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Application of a Sensitive Liquid Chromatography-Tandem Mass Spectrometric Method to Pharmacokinetic Study of Telbivudine in Humans

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Introduction

Telbivudine is a synthetic L-nucleoside analogue, which is phosphorylated to its active metabolite, 5'-triphosphate, by cellular kinases. The telbivudine 5'-triphosphate inhibits HBV DNA polymerase (a reverse transcriptase) by competing with the natural substrate, dTTP. Incorporation of 5'-triphosphorylated-telbivudine into viral DNA obligates DNA chain termination, resulting in inhibition of HBV replication. The objectives of the current studies were to develop a selective and sensitive LC-MS/MS method to determine of telbivudine in human plasma.

Method

Sample Preparation

(1) Add 100 µL of plasma into the polypropylene tube, add 40 µL of internal standard working solution (33 µg/mL, with thymidine phosphorylase) to all other tubes.
(2) Incubate the tubes for 1 h at 37 °C in dark.
(3) Add 200 µL of acetonitrile to all tubes, seal and vortex for 1 minutes.
(4) Centrifuge the tubes for 5 minutes at 13000 rpm.
(5) Transfer 200 µL supernatant to a clean glass bottle and inject into the HPLC-MS/MS system.

LC-MS/MS Analysis

The analysis was performed on a Shimadzu Nexera UHPLC instrument (Kyoto, Japan) equipped with LC-30AD pumps, CTO-30A column oven, DGU-30A$_5$ on-line egasser, and SIL-30AC autosampler. The separation was carried out on GL Sciences InertSustain C18 column (3.0 mmI.D. x 100 mmL.) with the column temperature at 40 °C. A triple quadruple mass spectrometer (Shimadzu LCMS-8050, Kyoto, Japan) was connected to the UHPLC instrument via an ESI interface.

Analytical Conditions

HPLC (Nexera UHPLC system)

Column	: InertSustain (3.0 mmI.D. x 100 mmL., 2 µm, GL Sciences)
Mobile Phase A	: water with 0.1% formic acid
Mobile Phase B	: acetonitrile
Gradient Program	: as shown in Table 1
Flow Rate	: 0.4 mL/min
Column Temperature	: 40 °C
Injection Volume	: 2 µL

Table 1 Time Program

Time (min)	Module	Command	Value
0.00	Pumps	Pump B Conc.	5
4.00	Pumps	Pump B Conc.	80
4.10	Pumps	Pump B Conc.	5
6.00	Controller	Stop	

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MS (LCMS-8050 triple quadrupole mass spectrometer)

Ionization	: ESI
Polarity	: Positive
Ionization Voltage	: +0.5 kV (ESI-Positive mode)
Nebulizing Gas Flow	: 3.0 L/min
Heating Gas Flow	: 8.0 L/min
Drying Gas Flow	: 12.0 L/min
Interface Temperature	: 250 °C
Heat Block Temperature	: 300 °C
DL Temperature	: 350 °C
Mode	: MRM

Table 2 MRM Parameters

Compound	Precursor *m/z*	Product *m/z*	Dwell Time (ms)	Q1 Pre Bias (V)	CE (V)	Q3 Pre Bias (V)
Telbivudine	243.10	127.10	100	-26	-10	-13
Telbivudine-D3	246.10	130.10	100	-16	-9	-25

Results and Discussion

Human plasma samples containing telbivudine ranging from 1.0 to 10000 ng/mL were prepared and extracted by protein precipitation and the final extracts were analyzed by LC-MS/MS. MRM chromatograms of telbivudine (1 ng/mL) and deuterated internal standard are presented in Fig. 1 (blank) and Fig. 2 (spiked). The linear regression for telbivudine was found to be >0.9999. The calibration curve with human plasma as the matrix were shown in Fig. 3. Excellent precision and accuracy were maintained for four orders of magnitude, demonstrating a linear dynamic range suitable for real-world applications. LLOQ for telbivudine was 1.0 ng/mL, which met the criteria for bias (%) and precision within ±15% both within run and between run. The intra-day and inter-day precision and accuracy of the assay were investigated by analyzing QC samples. Intra-day precision (%RSD) at three concentration levels (3, 30, and 8000 ng/mL) were below 2.5% and inter-day precision (%RSD) was below 2.5%. The recoveries of telbivudine were 100.6±2.5 %, 104.5±1.5% and 104.3±1.6% at three concentration levels, respectively. The stability data showed that the processed samples were stable at the room temperature for 8 h, and there was no significant degradation during the three freeze/thaw cycles at -20 °C. The reinjection reproducibility results indicated that the extracted samples could be stable for 72 h at 10 °C.

Application of a Sensitive Liquid Chromatography-Tandem Mass Spectrometric Method to Pharmacokinetic Study of Telbivudine in Humans

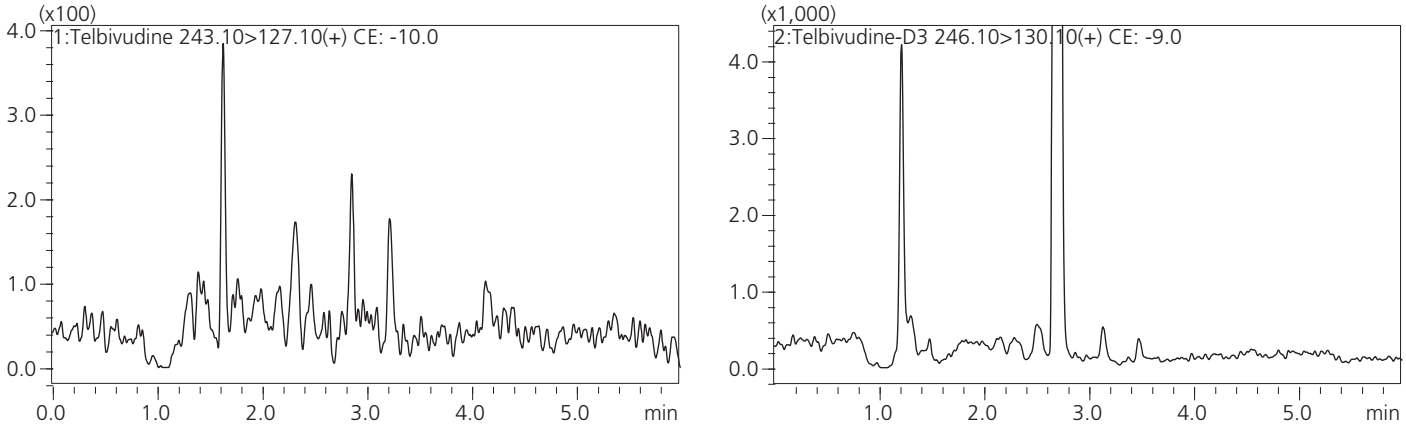

Figure 1 Representative MRM chromatograms of blank human plasma
(left: transition for telbivudine, right: transition for internal standard)

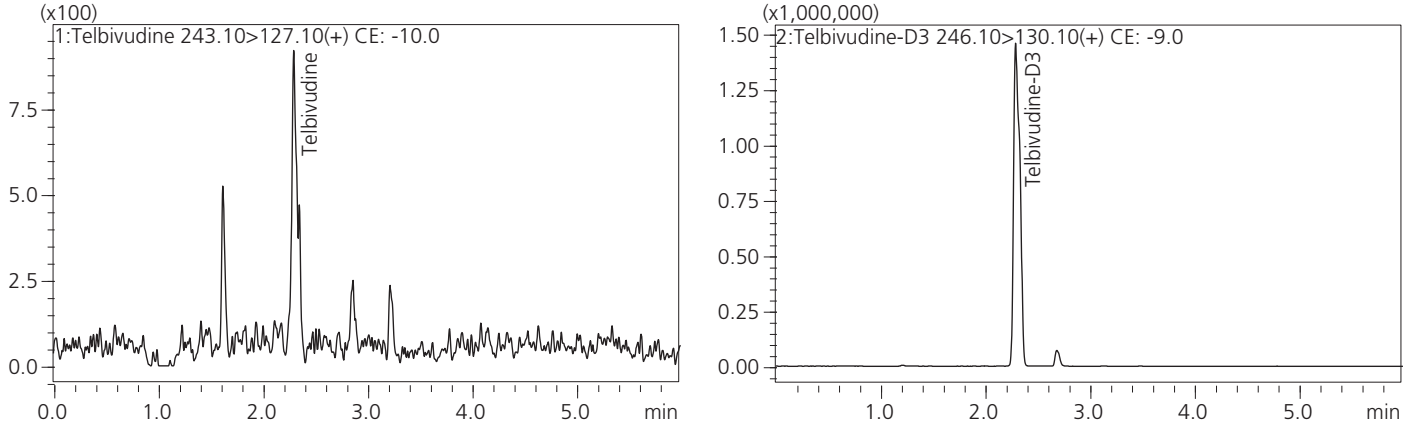

Figure 2 Representative MRM chromatograms of telbivudine (left, 1 ng/mL) and internal standard (right) in human plasma

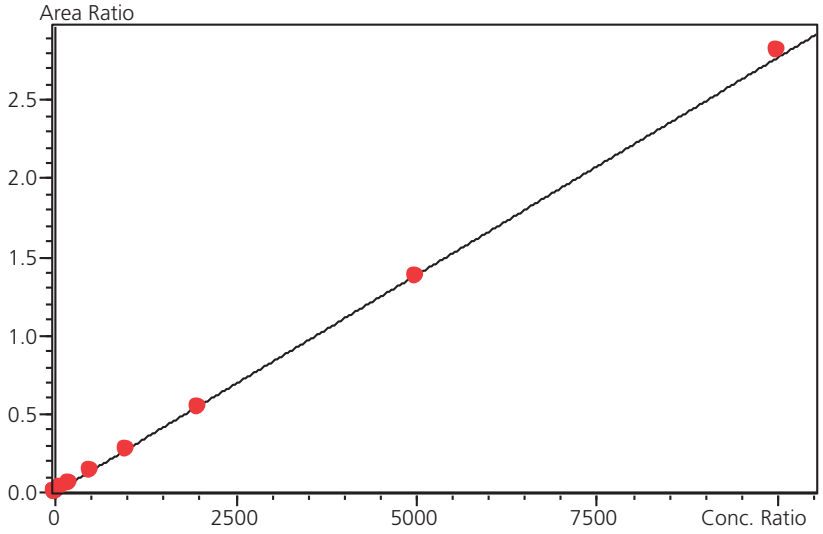

Figure 3 Calibration curve of telbivudine in human plasma

Application of a Sensitive Liquid Chromatography-Tandem Mass Spectrometric Method to Pharmacokinetic Study of Telbivudine in Humans

Compound	Calibration Curve	Linear Range (ng/mL)	Accuracy (%)	r
Telbivudine	$Y = (2.77\times10^{-4})X + (3.39\times10^{-5})$	1~10000	93.1~116.6%	0.9998

Table 3 Accuracy and precision for the analysis of amlodipine in human plasma (in pre-study validation, n=3 days, six replicates per day)

Added Conc. (ng/mL)	Intra-day Precision (%RSD)	Inter-day Precision (%RSD)	Accuracy (%)
3	2.18	2.11	107.7~114.4
400	1.52	1.58	91.6~95.9
8000	1.76	1.68	95.4~101.3

Table 4 Recovery for QC samples (n=6)

QC Level	Concentartion (ng/mL)	Recovery (%)
LQC	3	100.6
MQC	400	104.5
HQC	8000	104.3

Table 5 Matrix effect for QC samples (n=6)

QC Level	Added Conc. (ng/mL)	Matrix Factor	IS-Normalized Matrix Factor
LQC	3	82.3%	99.0%
MQC	400	81.7%	101.0%
HQC	8000	90.8%	101.5%

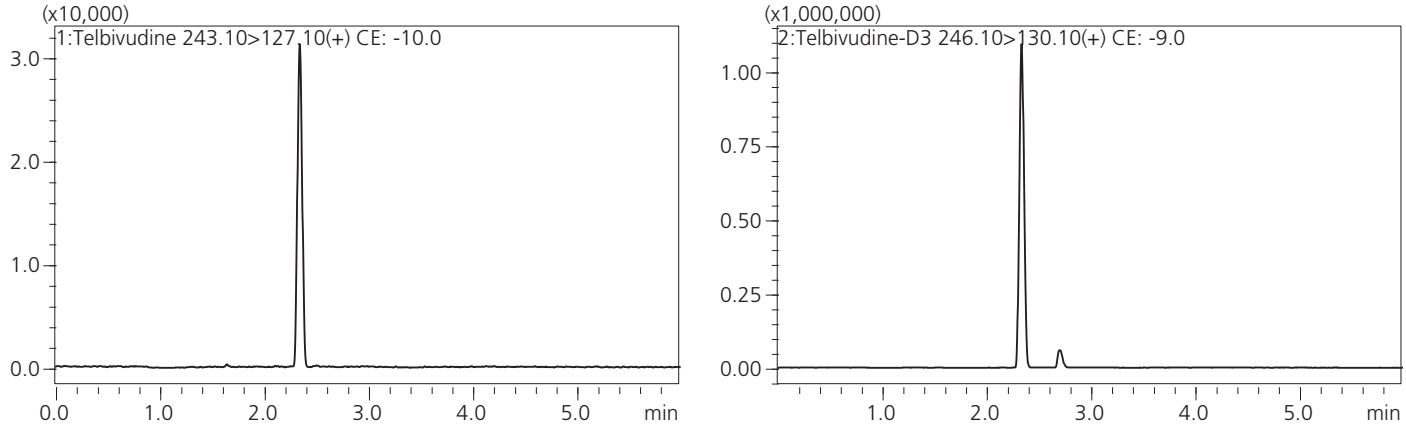

Figure 4 Representative MRM chromatograms of real-world sample

Application of a Sensitive Liquid Chromatography-Tandem Mass Spectrometric Method to Pharmacokinetic Study of Telbivudine in Humans

Conclusion

Results of parameters for method validation such as dynamic range, linearity, LLOQ, intra-day precision, inter-day precision, recoveries, and matrix effect factors were excellent. The sensitive LC-MS/MS technique provides a powerful tool for the high-throughput and highly selective analysis of telbivudine in clinical trial study.

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