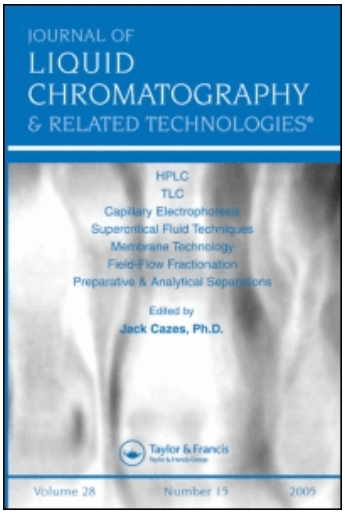

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Simultaneous Determination of Zalcitabine and Stavudine in Maternal Plasma, Amniotic Fluid, Placental, and Fetal Tissues Using Reversed Phase on Silica Liquid Chromatography/Tandem Mass Spectrometry

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Abstract: In order to study the placental transfer of nucleoside reverse transcriptase inhibitors, a quick and simple reversed phase high performance liquid chromatography with tandem mass spectrometry method has been developed and validated using an underivatized silica column for the separation and analysis of DDC and D4T from rat plasma, amniotic fluid, placental, and fetal homogenate. Extraction of DDC, D4T, and their internal standard lamivudine (3TC) from the matrices was processed by protein precipitation using ice cold acetonitrile. Chromatographic separation was achieved on a Waters Spherisorb S3W silica column (4.6 mm × 100 mm) equipped with a Phenomenex guard column. The mobile phase consisted of 20% methanol in 22 mM formic acid. The flow rate was 0.4 mL/min, and MRM was used for detection. The calibration curves for each day of validation showed good linear response over the range from 2 ng/mL to 2000 ng/mL. The absolute recoveries for all the drugs are all higher than 70%, and the matrix effects are all lower than 20%. All the intra- and inter- day assay precision and accuracy were better than 10% for all the matrices.

Keywords: Amniotic, Fetal tissues, Maternal plasma, Placental, Reversed phase, Silica LC, Stavudine, Tandem mass spectrometry, Zalcitabine

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INTRODUCTION

For children, about 90% of the human immunodeficiency virus (HIV) infections are due to vertical transmission from the infected mothers through blood, amniotic fluid, and/or breast milk.[1,2] This perinatal transmission of HIV occurs as a result of transplacental dissemination of the virus and intrapartum exposure to infected blood in the genital tract.[3–5]

With the number of HIV infections increasing, it is essential to determine the placental transfer of antivirals because of the increased resistance of HIV to zidovudine.[6,7] In addition, the use of multidrug therapies has become the rule rather than the exception in the treatment of patients with HIV infections.[8] The use of these drugs is important in the maintenance of maternal health through pregnancy and in possible prevention of vertical transmission of HIV.[7] While the use of combinations of antiviral drugs is popular, the impact of such combination therapies on placental transport is largely unknown.

Our previous studies showed substantial interactions between the antivirals AZT and acyclovir, AZT and 3TC, and between Zalcitabine (DDC) and Stavudine (D4T).[9–12] The data from these studies support a transporter mediated mechanism for placental transport. However, a series of studies by Unadkat and coworkers has reported the lack of interaction between several anti-HIV drugs when using the macaque as an animal model; their findings suggest passive diffusion as the primary mechanism for placental transport.[13–15] The differences between these studies may be related to the animal models, experimental design, or may be specific to the agents studied. More important, the dose used in our previous studies is several times higher than the dose used in human beings or the doses used in macaques from other studies. In order to obtain pharmacokinetic data, which is more directly comparable to other studies, we decided to conduct a pharmacokinetic study in rats with a dose of 5 mg/kg of the DDC/D4T combination. Several HPLC methods have been developed to determine the concentrations of DDC and D4T.[16–23] An HPLC-UV method was developed previously for the simultaneous determination of DDC and D4T in the pregnant rat model within a linear range from 0.1 μg/mL to 50 μg/mL.[12] However, these methods are either not sensitive enough or do not include all the necessary matrices we are going to use. Thus, a sensitive and specific analytical method was needed for this pharmacokinetic study. In this study, a rapid and sensitive HPLC tandem MS method was developed and validated, using reversed phase liquid chromatography on an underivatized silica column for the determination of concentrations in samples taken in a maternal fetal drug transfer study of DDC and D4T.

EXPERIMENTAL

Reagents and Chemicals

DDC and D4T were obtained from Sigma (St. Louis, MO, USA). The internal standard, 3TC, was obtained from GlaxoSmithKline (RTP, NC, USA). HPLC grade methanol and acetonitrile were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Reagent grade formic acid was from Sigma (St. Louis, MO, USA). The deionized water used was generated from a Continental Deionized Water System (Natick, MA, USA).

Instrumentation

An Agilent 1100 series HPLC system, consisting of a degasser, binary pump, autosampler, and thermostatted column compartment, was used in this study (Agilent, Palo Alto, CA, USA). The mass spectrometer utilized for this work was a Quattro Micro triple quadrupole mass spectrometer equipped with a Z-spray ion source (Waters, Manchester, UK). MS control and spectral processing were carried out using Masslynx software, version 4.0 (Waters, Beverly, MA, USA). Chromatographic separation was achieved on a Waters Spherisorb S3W silica column (4.6×100.0 mm, Milford, MA) equipped with a Phenomenex security guard C-18 guard column (4×3.0 mm, Torrance, CA).

Liquid Chromatographic and Mass Spectrometric Conditions

The mobile phase consisted of Solvent A (22 mM formic acid) and Solvent B (methanol) (80:20). The flow rate was set to be 0.4 mL/min, and the injection volume was 50 μL. The HPLC run time was 13 min for each run. The LC flow was diverted to waste from 0 to 3 minutes using a six port switching valve. The mass spectrometer was run in positive ion ESI mode using multiple reaction monitoring (MRM) to monitor the mass transitions. Nitrogen gas was used as the desolvation gas and was set to a flow rate of 500 L/h with a temperature of 375°C. The cone gas flow was set to 0 L/h. Argon was the collision gas and the collision cell pressure was 2.4×10^{-3} mbar. The precursor to product ion transitions along with the cone voltage and collision energy for each analyte and the internal standard were as follows: DDC m/z 212 → m/z 112, 13 V, 7 eV; D4T m/z 247 → m/z 149 (sodium adducts), 15 V, 10 eV; 3TC m/z 230 → m/z 112, 13 V, 7 eV. The source temperature and capillary voltage were set at 130°C and 2.5 kV, respectively.

Preparation of Standard Solutions

Individual DDC, D4T, and 3TC stock solutions were prepared in deionized water to give a final concentration of 10 mg/mL. Individual standard solutions of DDC and D4T with concentrations of 10, 50, 100, 500, 1000, 5000, and 10000 ng/mL were prepared by serial dilution with deionized water. Precision and accuracy standards with concentrations of 25, 250, and 2500 ng/mL were also prepared in the same manner. A 1 μg/mL 3TC standard solution was prepared with deionized water from the 10 mg/mL 3TC stock solution. The 10 mg/mL stock solutions were kept refrigerated and no degradation was observed during the period of this study. Fresh standard solutions were prepared for each day of analysis or validation.

Calibration Curves

Blank plasma was purchased from Innovative Research (Novi, MI, USA). Blank amniotic fluid, placenta, and fetal tissues were collected from untreated animals. The placental and fetal tissues were homogenized with two volumes of distilled water (v/w). Plasma, placental, and fetal calibration points were prepared by spiking 100 μL of the biological matrices with 20 μL of each DDC and D4T standard solution and 10 μL of the 1 μg/mL 3TC solution. Amniotic fluid calibration points were prepared by spiking 50 μL of the biological matrices with 10 μL of each DDC and D4T standard solution and 10 μL of the 1 μg/mL 3TC standard solution. The calibration curves of all the matrices were in the range of 2–2000 ng/mL with individual calibration points of 2, 10, 20, 100, 200, 1000, and 2000 ng/mL.

Precision and Accuracy

This method was validated using four QC points for each calibration curve. Five replicates of each QC point were analyzed each day to determine the intra-day accuracy and precision. This process was repeated 3 times in 3 days to determine the inter-day accuracy and precision. The QC points for all four matrics were 2, 5, 50, and 500 ng/mL.

Sample Preparation

All samples were prepared using protein precipitation. Ice cold acetonitrile (1 mL) was added to the samples (100 μL for plasma, placental,

and fetal homogenate, 50 μL for amniotic fluid). After being vortexed and centrifuged at 13,000 rpm for 10 min, the solid was discarded and the liquid was aspirated and dried under vacuum. Samples were then reconstituted in distilled water for injection. (100 μL for plasma, placental, and fetal homogenate, 60 μL for amniotic fluid).

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 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.

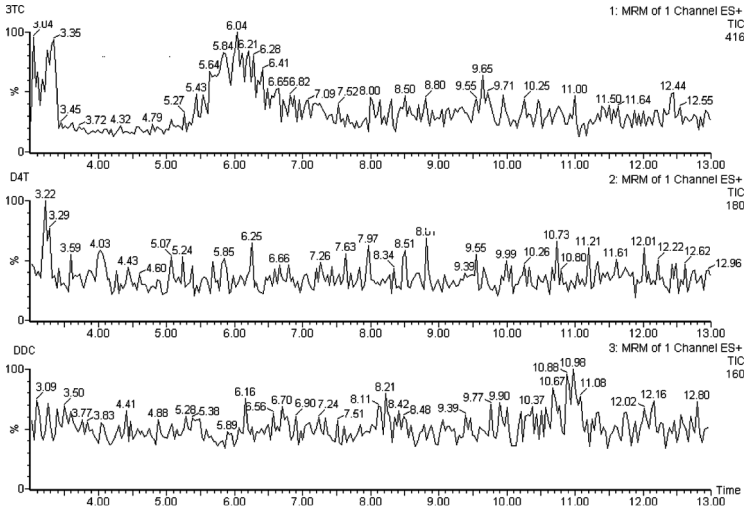
Three timed pregnant Sprauge–Dawley rats (Harlan, Indianapolis, IN, USA), weighing from 320 to 380 g, were anesthetized intramuscularly with ketamine:acepromazine:xylazine (50:3.3:3.4, mg/kg) and dosed on day 19 of gestation. For dosing and blood sampling purposes, a cannula was surgically implanted in the right jugular vein. For sampling of the pups (amniotic fluid, placenta, and fetal tissues), a laparotamy was performed. The rats were administered an i.v. bolus dose (5 mg/kg) of 5 mg/mL DDC and D4T 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, 15, 30, 45, 60, 90, 120, 180, and 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. Data was analyzed using WinNonlin (Version 5.2, Pharsight, Mountain View, CA, USA).

RESULTS AND DISCUSSION

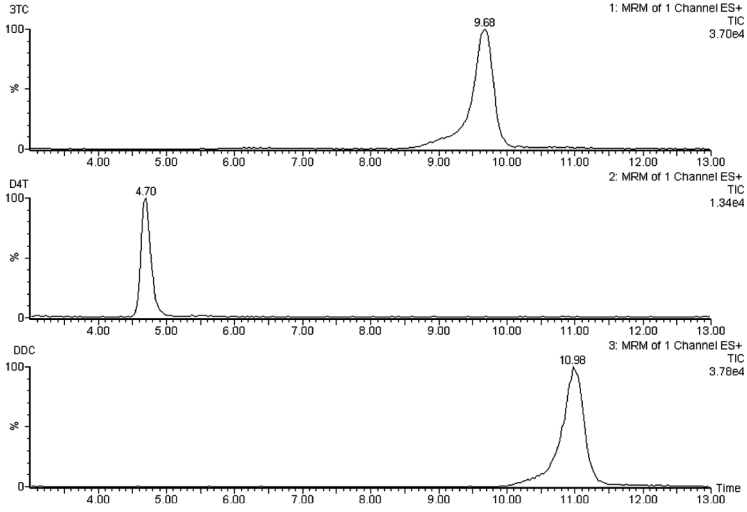
Method Development

Hydrophilic interaction chromatography (HILIC) is characterized by the presence of a high initial concentration of organic modifier to favor hydrophilic interaction between the solute and the hydrophilic stationary phase.[24] Briefly, HILIC based on silica columns is normal phase chromatography utilizing conventional reversed phase (RP) mobile phases. Thus, the retention times of highly polar compounds are increased as their hydrophilicity increases. Since both DDC and D4T are highly hydrophilic

compounds, HILIC is a good choice for retention. However, although D4T worked well under HILIC conditions (90% to 80% organic); DDC was permanently retained on the silica column when the percentage of organic solvent was higher than 30 percent. On the other hand, both DDC and D4T were well retained on the silica column with a high



(a)

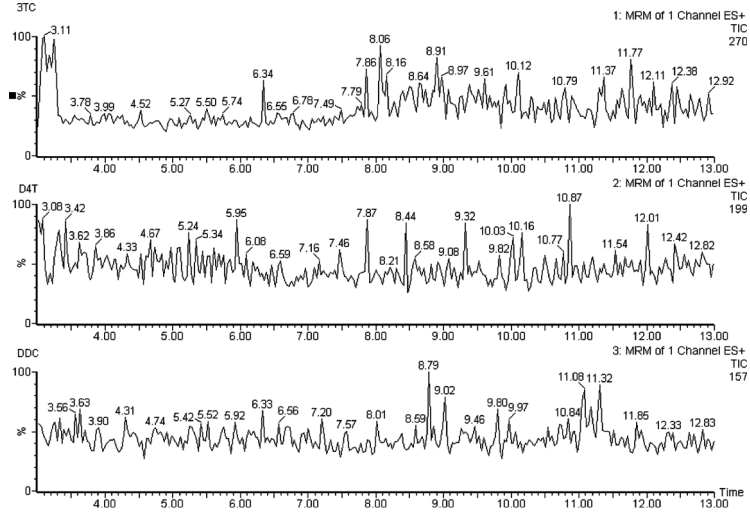


(b)

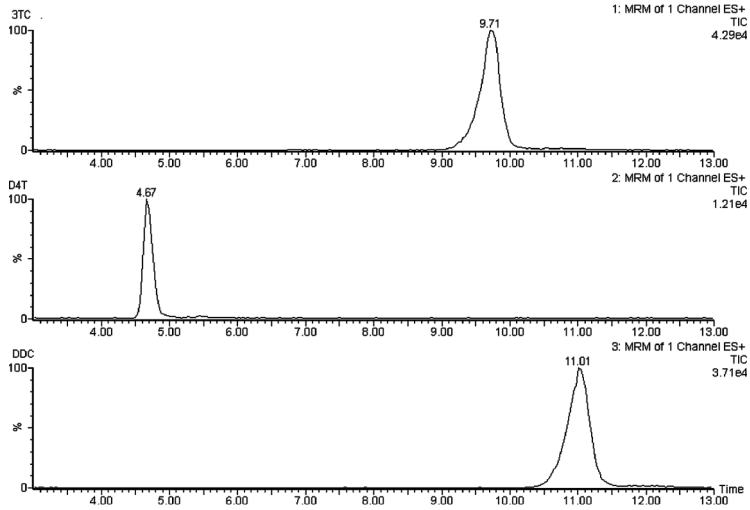
Figure 1. Chromatograms obtained from blank plasma (a) and plasma spiked with DDC (20 ng/mL), D4T (20 ng/mL), and 3TC (100 ng/mL) (b).

aqueous (10% to 20%) mobile phase. Thus, we chose to use 20% methanol on the silica column as our chromatographic conditions, which was different from both traditional reverse phase conditions and HILIC conditions.

During optimization of MS conditions, we found that the intensity of D4T plus sodium peak was 10 fold higher than the M + H peak, since D4T



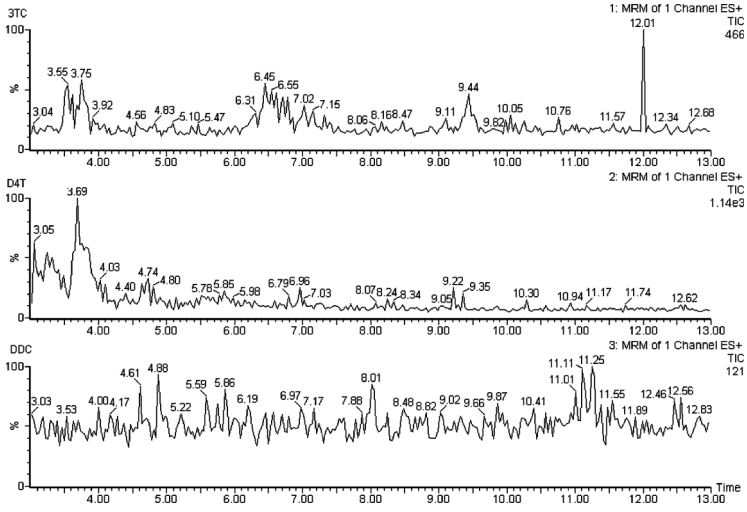
(a)



(b)

Figure 2. Chromatograms obtained from blank amniotic fluid (a) and plasma spiked with DDC (20 ng/mL), D4T (20 ng/mL), and 3TC (100 ng/mL) (b).

is a weak acid; it is rare to use adduct ions for quantitation, since the intensity of many are highly variable. However, our method meets validation requirements under FDA guideline.[25] Figures 1–4 show the representative chromatograms of each extracted blank matrix and extracted matrix spiked with DDC (20 ng/mL), D4T (20 ng/mlL) and 3TC (100 ng/mL).



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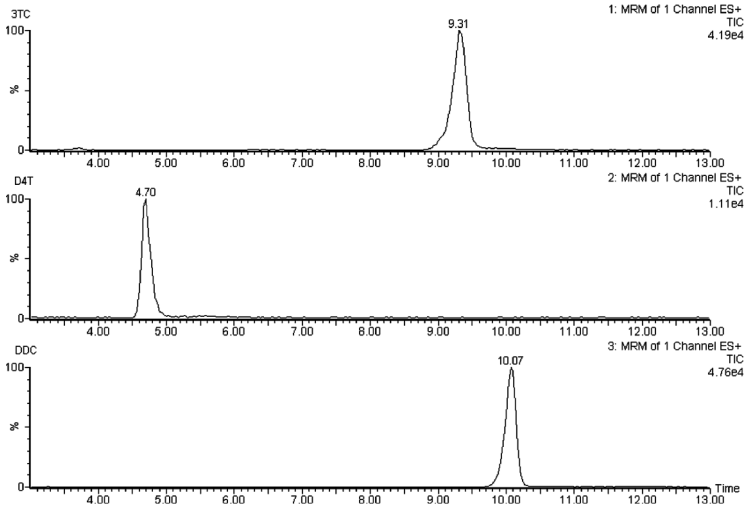
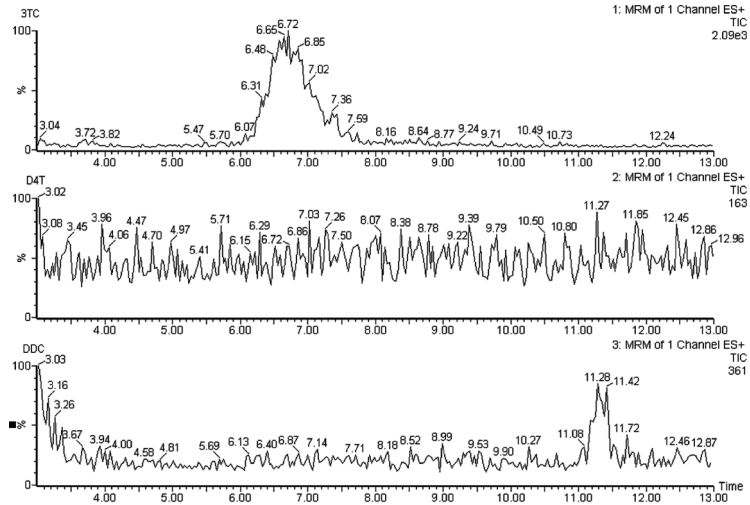
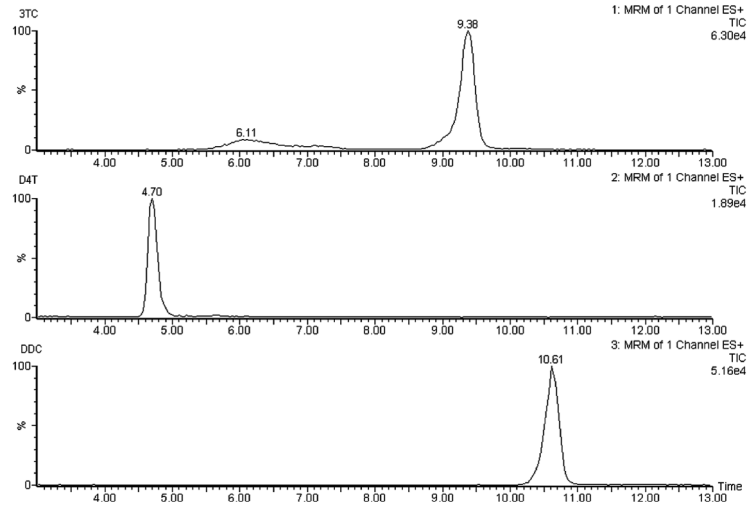


(b)

Figure 3. Chromatograms obtained from blank placental homogenate (a) and plasma spiked with DDC (20 ng/mL), D4T (20 ng/mL), and 3TC (100 ng/mL) (b).



(a)



(b)

Figure 4. Chromatograms obtained from blank fetal homogenate (a) and plasma spiked with DDC (20 ng/mL), D4T (20 ng/mL), and 3TC (100 ng/mL) (b).

Calibration Curves

The calibration curves for each day of validation and analysis showed good linear response ($R^2 = 0.992$–0.995) over the range of 2–2000 ng/mL mL for both drugs in all matrices (see Table 1). Microsoft Excel or JMP

Table 1. Linear regression fits generated from validation data from each matrix, ($n = 3$, for each matrix)

R^2	Maternal plasma	Amniotic fluid	Placental homogenate	Fetal homogenate
DDC	0.993 ± 0.002	0.995 ± 0.003	0.992 ± 0.001	0.993 ± 0.001
D4T	0.995 ± 0.001	0.994 ± 0.001	0.994 ± 0.001	0.992 ± 0.001

statistical software was used to generate linear regression equations for all calibration curves. A $1/x^2$ weighting scheme was used for each day of the validation and analysis for all four matrices.

Precision and Accuracy

Assay precision and accuracy were calculated for each matrix over 3 days. Precision, as expressed by % R.S.D., and accuracy as expressed by % error for DDC and D4T 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 pooled data over 3 days. Four QC points of concentrations 2, 5, 50, and 500 ng/mL were used for these calculations. Intra-day precision (% R.S.D.) and accuracy (% error) of DDC ranged from 1 to 7.5% and 0.48 to 9.46%, respectively. Inter-day precision and accuracy of DDC ranged from 1.32 to 9.6% and 0.06 to 6.2%, respectively. Intra-day precision and accuracy of D4T ranged from 0.5 to 7.2% and 0.4 to 8.11%, respectively. Inter-day precision and accuracy of D4T ranged from 1 to 9.2% and 0.48 to 6.69%, respectively. These results are shown in Table 2 and Table 3.

Recovery and Matrix Effect

Absolute recovery, relative recovery, and matrix effects for DDC, D4T, and 3TC are summarized in Table 4. Samples spiked with a drug concentration of 100 ng/mL were used for these calculations. Absolute recoveries ranged from 70.8% to 86.0%, and relative recoveries ranged from 84.8% to 98.5%. The suppression or the enhancement by the matrix was lower than 20% for all compounds.

Stability Studies

Stability testing was performed for DDC and D4T at 100 ng/mL concentration level. Spiked matrix samples were subjected to three consecutive

Table 2. The intra-day ($n = 5$, at each spiked concentration) and inter-day ($n = 15$, at each spiked concentration) precision (%R.S.D.) and accuracy (% error) of the LC-MS/MS method used to quantitate DDC in maternal plasma, amniotic fluid, placental and fetal homogenates

	Intra-day ($n = 5$)			Inter-day ($n = 15$)		
Concentration DDC added (ng/mL)	Concentration DDC found (ng/mL)	R.S.D. (%)	Error (%)	Concentration DDC found (ng/mL)	R.S.D. (%)	Error (%)
Maternal plasma						
2	2.14 ± 0.08	4	7	2.06 ± 0.15	7.5	3
5	4.87 ± 0.05	1	2.6	5.13 ± 0.12	2.4	2.6
50	51.64 ± 2.56	5.12	3.28	50.93 ± 3.43	6.86	1.86
500	509.24 ± 7.82	1.56	1.85	506.14 ± 7.92	1.58	1.23
Amniotic fluid						
2	2.05 ± 0.04	2	2.5	2.10 ± 0.06	3	5
5	5.19 ± 0.08	1.6	3.8	5.31 ± 0.16	3.2	6.2
50	52.93 ± 3.45	6.9	5.86	51.42 ± 4.21	8.42	2.84
500	514.01 ± 6.76	1.35	2.8	516.73 ± 8.02	1.6	3.35
Placental homogenate						
2	2.11 ± 0.02	1	5.5	1.99 ± 0.19	9.5	0.5
5	5.08 ± 0.07	1.4	1.6	5.26 ± 0.48	9.6	5.2
50	49.76 ± 0.59	1.18	0.48	49.97 ± 3.91	7.82	0.06
500	479.70 ± 5.97	1.19	4.06	497.44 ± 6.59	1.32	0.51
Fetal homogenate						
2	1.87 ± 0.15	7.5	6.5	2.10 ± 0.12	2.4	5
5	4.79 ± 0.24	4.8	4.2	5.01 ± 0.26	5.2	0.2
50	50.04 ± 1.98	3.96	0.8	49.68 ± 3.25	6.5	0.64
500	485.27 ± 9.16	1.83	9.46	496.66 ± 9.60	1.92	0.67

freeze/thaw cycles over the period of 4 days. Three samples were extracted and analyzed as described above. The remaining spiked matrix samples were stored at $-20°C$. Each of the following three consecutive days, the spiked matrix samples were thawed, and three more were extracted and analyzed. The freeze/thaw stability tests indicate both DDC and D4T were stable over three consecutive freeze/thaw cycles (See Table 5). The stability of extracted matrix samples in the autosampler was also evaluated. At time 0, one sample of each matrix was injected onto the HPLC column and analyzed. In another 24 h, the same sample from each matrix was injected again. The peak areas for DDC and D4T in each injection were compared. The error between each sample was $< 10\%$ for both compounds and there was no obvious changes in peak areas between each injection (See Table 6).

Table 3. The intra-day ($n = 5$, at each spiked concentration) and inter-day ($n = 15$, at each spiked concentration) precision (%R.S.D.) and accuracy (% error) of the LC-MS/MS method used to quantitate D4T in maternal plasma, amniotic fluid, placental and fetal homogenates

	Intra-day ($n = 5$)			Inter-day ($n = 15$)		
Concentration D4T added (ng/mL)	Concentration D4T found (ng/mL)	R.S.D. (%)	Error (%)	Concentration D4T found (ng/mL)	R.S.D. (%)	Error (%)
Maternal plasma						
2	2.15 ± 0.03	1.5	7.5	2.04 ± 0.03	1.5	2
5	5.31 ± 0.08	1.6	6.2	5.06 ± 0.10	2	1.2
50	52.77 ± 1.64	3.28	5.54	51.62 ± 2.59	5.18	3.24
500	519.28 ± 5.12	1.02	3.86	512.38 ± 16.27	3.25	2.48
Amniotic fluid						
2	2.06 ± 0.01	0.5	3	2.13 ± 0.18	9	6.5
5	5.08 ± 0.04	0.8	1.6	5.10 ± 0.36	7.2	2
50	51.69 ± 4.25	8.5	3.38	50.24 ± 3.55	7.1	0.48
500	508.43 ± 3.96	0.79	1.69	506.64 ± 20.47	4.09	1.32
Placental homogenate						
2	1.96 ± 0.16	8	2	1.89 ± 0.17	8.5	5.5
5	5.02 ± 0.13	2.6	0.4	4.85 ± 0.32	6.4	3
50	47.98 ± 3.47	6.94	2.02	47.69 ± 2.56	5.12	4.62
500	468.74 ± 15.28	3.06	6.25	487.66 ± 18.86	3.77	2.47
Fetal homogenate						
2	1.94 ± 0.04	2	3	1.88 ± 0.02	1	6
5	4.85 ± 0.36	7.2	3	4.75 ± 0.44	8.8	5
50	49.09 ± 0.87	1.74	1.82	48.45 ± 4.60	9.2	3.1
500	459.44 ± 12.68	2.54	8.11	466.56 ± 25.99	5.2	6.69

Animal Study

To demonstrate the utility of this assay, 3 pregnant rats were dosed with DDC and D4T at the level of 5 mg/kg. Maternal plasma, amniotic fluid, placenta, and fetal tissue were collected, extracted, and analyzed as described above. A calibration curve from each matrix was prepared on the day of analysis to calculate the concentration of DDC and D4T present in the real samples. Before analysis, each sample collected from the dosed pregnant rat was spiked to yield a concentration of 100 ng/mL of the internal standard 3TC. Figure 5 shows the mean concentration time profile of DDC and D4T in all four biological matrices of the pregnant rats. WinNonlin (Pharsight, Mountain View, CA, USA) was used to fit a non-compartment IV bolus model to the plasma data.

Table 4. The absolute, relative recovery ±S.D. ($n = 3$) and matrix effect of DDC, D4T and 3TC from maternal plasma, amniotic fluid, placental and fetal homogenates

Drugs and matrices	Absolute recovery (%)	Relative recovery (%)	Matrix effect (%)
100 ng/mL spiked maternal plasma			
3TC	78.6 ± 2.3	90.9 ± 2.6	−15.6
DDC	86.0 ± 3.7	98.5 ± 4.2	−14.5
D4T	76.8 ± 1.0	86.5 ± 1.3	−12.7
Amniotic fluid			
3TC	76.9 ± 1.5	92.1 ± 4.8	−19.8
DDC	84.6 ± 4.4	91.7 ± 3.1	−16.5
D4T	75.8 ± 3.2	89.3 ± 0.9	−17.8
Placental homogenate			
3TC	70.8 ± 4.2	82.9 ± 2.2	−17.1
DDC	72.9 ± 3.8	85.8 ± 1.4	−17.7
D4T	70.8 ± 1.9	84.8 ± 4.5	−19.8
Fetal homogenate			
3TC	79.6 ± 0.8	93.7 ± 4.7	−17.5
DDC	78.0 ± 1.3	87.6 ± 2.4	−12.3
D4T	77.9 ± 3.6	86.1 ± 2.8	−10.6

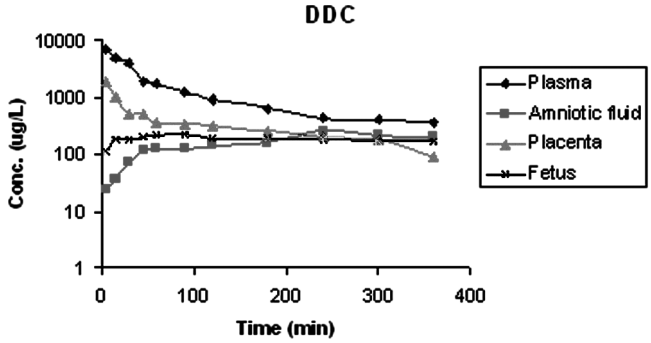
Table 5. Results of freeze/thaw stability of DDC and D4T in maternal plasma, amniotic fluid, placental and fetal homogenates ($n = 3$)

Freeze-thaw stability (3 cycles)	Observed conc. ±S.D. (ng/mL)	RSD (%)	Error (%)
100 ng/mL spiked maternal plasma			
DDC	102.5 ± 4.3	4.3	2.5
D4T	106.2 ± 2.8	2.8	6.2
Amniotic fluid			
DDC	105.7 ± 6.1	6.1	5.7
D4T	104.0 ± 8.4	8.4	4.0
Placental homogenate			
DDC	112.6 ± 6.8	6.8	12.6
D4T	113.2 ± 4.4	4.4	13.2
Fetal homogenate			
DDC	110.8 ± 8.3	8.3	10.8
D4T	108.9 ± 9.8	9.8	9.8

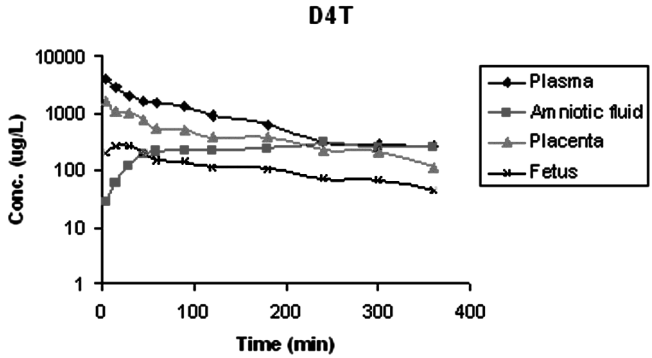
Table 6. Results of autosampler stability of DDC and D4T in maternal plasma, amniotic fluid, placental and fetal homogenates ($n = 3$)

Autosampler stability (24 h)	Observed conc. ±S.D. (ng/mL)	RSD (%)	Error (%)
100 ng/mL spiked			
Maternal plasma			
DDC	102.1 ± 3.3	3.3	2.1
D4T	101.2 ± 2.6	2.6	1.2
Amniotic fluid			
DDC	101.4 ± 4.5	4.5	1.4
D4T	100.9 ± 5.2	5.2	0.9
Placental homogenate			
DDC	105.8 ± 6.6	6.6	5.8
D4T	109.6 ± 4.8	4.8	9.6
Fetal homogenate			
DDC	106.7 ± 4.0	4.0	6.7
D4T	103.9 ± 3.6	3.6	3.9

DDC has a half-life of 155 min, a steady state volume of distribution of 1.38 L/kg, and total clearance of 0.64 L/h/kg based on the maternal plasma data. D4T has a half-life of 77 min, a steady state volume of distribution of 1.82 L/kg, and total clearance of 0.88 L/h/kg based on the maternal plasma data. Animal studies with an IV bolus administration of a dose of 25 mg/kg of DDC-D4T were also conducted for comparison to the DDC-D4T combination low dose. In that study, DDC has a half-life of 112 min, a steady state volume of distribution of 1.6 L/kg, and total clearance of 1.2 L/h/kg in the maternal plasma; D4T has a half-life of 113 min, a steady state volume of distribution of 0.6 L/kg, and total clearance of 0.34 L/h/kg in the maternal plasma. Therefore, the clearance for DDC at the higher dose was about 2 fold higher than at the lower dose but it was the opposite for D4T, which showed a 2 fold decrease in clearance at the higher dose. The steady state volume of distribution for D4T also decreased approximately 2–3 fold at the higher dose. This preliminary data showed that D4T may be up regulating transporters for DDC in the kidney; on the other hand, DDC may inhibit the active tubular secretion of D4T in the kidney. Significant differences in relative exposures ($AUC_{tissue}/AUC_{maternal\ plasma}$) of the placenta were also noticed for DDC and D4T at the two doses. The relative exposure of the placenta was significantly lower for DDC at the lower dose (0.28 with the lower dose vs. 0.6 with the higher dose). However, the opposite effect was observed for D4T, which had a higher relative exposure for the placenta at the lower dose (0.46 with the lower dose vs. 0.29 with the higher dose). No significant difference was noted in



(a)



(b)

Figure 5. Concentration vs. time profile of DDC and D4T in maternal plasma, amniotic fluid, placenta, and fetus after 5 mg/kg i.v. bolus dose of DDC and D4T ($n = 3$).

the relative exposures of the amniotic fluid for DDC and D4T. The relative exposures of both DDC and D4T in the fetus were increased with the increase in dose. The results suggested that with the increase in dose, there is an increase in the distribution of DDC into the placenta and fetus. The increase in fetal levels of D4T may due to the saturation of the efflux transporters from fetus to placenta. This preliminary data suggests that the interactions between DDC and D4T are not linear at different doses, which suggests that active transporters may play a role in placental transfer for both DDC and D4T. The fact that the transport of nucleosides appears to be non-linear also suggest that the differences may be related to regulation of the transporters. However, additional in vivo and in vitro studies will be needed to fully elucidate this mechanism.

CONCLUSION

A sensitive, efficient, and accurate LC MS/MS method was developed and validated for the simultaneous quantification of DDC and D4T in rat plasma, amniotic fluid, placental, and fetal tissues. This method was useful for pharmacokinetic studies to investigate the distribution of DDC and D4T in the maternal and fetal compartment of rats.

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