

Overview

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

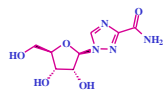
In this method, the drug was extracted from a 0.1 mL plasma sample using a protein precipitation extraction method. Separation was performed on a reverse phase C18 column. Detection was achieved using a AB/SCIEX API-4000 tandem mass spectrometer employing turbo-ion spray ionization in the positive ion mode along with multiple reaction monitoring (MRM). The lower limit of quantitation was 5 ng/mL.

The method has been successfully applied to pharmacokinetic studies in human plasma samples.

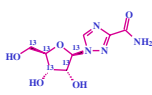
Introduction

Ribavirin, a purine nucleoside analog, has broad-spectrum activity against a variety of DNA and RNA viruses. Ribavirin/pegylated interferon- α combination has been widely used for the treatment of chronic hepatitis C disease. So far many analytical methods have been reported by using HPLC-UV for the quantitation of ribavirin in human plasma. The HPLC analysis cycle time was typically greater than 20 min. The reported sample preparation required many steps of manipulation. To support clinical trials, short sample preparation and analysis times are desirable. We report a rapid and specific LC-MS/MS method for quantifying ribavirin from 0.1 mL of human plasma with a limit of quantitation at 5 ng/mL.

Structures



Ribavirin



Ribavirin- $^{13}\text{C}_5$

Experimental

Sample Preparation

100 μL aliquots of plasma spiked with 50 μL internal standard working solution were transferred into the corresponding well of a 96 deep-well plate. After the addition of 300 μL of acetonitrile, the 96 deep-well was sealed with the sealing mat, followed by vortexing at high speed. The samples were centrifuged at approx. 4,000 rpm for 10 min. Approximately 300 μL of the supernatant was transferred into a well on a 96 deep-well plate using a TOMTEC liquid handling system, which was followed by evaporation of the solvent using SPE-Dry 96 ® with the temperature set at 40 $^\circ\text{C}$ and a nitrogen stream pressure of 45 psi.

The residues in the 96 deep-well plate were reconstituted with 200 μL of water for each well and sealed with sealing mat. The plate was vortexed at high speed and centrifuged at approx. 4,000 rpm prior to LC-MS/MS analysis.

Liquid Chromatography:

HPLC System Shimadzu LC-10AD
Analytical Column: C8 column, 4.6 x 150 mm, 3 μm
Mobile Phase A) Water with 0.1% HCOOH
Mobile Phase B) Methanol with 0.1% HCOOH
Gradient
Flow rate: 0.8 mL/min
Injection Volume: 20 μL

Mass Spectrometry

MS System: AB Sciex API-4000
Condition: LC/(+)-ESI-MS/MS (MRM)
MRM Transition:
Ribavirin: 245.1 \longrightarrow 113.1
Ribavirin- $^{13}\text{C}_5$: 250.1 \longrightarrow 113.1

Results and Discussion

Table I. Validation Data Summary

Calibration Range		5 to 500 ng/mL	
Accuracy & Precision	QC Conc. (ng/mL)	Accuracy RES	Precision CV%
Inter-Batch (n=18)	LLOQ	5	10.7
	Low	15	5.35
	Medium	200	2.39
	High	450	2.18
	Condition	Accuracy RES	
Freeze/Thaw	3 Cycles, -70°C	<10.0	
Bench-To	24 hrs, Room Temperature	<5.33	
Autosampler Extract Stability	3 Days, Room Temperature	<8.67	
Long-Term Storage Stability	31 Days, -70°C	<6.44	

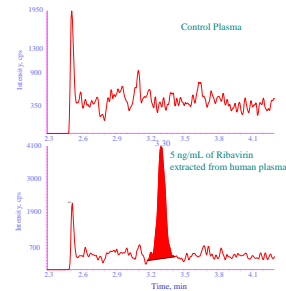


Figure 1. Ion chromatograms of blank plasma sample (A) and 5 ng/mL ribavirin extracted from plasma (B)

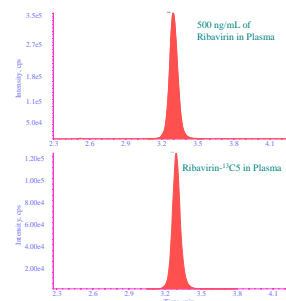


Figure 2. Ion chromatograms of 500 ng/mL ribavirin extracted from plasma (A), and the internal standard extracted from plasma (B)

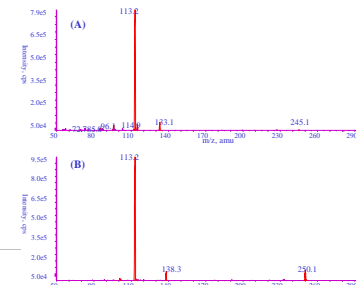


Figure 3. LC/(+)-ESI-MS/MS product ion spectra of ribavirin (A) and ribavirin- $^{13}\text{C}_5$ (B)

- In most previous work, sample preparation required many steps of manipulation including: dilution with water, filtration with 30,000 Da cut-off, extraction with dichloromethane, filtration through a double-bed column, elution, evaporation to dryness and reconstitution. These methods required more time and would be more susceptible to more error in the preparation procedure.

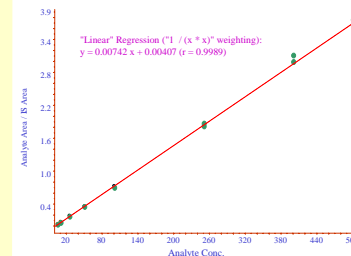


Figure 4. Representative calibration curve for the determination of ribavirin in the range from 5 ng/mL to 500 ng/mL in human plasma

- The current method used protein precipitation, a 96-well plate format with the TOMTEC liquid handling system to make sample preparation quick and consistent.

- Excellent linearity was obtained with a correlation coefficient greater than 0.9989. The inter-day precision (CV%) and accuracy (RE%) for all QC plasma samples, including LLOQ were $\leq 10.7\%$ and $\leq 8.00\%$, respectively (Table I). Three freeze/thaw cycles, ambient temperature storage for up to 24 h prior to analysis, and 1 month long-term storage at -20°C appeared to have little effect on the quantitation.

Conclusions

A rapid and specific LC-MS/MS method was developed and validated for quantifying ribavirin with a lower limit of quantitation of 5 ng/mL from a 0.1 mL human plasma sample.