

A Rapid LC-MS/MS Method for the Determination of Telbivudine in Human Plasma

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Overview

A rapid and specific liquid chromatographic-tandem mass spectrometric (LC-MS/MS) method capable of quantifying telbivudine in human plasma is described.

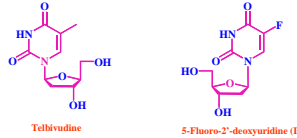
In this method, the drug was extracted from a 0.1 mL sample of plasma using a protein precipitation method. Chromatography separation was performed on a reverse phase C18 column. Detection was achieved using an AB SCIEX API-4000 tandem mass spectrometer employing turbo-ion spray ionization in the positive ion mode along with multiple reaction monitoring (MRM). The lowest limit of quantitation is 10 ng/mL.

The method has been successfully validated.

Introduction

Telbivudine, marketed as Tyzeka, is a recently approved L-nucleoside with potent and specific antiviral activity against hepatitis B virus (HBV). Compared with older lamivudine (Epivir-HBV), a significantly higher proportion of HBeAg positive patients receiving telbivudine experienced a therapeutic response. Several analytical methods have been developed for pharmacokinetic studies or clinical trials. In these methods, solid phase extraction was used in sample preparation with longer LC-MS/MS run times. To support clinical trials, a short sample preparation time and lower detection limit were required. We now report a rapid and specific liquid chromatographic-tandem mass spectrometric (LC-MS/MS) method capable of quantifying telbivudine from 0.1 mL of human plasma with a curve range from 10 to 5000 ng/mL.

Structures



Experimental

Sample Preparation

100 µL aliquots of plasma sample with 20 µL internal standard working solution were transferred into the corresponding well of a 96 deep-well plate. After the addition of 50 µL of water, the 96 deep-well was sealed with the sealing mat, followed by vortexing for about 3 min.

About 400 µL of methanol was then added followed by vortexing at high speed and centrifugation at approximately 4,000 rpm for about 10 min. Approximately 400 µL of the supernatant was transferred into a newly labeled 96 deep-well plate using a Tomtec® liquid handling system, followed by evaporation of the solvent using SPE-Dry 96® with temperature set at 35°C and nitrogen stream pressure at 45 psi.

The residues in the 96 deep-well plate were reconstituted with 200 µL of methanol:water (2:8) followed by sealing with sealing mat, vortexing at high speed and centrifugation at approximately 4,000 rpm.

Liquid Chromatography:

HPLC System Shimadzu LC-10AD
Analytical Column: Luna PFP column, 4.6 x 100 mm, 5 µm.
Mobile Phase A) Water with 10 mM ammonium formate, pH 3.5.
Mobile Phase B) Methanol
Gradient
Flow rate: 1 mL/min
Injection Volume: 10 µL

Mass Spectrometry

MS System: AB Sciex API-4000
Condition: LC/(+)-ESI-MS/MS (MRM)
MRM Transition:
 Telbivudine: 243.1 → 127.1
 5-Fluoro-2'-deoxyuridine (IS): 264.1 → 131.1

Results and Discussion

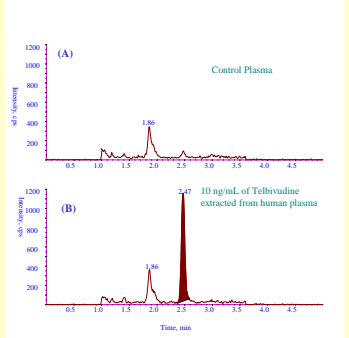


Figure 1. Ion chromatograms of blank plasma sample (A) and 10 ng/mL telbivudine extracted from plasma (B)

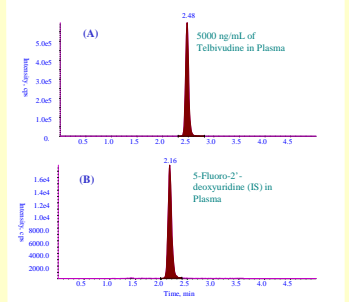


Figure 2. Ion chromatograms of 5000 ng/mL telbivudine extracted from plasma (A), and the internal standard extracted from plasma (B)

Table I. Validation Data Summary

		10 to 5000 ng/mL	
		0.9561 to 0.9987	
Accuracy & Precision	QC	Conc. (ng/mL)	Accuracy RE%
	LLOQ	10	2.00
	Low	30	-1.33
	Medium	2000	-2.50
	High	4000	0.00
			Precision CV%
			4.08
			5.54
			2.57
			2.06
Method Recovery	Compared with Nominal Value (%)		
	104.61 - 108.21		
Freeze/Thaw Stability	Condition		Accuracy RE%
	3 Cycles, 20°C		-5.67 - 7.25
Bench-Top Stability	4 hrs, Room Temperature		-3.00 - 0.75
	4 Days, Room Temperature		-7.00 - 0.58
Autosampler Extract Stability	42 Days, -20°C		-3.33 - 2.50
Long-Term Storage Stability			

Atmosphere pressure chemical ionization (APCI) and electrospray ionization (ESI) modes were tested for their response; Positive ESI was found to provide better sensitivity. The precursor ion mass spectrum and product ion mass spectrum are shown in Figure 3. The analyte and internal standard MRM transitions are listed under the LC/MS conditions. The instrument was tuned to give maximum abundance of each compound's product ion. Figure 1 presents two traces: the first shows a typical blank human plasma chromatogram, and the second shows a human plasma sample with telbivudine (10 ng/mL).

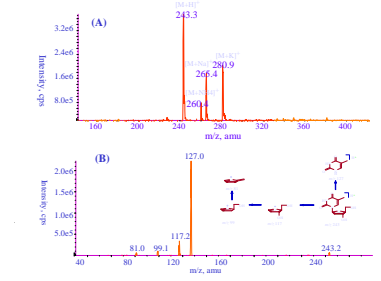


Figure 3. LC(+)-ESI-MS spectrum (A) and product ion scan (B) of telbivudine

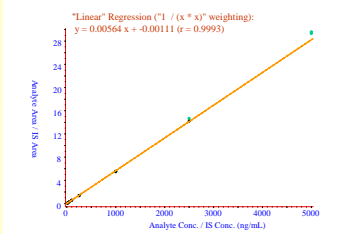


Figure 4. Representative calibration curve for the determination of telbivudine in the range from 10 ng/mL to 5000 ng/mL in human plasma

A simple, robust protein precipitation extraction method was used to prepare the samples for analysis. Sample preparation could be done quickly and consistently through the use of 96-well plates with the Tomtec® liquid handling system.

Excellent linearity was obtained with a correlation coefficient equal to or greater than 0.9961. The inter-day precision (CV%) and accuracy (RE%) was ≤5.54% and ±2.00%, respectively for all QC samples, including the lower limit of quantitation (LLOQ) (Table I). Telbivudine was found to be stable during assessment of QC samples over the following conditions: (1) Three freeze/thaw cycles and (2) storage at -20 °C for about 3 months. Extracted samples were found to be stable at room temperature for ~4 days.

Conclusions

A rapid, sensitive and selective LC-MS/MS method was developed and validated for quantifying telbivudine in human plasma. A lower limit of quantitation of 10 ng/mL was established utilizing a plasma sample volume of 0.1 mL. This method has been successfully validated.