CAPSULES notes and applications from BASi

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Rifampin

Method for LC-MSMS Analysis of Rifampin in Human Plasma Using Liquid-Liquid Extraction

Introduction

BASi has developed and validated an original LC-MSMS method for the analysis of rifampin in human plasma over the range of 200 - 20,000 ng/mL This method uses liquid-liquid extraction and LC-MSMS. The result is a reliable, robust method that provides accurate results with quick turnaround.

Rifampin is an antibiotic used in the treatment of tuberculosis. It is also used to eliminate the meningococcus microorganism from carriers and to treat leprosy, or Hansen's disease. Rifampin, or rifampicin, acts by inhibiting protein synthesis in sensitive cells. Because resistant microorganisms emerge during treatment, rifampin is used along with other drugs, e.g., with isoniazid for tuberculosis treatment and with dapsone and clofazimine in the treatment of leprosy.

It is a common practice to add ascorbic acid, an antioxidant, to the plasma samples for rifampin stability. BASi's method is validated with and without the presence of ascorbic acid. Results indicate that at higher concentrations, ascorbic acid is actually detrimental to the stability of rifampin.

This method has been used to successfully assay over 800 samples from clinical studies.

Method Summary

Plasma samples are extracted with methyl-*t*-butyl ether after the addition of internal standard and phosphate buffer. The organic layer is evaporated under nitrogen and the samples reconstituted. The analytes are separated isocratically on a C18 column using a 55% methanol in ammonium formate mobile phase at a flow rate of 0.5 mL/min. Detection is by MSMS with an electrospray interface in positive ion mode.

Method Results

Plasma samples with and without the addition of ascorbate were evaluated for accuracy and precision over the range of 200 - 20,000 ng/mL. The validation data indicate excellent precision and accuracy without the need to add ascorbate as shown in **T1** and **T2**.

Quality control (QC) samples were prepared with rifampin at five different levels over the range of the assay. QC samples (LLOQ, 3x LLOQ, one mid-range, one high-level, and ULOQ) were injected (n = 6) in each of the three validation runs. Excellent accuracy and precision were observed for these QC samples as shown in **T3**.

Ruggedness and Selectivity

The ruggedness of the rifampin method was examined by subjecting spiked QC samples to normal sample handling challenges. Frozen, freeze-thaw, bench top, heat treatment (for deactivation of the HIV virus), and autosampler stability were examined. In all cases the method maintained the requisite precision and accuracy. Over-curve samples were diluted 10-fold into the range of the curve with excellent accuracy and precision.

It is important to develop methods that are specific for the analyte of interest. Our method for rifampin was examined for selectivity and possible interferences. No matrix interferences were observed in six different lots of blank human plasma. In addition, isoniazid and ascorbate along with a panel of over-the-counter drugs did not interfere with the assay. Representative chromatograms are shown below in **F1** (on back).

T1. Between-run (n = 6) accuracy and precision for extracted standards.

	Rifampin	
Concentration (ng/mL)	**Mean**	**CV**
200	110%	3.3%
300	98.3%	9.1%
500	92.4%	7.4%
1000	96.2%	6.9%
3000	10.1%	7.9%
5000	101%	7.1%
10,000	99.7%	6.1%
20,000	100%	2.9%

T2. Coefficient of determination for standard curves.

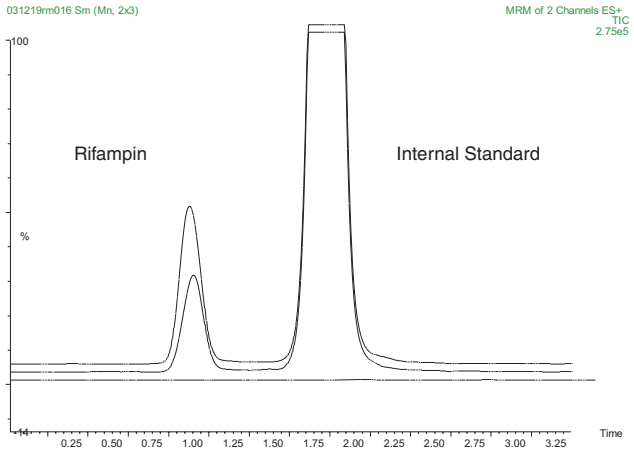
	r^2
Run 1	0.999
Run 2	0.997
Run 3	0.998

T3. Between-run accuracy and precision for extracted QC samples.

	Rifampin	
Concentration (ng/mL)	**Mean**	**CV**
200	102%	16%
500	94.0%	12%
5000	104%	7.2%
15,000	104%	7.6%
20,000	100%	6.8%

F1. Example chromatograms.

Overlay (Blank, LLOQ, 3x LLOQ)



ULOQ Standard (20,000 ng/mL)