CAPSULES notes and applications from BASi

October 2004

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Rapamycin

Method for LC/MS/MS Analysis of Rapamycin (Sirolimus) in Human Whole Blood Using Protein Precipitation and Column Switching

Introduction

BASi has developed and validated an original LC/MS/MS method for the analysis of rapamycin in human whole blood. This method uses protein precipitation and column switching for quick sample preparation. Processing a sample volume of only 0.1 mL yields a detection limit of 0.1 ng/mL.

Rapamycin, also known as sirolimus, is a second-generation immunosuppressive agent marketed as Rapamune® used for transplant pharmacotherapy. Structurally, rapamycin is a macrocyclic lactone resembling tacrolimus but with a novel mechanism of action. Therefore, rapamycin is believed to act in synergy with cyclosporin (or tacrolimus) in suppressing the immune system.

Rapamycin is often administered, with cyclosporin and corticosteroids, for the prophylaxis of organ rejection in patients receiving renal transplants. This drug has been in trials investigating its use in the prophylaxis of rejection for other organ transplants, such as the heart, lung, skin and bone marrow. Prolongation of allograft survival in mice, rats, pigs, and/or primates has been shown with studies in experimental models. Rapamycin has been shown to reverse acute rejection of heart and kidney allografts in rats.

BASi chose whole blood as the sample matrix for this highly sensitive LC/MS/MS analysis method. The distribution of macrocyclic lactones such as rapamycin is highly temperature-dependent. The fraction of total blood macrocyclic lactone concentration in plasma increases with temperature. Considering this temperature dependency, whole blood is presently the preferred matrix for the therapeutic monitoring of total macrocyclic lactone immunosuppressive agents because storing blood at different temperatures does not alter the total blood concentration.

Protein precipitation with online column switching makes for simple sample preparation, a robust and reliable method, a lower limit of quantitation (lower than other methods previously reported in the literature), and accurate results with fast data generation.

Method Summary

The method for the analysis of rapamycin in human whole blood involves internal standard (IS) / precipitating solution to 100 µL of whole blood. Samples are vortexed and centrifuged. Supernatant is analyzed by injection onto a reversed-phase guard column for cleanup. Following the elution to waste of the major polar components of the sample the flow is back-flushed through the guard column to the analytical column. After peak elution, the injector flow is switched back to waste and the system is re-equilibrated. The mobile phase contains low molar NaCl to favor the formation of the sodium adduct of rapamycin and the internal standard in the MS interface. Run time is about 6 min. The range of the assay is 0.1 – 100 ng/mL in human whole blood.

Method Results

Data in **T1** show that standard calibrator accuracy, achieved by a quadratic curve fit with $1/x^2$ weighting, was excellent and well within the 15% guidelines. Calibration parameters remained steady over the course of seven independent sequences.

The between-run accuracy and precision of validation samples, prepared at four different levels (LLOQ, 3 X LLOQ, mid-level, ULOQ) with an n=6 replicates for 3 different sequences, were excellent as shown in **T2**.

T1. Between-run (n=14) accuracy and precision for standard calibrators.

Concentration	Rapamycin	
(ng/mL)	Mean (ng/mL)	CV (%)
0.1	0.102	3.4
0.3	0.287	5.3
0.75	0.728	4.3
1.0	0.947	4.9
3.0	3.10	4.9
7.5	7.49	4.4
10.0	10.3	3.7
30.0	31.4	5.4
75.0	76.2	3.4
100.0	97.5	3.6

T2. Between-run (n=18) accuracy and precision for validation samples.

Concentration	Rapamycin	
(ng/mL)	Mean (ng/mL)	CV (%)
0.1	0.104	14.0
0.3	0.305	10.0
10.0	10.8	5.4
100.0	96.9	5.0

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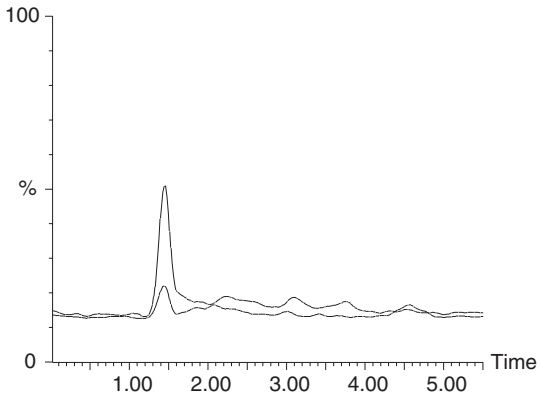
Ruggedness and Selectivity

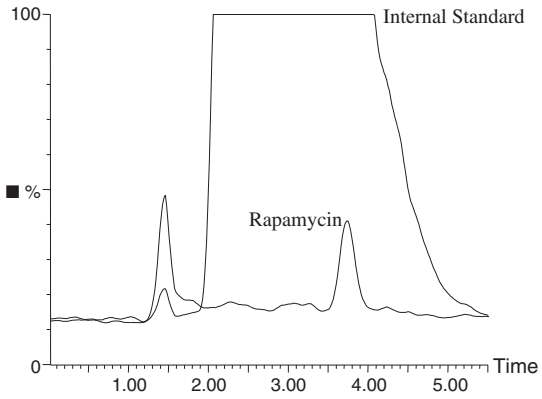
The ruggedness tests of freeze/thaw stability, heat treatment stability (for deactivation of the HIV virus), short-term room temperature stability, and autosampler stability were performed with acceptable accuracy and precision. Dilutions for over-curve samples were made at 100-fold with blank matrix and 5-fold smaller sample size with acceptable accuracy and precision.

The selectivity of the method was examined by testing six different lots of whole blood, and a panel of eight over-the-counter (OTC) drugs. No matrix interferences were detected from between the six different lots of whole blood and no interference was detected for any of the OTC drugs. Representative chromatograms are shown in **F1** through **F4**.

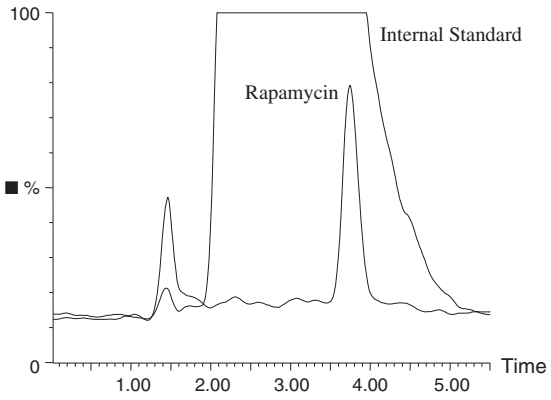
F1. Extracted Blank (Same Scale as LLOQ)



F2. LLOQ Validation Sample



F3. 3X LLOQ Sample (0.3 ng/mL)



F4. Intermediate Validation Sample