped with a Phenomenex C_{18} guard column (Torrance, CA, USA) was used to perform all chromatographic separations.

The mobile phase consisted of 8% acetonitrile in 5 mM 1-heptane sulfonic acid dissolved in 30 mM ammonium formate buffer (pH 3.3). The flow rate was 0.2 mL/min and the detection wavelength was 254 nm. The injection volume was 15 μL.

Preparation of standard solutions. Stock solutions of 1 mg/mL AZT and 3TC were individually prepared in distilled water. Standard solutions of AZT and 3TC were prepared by mixing and diluting the appropriate amounts from the individual stock solutions. The final concentrations of the standard solutions were 500, 400, 250, 100, 10, 7.5, 5, 2.5 and 2 μg/mL. Two albuterol stock solutions of concentrations 1 and 3 mg/mL were prepared in distilled water.

Calibration curves. Blank plasma, amniotic fluid and placental and fetal tissues were harvested from untreated animals. The placental and fetal tissues were homogenized with 2 vols of distilled water (v/w). Plasma, placenta and fetus calibration points were prepared by spiking 100 μL of the biological matrices with 10 μL of each 3TC–AZT and albuterol standard solution. Amniotic fluid calibration points were prepared by spiking 50 μL of the biological matrix with 5 μL of each 3TC–AZT and albuterol standard solution.

Two different internal standard (albuterol) solutions were used to prepare the calibration curves of the four matrices. An internal standard stock solution with a concentration of 1000 μg/mL was used for the plasma and amniotic fluid matrices. For the fetal and placental homogenates, an internal standard stock solution of 3000 μg/mL was used.

The calibration curves of all the four matrices were in the range of 0.2–50 μg/mL with individual calibration points of 50, 25, 10, 1, 0.75 and 0.2 μg/mL. The internal standard concentration was 100 μg/mL in plasma and amniotic fluid, while it was 300 μg/mL for the fetal and placental tissue homogenates.

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 0.25, 0.5, 5 and 40 μg/mL.

Sample preparation. Several liquid–liquid and solid-phase extraction techniques were investigated for sample clean-up. SPE using C_{18}, C_8, C_2, silica, cyano, phenyl, amino and Oasis HLB cartridges was investigated. The SPE procedure included conditioning the samples with 1 mL of methanol followed by 1 mL of water. The spiked samples were then

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loaded onto the conditioned cartridges and washed with 75 μL of distilled water. The analytes were eluted with 1 mL of methanol and evaporated under vacuum. Finally, samples were reconstituted in 100 μL of distilled water.

Protein precipitation using perchloric acid or acetonitrile was also investigated: 20 μL of 2 M perchloric acid or 400 μL of acetonitrile were added to 100 μL of the spiked samples. Samples were vortexed and centrifuged at 13,000 rpm for 10 min. The supernatant was aspirated and evaporated under vacuum. Samples were then reconstituted in 100 μL of distilled water.

Finally, 3TC, AZT and albuterol extraction from the different matrices was attempted by the addition of a high concentration of salt. In these experiments, 200 μL of saturated ammonium sulfate and 400 μL of cold acetonitrile were added to the spiked samples. Samples were vortexed and centrifuged at 13,000 rpm for 10 min. The upper organic layer was aspirated and dried under vacuum. Samples were then reconstituted in 100 μL of distilled water.

Animal experiment. 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 University of Georgia College of Pharmacy animal facility (AALAC accredited). The environment was controlled (20–22°C, 14 h of light per day) with daily feedings of standard chow pellets and water ad libitum.

A timed pregnant Sprague–Dawley rat (Harlan, Indianapolis, IN, USA) weighing 335 g was anesthetized intramuscularly with ketamine:acepromazine:xylazine (50:3.3:3.4 mg/kg) and dosed on day 19 of gestation. During anesthesia, the animal was given atropine (0.5 mg/kg) subcutaneously. For dosing and blood sampling purposes, a cannula was surgically implanted in the right jugular vein. For sampling of the pups (amniotic fluid, placenta and fetal tissues), a laparotomy was performed. The rats were administered an i.v. bolus dose of AZT (25 mg/kg) and 3TC (25 mg/kg) dissolved in 0.1 N NaOH in physiological saline (pH 7.4) via the jugular cannula. Blood samples were collected at 5, 15, 30, 45, 60, 90, 120, 180, 240, 300 and 360 min after dosing into heparinized tubes and centrifuged at 10,000 rpm for 10 min to enable plasma collection. Amniotic fluid, placenta and fetus samples were collected at 5, 10, 15, 30, 45, 60, 90, 120, 180, 240, 300 and 360 min. Placental and fetal tissue samples were homogenized in two volumes of deionized water. All samples were stored at −20°C until analysis.

RESULTS AND DISCUSSION

Development of HPLC assay

Structures of 3TC, AZT and albuterol are shown in Fig. 1. 3TC is a hydrophilic weak base with a pK_a = 4.3 while AZT (pK_a = 9.7) is much more lypophilic than 3TC (Fridland *et al.*, 2000). Under reverse-phase chromatographic conditions, using a phosphate buffer mobile phase, very little methanol was needed to separate 3TC from the polar endogenous compounds in the different biological matrices. Using a low percentage of methanol, AZT eluted at 40 min while 3TC eluted at 13 min.

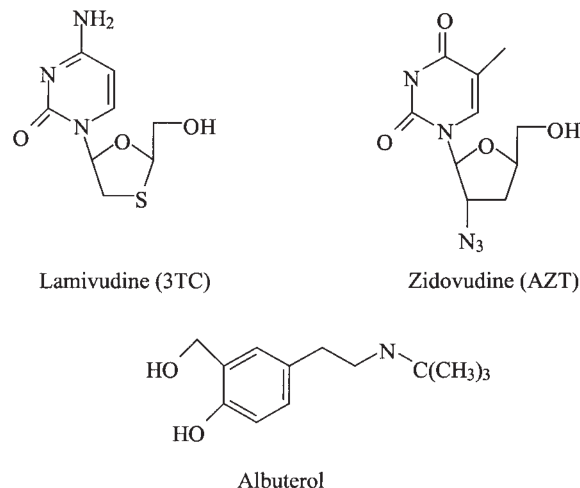

Figure 1. Chemical structures of lamivudine, zidovudine and albuterol.

To force AZT to elute earlier, a gradient method increasing the methanol percentage in the mobile phase over time was employed. Combinations of continuous and step gradient techniques were attempted, however the baseline drift was too significant for effective quantification. This baseline drift made it hard to reproducibly integrate the baseline for the AZT peak, causing the method to fail several validation days.

Therefore, an alternative method using ion-pairing chromatography was considered. 3TC is a weak base and carries a negative charge at acidic pHs, while AZT is hydrophobic enough to be retained in the column for a longer period of time. Therefore, we used a lower concentration of an anionic ion-pairing reagent under acidic conditions. The ionic interaction between the negatively charged ion pair and the positively charge 3TC increases the retention of 3TC. At the same time, the negative charge on the ion pair decreases the hydrophobic interaction between AZT and the phenyl column and therefore decreases the retention of AZT.

Pentane, heptane and octane sulfonic acids were investigated to serve as ion pairing reagents. The retention time of 3TC increased by increasing the length of the hydrocarbon chain or the concentration of the ion pair reagent. The retention time of AZT decreased from 40 to 15 min after the addition of any of these anionic ion pairing agents. Neither the hydrocarbon chain length nor the concentration of the ion pairing reagent made a significant difference to the AZT retention time. This behavior suggests that the retention of 3TC was due to electrostatic interaction with the ion pair while the retention of AZT was primarily due to hydrophobic interaction with the phenyl column. This hydrophobic interaction was decreased in the presence of the ion pairing agent and resulted in a greater than 60% decrease of the retention time from that observed in the absence of the ion pair agent. Five millimolar

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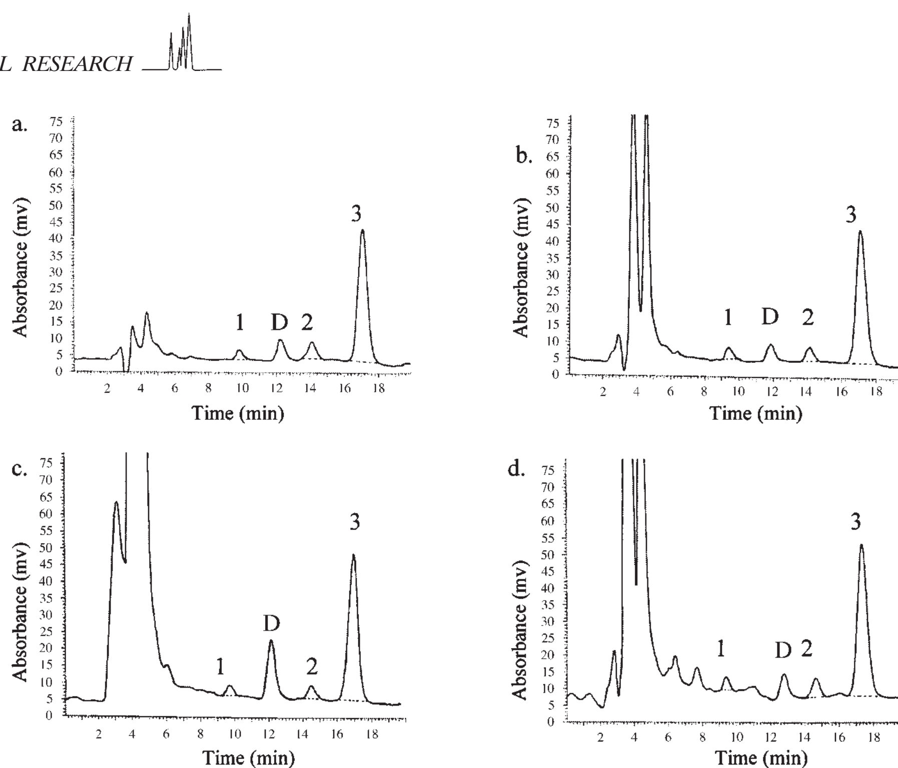

Figure 2. Representative chromatograms of 3TC (peaks marked '1'), AZT (peaks marked '2') and albuterol (peaks marked '3') in (a) fetus, (b) amniotic fluid, (c) placenta and (d) plasma. Concentration of AZT and 3TC in all the chromatograms is 10 μg/mL. Concentration of albuterol is 100 μg/mL in plasma and amniotic fluid and 300 μg/mL in placental and fetal tissues. Note: the peak marked D is an endogenous peak.

heptane sulfonic acid provided the condition to achieve baseline resolution between the analytes and the endogenous peaks. Representative chromatograms of 3TC, AZT and albuterol in the four biological matrices are shown in Fig. 2. 3TC, AZT and albuterol eluted in 9, 15 and 17 min, respectively.

The pH of the mobile phase was a critical factor to achieve a successful chromatographic separation. In order to retain 3TC, the pH should be lower than the pK_a (4.7) of 3TC. Increasing the pH gradually from 3 to 7 decreased the retention time of 3TC. Interestingly, pH also had a great effect on the retention time of an endogenous peak, marked 'D' in the chromatograms. AZT and this endogenous peak formed the critical pair of peaks for the separation. A pH of 3.3 was found to achieve baseline resolution between all the compounds of interest in the chromatogram.

Methanol and acetonitrile were investigated for the use as organic modifiers. Acetonitrile produced better peak shapes than methanol. This may be due to higher rates of mass transfer of the analytes between the mobile and the stationary phases, which results from the higher strength of acetonitrile as an organic solvent. Using 8% acetonitrile produced baseline resolution of all analytes of interest.

The main concern in the search for an internal standard was to find a compound to elute within 20 min without coeluting with any of the analytes or the endogenous compounds under our chromatographic conditions. Albuterol was found to elute in 18 min and was therefore chosen as an internal standard.

Extraction procedures

None of the liquid–liquid extraction or protein precipitation techniques produced samples clean enough to work out chromatographic separation between the analytes and the peaks resulting from the endogenous compounds in the different biological matrices. Therefore, SPE was the remaining choice for our method.

Among the SPE cartridges investigated, C2 cartridges produced the cleanest samples with chromatograms that contained very few endogenous peaks. The amount of water used to wash the cartridges after loading the sample as well as the amount of methanol used to elute the analytes was investigated to optimize the extraction efficiency of the analytes. A wash step using 0.75 mL of water was enough to remove all the undesirable endogenous compounds and produce a reasonable recovery of the analytes. One milliliter of methanol eluted the analytes with the highest recovery. Further increases in the methanol amount did not result in higher recovery of the analytes.

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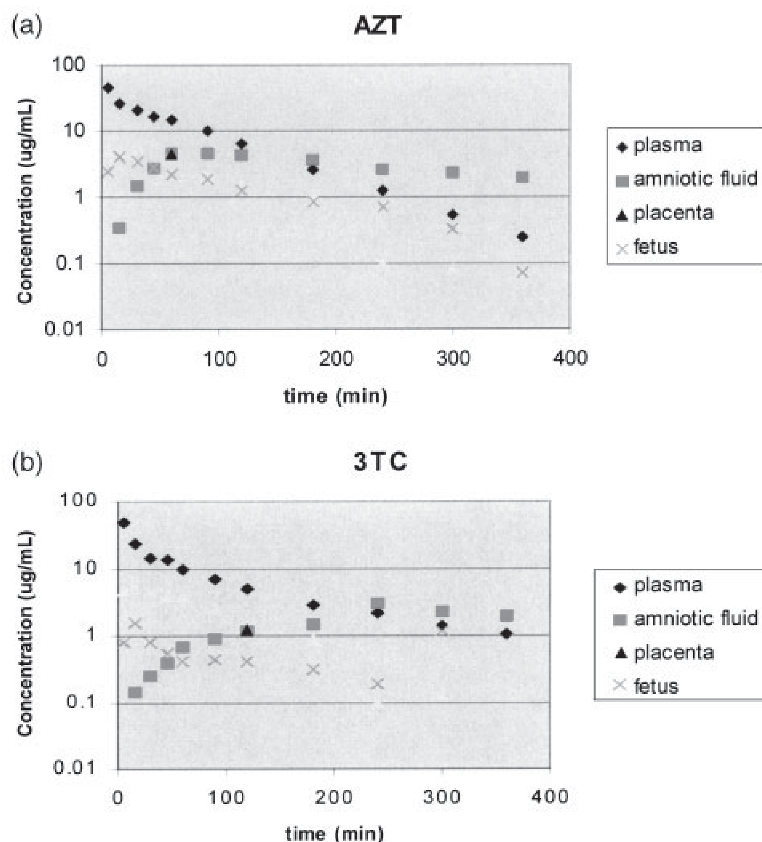

Figure 3. Concentration-time profile of (a) AZT and (b) 3TC in plasma, amniotic fluid, placental and fetal homogenates when administered as a combination therapy.

The recoveries of 3TC, AZT and albuterol from the four biological matrices are shown in Table 1. 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). 3TC recoveries ranged from 60 to 78% while AZT recoveries ranged from 77 to 90% in the different biological matrices. The internal standard recovery was lower and ranged from 12 to 40%.

Accuracy and precision

Assay precision and accuracy were calculated for each matrix over 3 days. Precision, as expressed by %RSD, and accuracy as expressed by percentage error for 3TC and AZT in the four biological matrices are shown in Table 2. Intra-day (n = 5) precision and accuracy were calculated from the measurement of five samples at each QC point on three separate days. Inter-day (n = 15) precision and accuracy were calculated from the pooled data from the 3 days. Four QC points of concentrations 40, 5 and 0.5 μg/mL and the lowest concentration in the calibration curve (0.25 μg/mL) were selected to validate the method. Intra-day precision (%RSD) and accuracy (percentage error) for 3TC ranged from 0.6 to 13.5 and 0.9 to 14.1%, respectively, while for AZT it ranged from 0.7 to 12.9 and 1.4 to 12.3%, respectively. Inter-day precision and accuracy for 3TC ranged from 1.23 to 10.8 and 1.87 to 11.7%, respectively, while for AZT it ranged from 1.0 to 10.2 and 1.7 to 10.3%, respectively. The calibration curves showed acceptable linearity ($r^2 > 0.99$) over the range 0.25–50 μg/mL for plasma, amniotic fluid, placental and fetal homogenates.

Animal study

To demonstrate the application of this method in animal studies, a female rat was dosed with a combination of 3TC and AZT IV bolus (25 mg/kg for each compound). Plasma, amniotic fluid, placental and fetal tissues were processed and analyzed as mentioned. Figure 3 shows the concentration–time profile of 3TC and AZT in all matrices. The plasma data was analyzed using WinNonlin (Pharsight, Mountain View, CA, USA). For 3TC, half-life, volume of distribution at steady state, area under the curve (AUC) and clearance were calculated to be 89 min, 1160 mL/kg, 2490 min mg/L and 10 mL/min/kg, respectively. For AZT, half-life,

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Table 1. Absolute recoveries of 3TC, AZT and albuterol in plasma, amniotic fluid, placenta and fetus ($n = 15$)

Concentration	Analyte	Plasma	Amniotic fluid	Fetal tissue	Placental tissue
40	3TC	76 ± 5.4	74 ± 6.2	65 ± 4.9	61 ± 7.2
	AZT	84 ± 6.3	85 ± 3.8	80 ± 7.0	77 ± 4.2
5	3TC	77 ± 7.0	76 ± 5.8	66 ± 2.4	61 ± 5.6
	AZT	89 ± 3.9	90 ± 8.1	81 ± 6.6	78 ± 4.4
0.5	3TC	76 ± 5.8	78 ± 7.1	62 ± 4.6	61 ± 1.9
	AZT	82 ± 4.3	86 ± 6.6	77 ± 5.5	77 ± 8.7
0.2	3TC	72 ± 3.9	74 ± 5.3	66 ± 6.1	60 ± 5.1
	AZT	90 ± 6.0	88 ± 4.7	80 ± 3.9	87 ± 7.8
300	Albuterol	—	—	16 ± 1.6	12 ± 1.1
100		38 ± 3.6	40 ± 2.6	—	—

Table 2. Intra-day ($n = 5$) and inter-day ($n = 15$) precision (%RSD) and accuracy (percentage error) measured for four QC points for AZT and 3TC in plasma, amniotic fluid, placental and fetal tissues

		Day 1			Day 2			Day 3			Inter-day		
	TC	EC	RSD	Error	EC	RSD	Error	EC	RSD	Error	EC	RSD	Error
							Plasma						
3TC	40	41.6	5.8	4.2	40.9	3.4	6.1	43.2	5.2	5.8	41.9	4.92	5.37
	5	4.91	7.9	0.9	4.42	1.2	1.5	4.69	3.5	3.2	4.67	3.41	1.87
	0.5	0.48	10.9	9.2	0.47	8.9	7.3	0.45	6.3	10.9	0.47	7.24	9.13
	0.25	0.28	6.3	11.3	0.26	7.3	9.2	0.23	9.9	8.1	0.26	6.69	9.53
AZT	40	43.4	7.2	8.4	44.6	9.2	6.5	45.1	4.2	4.9	44.4	4.95	6.6
	5	5.23	0.9	1.5	5.64	0.7	2.2	5.51	1.3	1.4	5.46	1.00	1.7
	0.5	0.53	2.1	7.6	0.55	6.1	7.3	0.54	5.8	6.8	0.54	3.81	7.23
	0.25	0.27	12.2	10.2	0.26	9.2	12.3	0.28	8.8	7.8	0.27	9.12	10.1
							Amniotic fluid						
3TC	40	42.3	8.2	5.2	44.9	6.3	3.1	43.9	6.8	3.9	43.7	6.54	4.07
	5	5.15	2.1	2.1	5.34	0.6	2.5	5.11	5.1	6.8	5.20	1.23	3.27
	0.5	0.55	9.4	6.2	0.52	6.5	8.3	0.49	4.9	3.4	0.52	5.23	5.97
	0.25	0.24	12.5	13.3	0.25	10.1	11.6	0.23	13.0	7.9	0.24	10.8	10.9
AZT	40	43.8	6.9	4.6	45.1	5.5	9.4	39.6	5.6	7.2	42.8	5.74	7.07
	5	4.86	1.6	5.9	5.36	4.3	7.3	5.16	3.3	5.5	5.13	1.92	6.23
	0.5	0.45	7.3	8.9	0.53	6.9	6.5	0.46	8.5	8.4	0.48	7.12	7.93
	0.25	0.23	11.6	9.9	0.24	8.7	10.2	0.24	9.1	8.1	0.24	8.95	9.4
							Placenta						
3TC	40	42.6	6.2	8.3	44.1	4.56	7.46	40.7	3.87	3.87	42.5	4.01	6.54
	5	4.91	1.9	0.98	5.34	6.14	3.54	5.14	1.45	1.45	5.13	2.16	3.22
	0.5	0.52	8.3	4.7	0.54	5.54	7.47	0.53	2.55	2.55	0.53	4.68	4.91
	0.25	0.26	13.5	11.8	0.26	8.37	9.40	0.24	6.79	6.79	0.25	7.97	9.33
AZT	40	44.0	9.6	6.9	38.8	6.89	3.67	37.2	9.49	4.58	40.0	7.14	5.05
	5	4.86	3.13	4.12	4.79	5.24	7.38	4.58	2.47	3.63	4.74	2.67	5.04
	0.5	0.54	5.24	8.76	0.52	6.99	7.35	0.44	8.25	9.67	0.50	7.02	8.59
	0.25	0.28	8.7	12.0	0.27	10.14	8.56	0.22	9.59	8.14	0.26	9.16	9.57
							Fetus						
3TC	40	43.6	6.47	5.35	42.5	9.56	7.12	45.1	7.35	4.67	43.7	7.24	5.71
	5	5.34	1.65	2.43	5.35	4.18	1.37	5.14	5.12	3.98	5.28	2.19	2.59
	0.5	0.51	7.98	8.79	0.45	6.87	9.47	0.46	8.97	7.38	0.47	7.24	8.55
	0.25	0.22	8.98	11.2	0.24	9.17	9.98	0.26	10.1	14.1	0.24	9.43	11.7
AZT	40	44.1	8.96	6.87	41.9	5.98	6.45	43.6	8.34	5.35	43.2	6.31	6.22
	5	4.93	6.10	1.45	4.45	1.36	7.34	5.15	2.56	2.68	4.84	2.16	3.82
	0.5	0.46	9.34	8.93	0.48	8.85	7.92	0.48	7.82	9.43	0.47	8.33	8.76
	0.25	0.26	12.4	9.37	0.26	12.91	10.3	0.23	8.14	11.2	0.25	10.2	10.3

Note: TC stands for theoretical concentration and EC stands for the actual measured (experimental) concentration.

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volume of distribution at steady state, the AUC and clearance were calculated to be 51 min, 650 mL/kg, 2620 min mg/L, and 9.5 mL/min/kg, respectively.

The pharmacokinetic values for 3TC had interesting differences when compared with the parameter estimates obtained when the compound was administered as a single agent. Lamivudine showed an increased half-life (64 min) and AUC (1530 min mg/L) and maintained a similar volume of distribution at steady state (1250 mL/kg). The clearance of 3TC decreased by 40% from 16.3 mL/min/kg. However, zidovudine did not show much change in pharmacokinetic parameters, with the possible exception of half-life (88 min). The volume of distribution at steady state (880 mL/kg), AUC (2600 min mg/mL) and clearance (9.7 mL/min/kg) was very similar when zidovudine was administered as a single agent or in combination. This data combined with the fetal and placental compartment data suggests that the transport of 3TC was increased by the presence of AZT. However, this interaction requires further study before any definitive conclusions can be drawn, but the results are similar to the interaction between acyclovir and zidovudine (Brown *et al*., 2003).

CONCLUSION

A sensitive, efficient and accurate method was developed and validated for the simultaneous quantification of 3TC and AZT in rat plasma, amniotic fluid, placental and fetal tissues. The SPE procedure produced samples with few remaining endogenous compounds. This efficient sample clean-up facilitated the method development by expediting the development of the chromatographic conditions. This method is useful for pharmacokinetic studies to investigate the distribution of 3TC and AZT in the maternal and fetal compartments of rats, where the preliminary data suggests that there is a significant interaction between these two compounds.

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