Validation of an LC-MS/MS Method for the Determination of Ribavirin in Human Plasma (EDTA)

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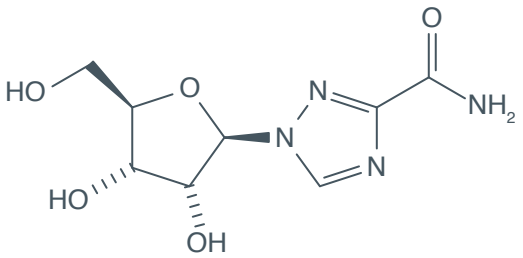
Overview

- A method for the measurement of ribavirin in human plasma (EDTA) has been validated
- A phenylboronic acid (PBA) SPE was used for the selective extraction of ribavirin
- A HILIC mode of separation was used for the chromatographic separation
- Chromatographic separation of ribavirin from an endogenous isomer was achieved
- The method was shown to be selective against peginterferon alpha-2a

Ribavirin



Introduction

Ribavirin is a purine nucleoside analog that exhibits broad activity against DNA and RNA viruses. Ribavirin is typically co-administered with peginterferon alpha-2a and is considered first line therapy in the treatment of chronic hepatitis C. There are numerous reports of methods for ribavirin analysis in plasma. Most of these methods use reversed phase separations for both sample extraction and chromatographic separation. Due to the high polarity of ribavirin, the conditions for reversed phase retention mechanisms are not always ideal. Protein precipitation is a more convenient approach for sample extraction but it can lead to ion matrix effects in the chromatography. To address the concerns stated above, a rugged, robust, accurate and precise method for the measurement of ribavirin in human plasma was developed employing an automated highly specific solid phase extraction procedure and HILIC chromatography.

Methods

Sample Preparation

1. Condition Varian Bond Elut PBA, 100 mg/well SPE plate with acetonitrile and a pH 9.0 buffer
2. Load sample (100 µL sample, 50 µL ISTD and 1 mL pH 9.0 buffer)
3. Wash SPE with acetonitrile and an acetonitrile/water mixture
4. Elute SPE with acidified methanol
5. Drydown extracts and reconstitute
6. Submit for LC-MS/MS analysis

HPLC

Column: Waters Atlantis HILIC

Mobile Phase: ACN:H_2O:HCOOH

Run time: 2.5 minutes

Retention time: 1.7 minutes

LC-MS/MS

Mass spectrometer: API 4000

Source: ESI+

Resolution: Unit

Ions monitored: Ribavirin (245.1 – 113.1 m/z)
^{15}N-d_2-Ribavirin (248.1 – 114.1 m/z)

Results

Table 1.

Inter-Batch Statistics for Precision and Accuracy

	LLOQ QC 10.0 ng/mL	QC A 30.0 ng/mL	QC B 200 ng/mL	QC C 1500 ng/mL
Inter-Batch Mean	10.6	30.5	205	1510
Inter-Batch SD	1.01	1.48	4.93	27.4
Inter-Batch % CV	9.5	4.9	2.4	1.8
Inter-Batch % Bias	6.0	1.7	2.5	0.7
n	30	30	30	30

Table 2.

Matrix Effect for Ribavirin in Human Plasma (EDTA)

		LLOQ		High	
	Lot#	10.0 ng/mL	% Bias	1500 ng/mL	% Bias
	1	9.38	-6.2	1470	-2.0
	2	9.47	-5.3	1460	-2.7
	3	9.16	-8.4	1480	-1.3
	4	10.2	+2.0	1460	-2.7
	5	9.83	-1.7	1470	-2.0
	6	9.62	-3.8	1450	-3.3
	7	9.58	-4.2	1490	-0.7
	8	9.58	-4.2	1460	-2.7
	9	9.38	-6.2	1530	+2.0
	10	8.81	-11.9	1500	+0.0
Mean		9.50		1480	
% CV		3.9		1.6	
% Bias		-5.0		-1.3	
n		10		10	

Table 3.

Co-Administered Compound Evaluation of the Method for Ribavirin in Human Plasma (EDTA) Against Peginterferon Alpha-2a

	30.0 ng/mL	1500 ng/mL
	27.9	1560
	29.5	1550
	28.5	1550
	32.0	1550
	30.3	1560
	29.6	1540
Mean	29.6	1550
%CV	4.9	0.5
% Bias	-1.3	3.3
n	6	6

Figure 1. Representative Chromatograms of Ribavirin and $^{15}N,d_2$-Ribavirin (IS)

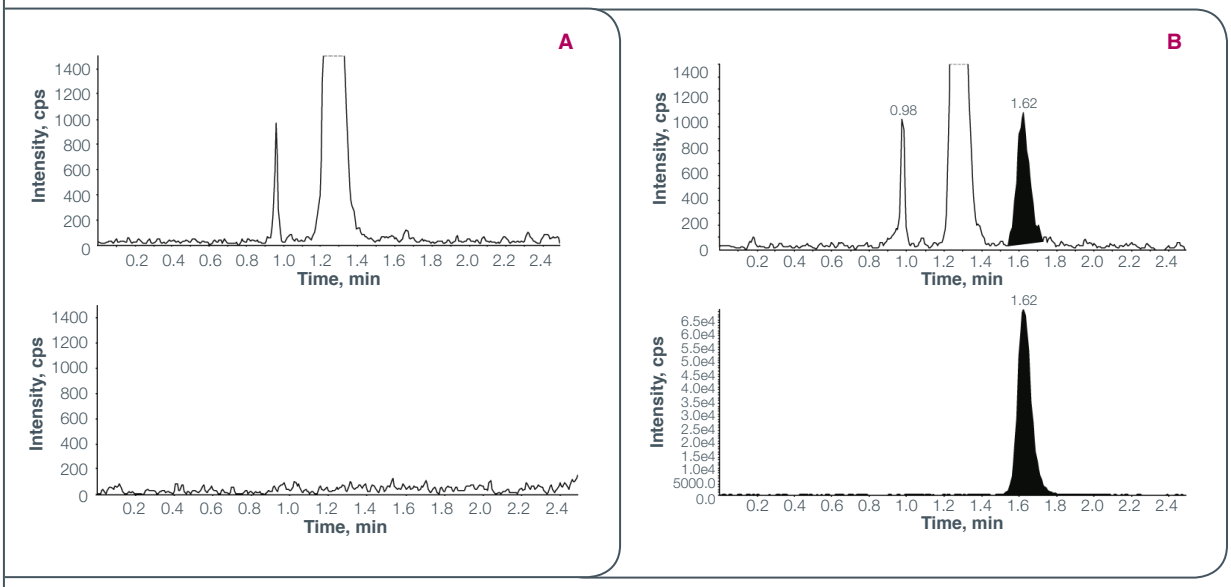
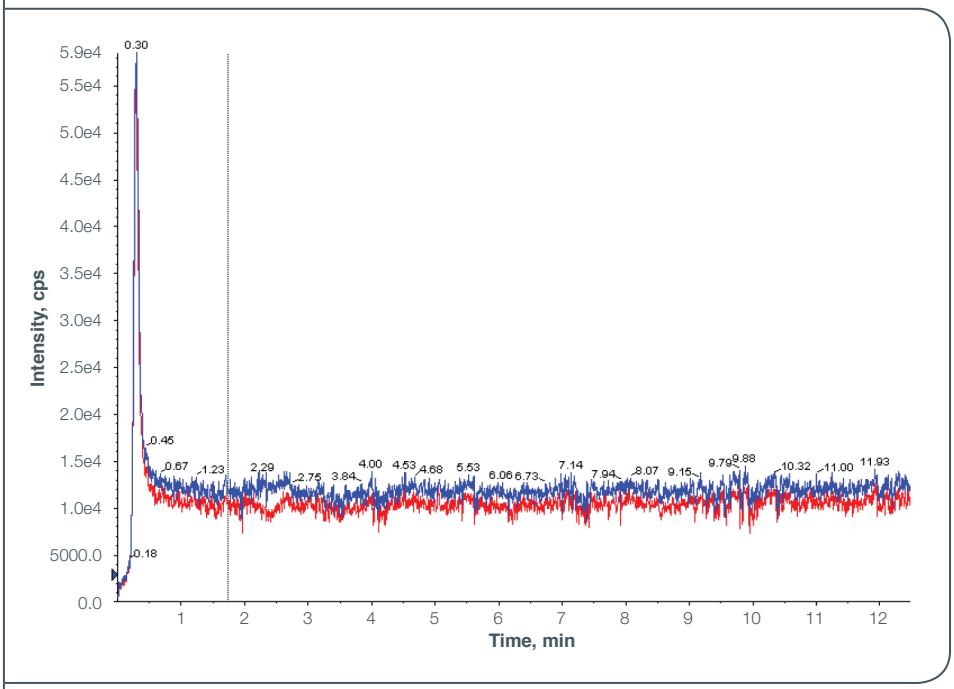
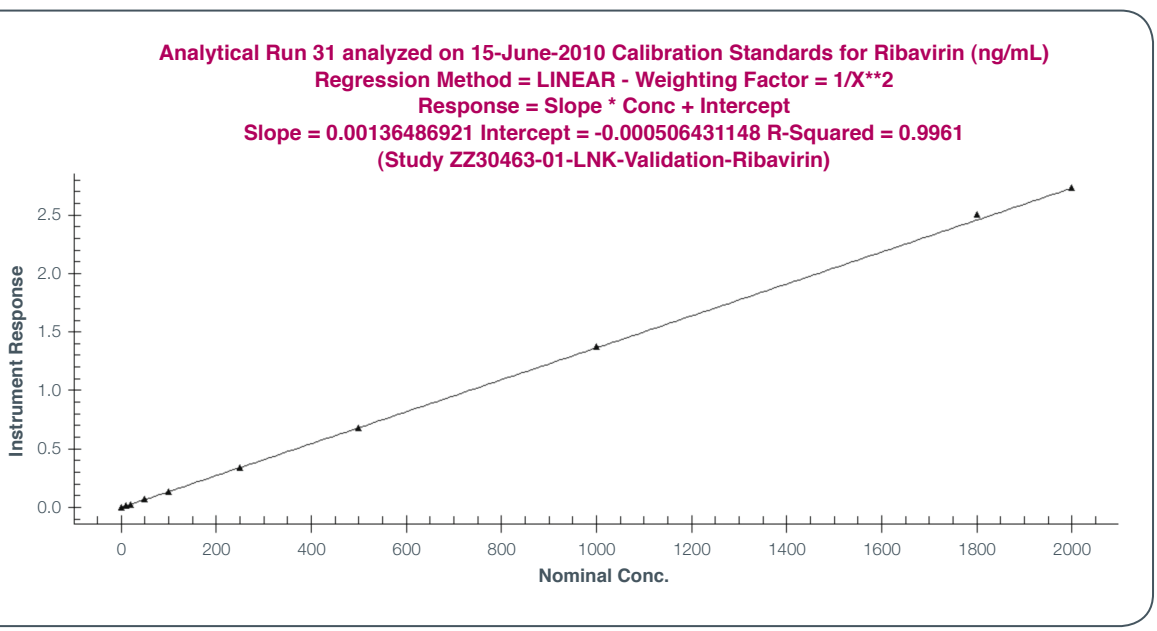


Figure 2. Post-Column Matrix Infusion Test for Ribavirin and $^{15}N,d_2$-Ribavirin (IS) from an Extracted Human Plasma (EDTA) Blank Sample





Validation Summary

Assay Volume Required	0.100 mL
Standard Curve Range	10.0 – 2000 ng/mL
Dilution Integrity	Up to 10,000 ng/mL
Regression Type	Linear (1/concentration^2)
Batch Size	192 injections
Mean Extraction Recovery	55%
Short-term Stability	Ambient Temperature, 52 hours
Freeze and Thaw Stability	6 cycles in polypropylene tubes at -80°C
Processed Sample Integrity	123 hours in polypropylene 96 well plate at 5°C
Post-preparative Stability	154 hours in polypropylene 96 well plate at 5°C
Sample Collection and Handling Stability	Up to 90 minutes on ice-water bath Up to 60 minutes at 5°C

Conclusion

A rugged, robust, accurate and precise method for the measurement of ribavirin in human plasma was developed. This method was further enhanced using robotics for the sample processing that greatly increased method throughput. The PBA extraction provided excellent sample clean-up whereby no significant ion matrix effects were observed. The HILIC chromatography provided adequate separation of ribavirin from other endogenous analogs of ribavirin. This method has successfully been used to support clinical studies.